# Kānuka Honey for the Treatment of Herpes Simplex Labialis within a Novel Community Pharmacy-Based Network

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# ABSTRACT

## BACKGROUND

Herpes simplex virus infection is common with an estimated global prevalence of over 90%. Of those harbouring the dormant virus, around one third suffer from recurrent episodic reactivation on the vermillion border of the lip, leading to herpes simplex labialis (HSL), or a 'cold sore'. The painful lesions associated with HSL are treated with a range of therapeutic approaches globally, from pharmaceuticals to complementary and alternative medicines (CAM), many of which have a limited or absent evidence base for efficacy and safety. Honey is one such CAM product with growing evidence for efficacy in wound healing, often applied topically for various dermatological ailments and of interest in the management of HSL.

Obtaining robust evidence of the efficacy and safety of novel therapies for HSL is a complex and costly undertaking, traditionally requiring large randomised controlled trials (RCT), and as such has been limited to pharmaceutical funded drug development programmes. Given the paucity of high-quality evidence for many CAM therapies there is a clear need for a novel research methodology to overcome the existing barriers and ensure the timely recruitment of participants with an acute episode of HSL and the collection of valid, standardised outcome data at a modest cost.

## RESEARCH AIMS

The primary aim was to investigate the effectiveness, safety and tolerability of a kānuka honey/glycerin cream compared to 5% aciclovir cream in the treatment of HSL. The secondary aim was to establish whether it was feasible to conduct an adequately

powered, interventional RCT which includes electronic capture and transmission of data within a New Zealand wide network of community pharmacies, the Pharmacy Research Network (PRN).

## METHODS

A phase III, open label, RCT of adults aged 16 and over presenting to a community pharmacy with an acute episode of HSL was conducted between September 2015 and December 2017. Participants were recruited within 72 hours of the onset of symptoms, and randomly allocated to either the kānuka honey/glycerin formulation or 5% aciclovir cream, to be applied five times daily until skin returned to normal or study day 14, whichever occurred first. All outcome data were collected remotely using a customised digital system. The primary outcome was time taken for skin to return to normal at the site of the HSL lesion, analysed by Kaplan-Meier estimates with 95% Confidence Internal (CI). The PRN was established for the purpose of undertaking this trial.

## FINDINGS

952 participants with HSL presenting to one of the 76 pharmacies within the PRN were randomised. For the primary outcome variable of time for skin to return to normal, there was no significant difference between kānuka honey/glycerin and 5% aciclovir cream, 9 (95% CI 8 to 9) vs 8 (95% CI 8 to 9) days respectively, Hazard Ratio (HR) (95% CI) 1.06 (0.92 to 1.22) P=0.56. There was no difference between treatments for all secondary outcomes, including healing time to ulceration, healing time from ulceration to resolution, time to resolution of pain, maximal pain, acceptability and adverse events.

The PRN was shown to be a robust clinical research infrastructure, able to fully recruit a large-scale randomised trial at fractional cost of traditional models with acceptable recruitment, attrition and deviation rates.

## Conclusion

In one of the largest RCTs of a therapy in HSL, there was no evidence of superior effectiveness for either kānuka honey/glycerin or 5% aciclovir cream. This unique, real-world PRN using electronic capture and transmission of data provides a model for future research of CAM in the community setting.

# CONTRIBUTIONS

I have drafted the thesis presented within this document in its entirety. The concept of an established Pharmacy Research Network (PRN) placed to conduct randomised trials in the community setting was my own, as was the responsibility in progressively setting up the required infrastructure as part of this doctorate. I developed and drafted all study related materials including the protocol, case-report forms, participant information sheet and consent form, participant diaries and recruitment paraphernalia, both in paper and digital format. I drafted and controlled the regulatory submissions and reports to the New Zealand Health and Disability Ethics Committee, Standing Committee On Therapeutic Trials and registered the study with the Australia New Zealand clinical Trials Registry. I conceived and developed the digital process for data collection, configuring and maintaining all systems. I conducted all site initiations in person at all the 76 pharmacies active during the recruitment phase of the study and fostered these relationships to establish the PRN as a long-term capability. In a shared capacity with my colleague and friend Joe Singer, who joined the study team mid-way, I undertook responsibility for daily data integrity checking and participant follow-up. Together, Joe and I closed out all study sites in person. As study physician I undertook responsibility for the medical aspects of the study and directly reviewed all adverse events and concomitant medications, consulting with participants directly where required, drawing on the expert supervision of Professors Richard Beasley and Irene Braithwaite. Professor Mark Weatherall, the study biostatistician, undertook the statistical analysis of the randomised trial following which I undertook responsibility for interpretation of publication of the data. The manuscript publishing the results of the randomised trial was drafted by myself with input and review from Joseph Singer, Irene Braithwaite, Nick Shortt, Darmiga Thayabaran, Melanie McConnell, Mark Weatherall and Richard Beasley.

# PUBLICATIONS

The following manuscripts have been published to date as a direct output from this thesis:

- Semprini, A. Singer, J. Braithwaite, I. Shortt, N. Thayabaran, D. McConnell, M. Weatherall, M. Beasley, R. Kānuka honey versus aciclovir for the topical treatment of herpes simplex labialis: a randomised controlled trial. BMJ Open. 2019 May 14;9(5):e026201

- Semprini, A. Singer, I. Shortt, N. J. Braithwaite. I. Beasley, R. Protocol for a randomised controlled trial of 90% kānuka honey versus 5% aciclovir for the treatment of herpes simplex labialis in the community setting. BMJ Open. 2017 Aug 3;7(8):e017766.

- Armour M, Semprini A, Ee C, MacCullagh L, Shortt N. Efficacy of a topical herbal and mineral formulation (Dynamiclear) for the treatment of herpes simplex labialis in the community setting: study protocol for a randomised, double-blind placebo-controlled trial. BMJ open. 2020 Jan 12;10(1):e031876

In addition, the following two papers were published during the PhD period, focused on the same kānuka honey and glycerin formulation, Honevo.

- Semprini A, Fingleton J, Hunt A, Riley J, Corin A, Tofield C. A single-blind randomised controlled trial of topical kānuka honey for the treatment of nappy rash. Focus on Alternative and Complementary Therapies. 2015;20(3–4):187–8.
- Semprini A, Braithwaite I, Corin A, Sheahan D, Tofield C, Helm C, et al. Randomised controlled trial of topical kānuka honey for the treatment of acne. BMJ open. 2016 Feb 1;6(2):e009448

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To my family in the UK and beyond who have maintained interest and pride in the small wins from the last few years, in particular my parents Mandy and Chris who carried me through some dark rifts and ensured there was always a means to re-board the academic ship when I fell off and in 2005 for bringing me back from drowning. They can finally relax after watching 35 years of study. Until the MBA. Thank you.

To all my friends at Kenilworth and NA around the world, without which I would not have the recovery and identity it helped transform. Thank you.

Most importantly my wife Ruth, who put up both with my away with the fairies thinking in conjuring up new directions, excuses and procrastination, creating me the child free space to travel and write whilst completing her own PhD, taking the New Zealand clinical exams and having three under threes. To my three soon be over threes, Jack and his twin sisters Rosalie and Matilda, who provided a reality check as to that which is important and balanced my propensity for imbalance. It seems fitting that Jack starts school the month I submit. Thank you.

A Semprini, August 2020.

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| | |
|---|---|
| ACV | Aciclovir |
| AE | Adverse Event |
| ANZCTR | Australian New Zealand Clinical Trials Registry |
| APC | Annual Practicing Certificate |
| API | Application Programming Interface |
| CAM | Complementary and Alternative Medicine |
| CD4 | Cluster of Differentiation-4 |
| CDISC | Clinical Data Interface Standards Exchange |
| CI | Confidence Interval |
| CONSORT | Consolidated Standards of Reporting Trials |
| CTFA | Cosmetic Toiletry and Fragrance Association |
| DDS | Dioctyl Sodium Sulphate |
| DNA | Deoxyribonucleic Acid |
| DSMB | Data and Safety Monitoring Board |
| eCRF | Electronic Clinical Case Report Form |
| ECRIN | European Clinical Research Infrastructure Network |
| EMA | European Medicine Authority |
| FDA | Food and Drug Administration |
| GCP | Good Clinical Practice |
| GMP | Good Manufacturing Practice |
| HDEC | Health and Disability Ethics Committee |
| HIV-1 | Human Immunodeficiency Virus-1 |
| HPN | High Performance Network |
| HR | Hazard Ratio |
| HSL | Herpes Simplex Labialis |
| HSV | Herpes Simplex Virus |
| ICH | International Conference for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use |
| IFN | Interferon |

| IL | Interleukin |
|---|---|
| IQR | Inter-Quartile Range |
| LAT | Latency Associated Transcript |
| MGO | Methylglyoxal |
| MRINZ | Medical Research Institute of New Zealand |
| mRNA | Messenger Ribonucleic acid |
| PDF | Portable Document File |
| PI | Principal Investigator |
| PISCF | Participant Information Sheet and Consent Form |
| PRN | Pharmacy Research Network |
| RAG-M | Research Advisory Group-Māori |
| RCT | Randomised Controlled Trial |
| RR | Risk Ratio |
| SAE | Severe Adverse Event |
| SCOTT | Standing Committee On Therapeutic Trials |
| SGAT | Subjective Global Assessment of Treatment |
| SMS | Short Messaging Service |
| TGA | Therapeutic Goods Authority |
| UMF | Unique Mānuka Factor |
| UV | Ultra Violet |
| VCZ | Varicella Zoster |
| WHO | World Health Organisation |
| WTTCGE | Wisteria floribunda, Trapa natans, Terminalia chebulae, Coicis lachryma-jobi, Ganoderma lucidum and Elfuinga applanata |

# 1 CHAPTER ONE - INTRODUCTION

## 1.1 THESIS OUTLINE

This work is formed of two main sections, each of which tests the hypothesis: that an adequately powered randomised controlled trial (RCT) to assess the effectiveness of a medicinal grade kānuka honey in the management of herpes simplex labialis (HSL) is feasible within a novel, community pharmacy-based research network.

First, the pathophysiology and management strategies of HSL, colloquially known as a cold sore, are reviewed with a specific focus on topical aciclovir and complementary and alternative (CAM) therapies, in particular the medicinal use of honey. This forms the rationale behind the RCT subsequently presented which seeks to test whether New Zealand kānuka honey offers superior effectiveness in the topical management of HSL compared to a standard treatment, 5% aciclovir cream.

Second, the methodological barriers to conducting robust, randomised research into the use of CAM therapies for over-the-counter ailments are discussed and how these led to the inception and evolution of a globally unique, pharmacy research network (PRN) anchored in 76 community pharmacies throughout the north and south islands of New Zealand. The development of this network formed the methodological basis of the hypothesis, that an RCT was feasible within a remotely managed, national network of research trained community pharmacies.

With the results of the clinical study dependent on the success of the novel methodology the two are intertwined and described throughout.

## 1.2 Herpes simplex labialis

Herpesviridae are perhaps the most studied of viruses. As with many other contemporary disease terminologies, the term *herpes* was originally used by Hippocrates around 1500 BC and in Greek literally means to creep, likely referencing his observations of the natural history of infection.[1] Progressive study of genitourinary disease over the past two centuries established that Herpes Simplex Virus (HSV) was both infectious and lifelong, followed by the discovery of two distinct subtypes and their taxonomic classification as herpes simplex viruses 1 and 2 (HSV-1 and HSV-2).[2]

HSV-1 and HSV-2 are members of the alpha-subfamily of herpesviruses that also includes another important human pathogen, varicella-zoster (VCZ). These highly virulent organisms are responsible for commonly encountered clinical manifestations such as facial herpes (primarily HSV-1), genitourinary herpes (primarily HSV-2), chicken pox (VCZ) and shingles (VCZ). Structurally HSV-1 and -2 each contain a double stranded deoxyribonucleic acid (DNA) core, an icosahedral protein shell or capsid and an outer lipid layer within which glycoproteins sit, responsible for host cell interaction and subsequent penetration. Once located intracellularly, the HSV virion migrates toward the nucleus where viral DNA is released for transcription by the host machinery. Viral messenger ribonucleic acid (mRNA) is translated into viral proteins and capsid assembly proceeds with incorporation of replicated viral DNA and glycoprotein embedded plasma membrane endocytosis resulting in complete virion release from the host cell. The overall process (Figure 1) takes in the region of 18 to 20 hours.[3]
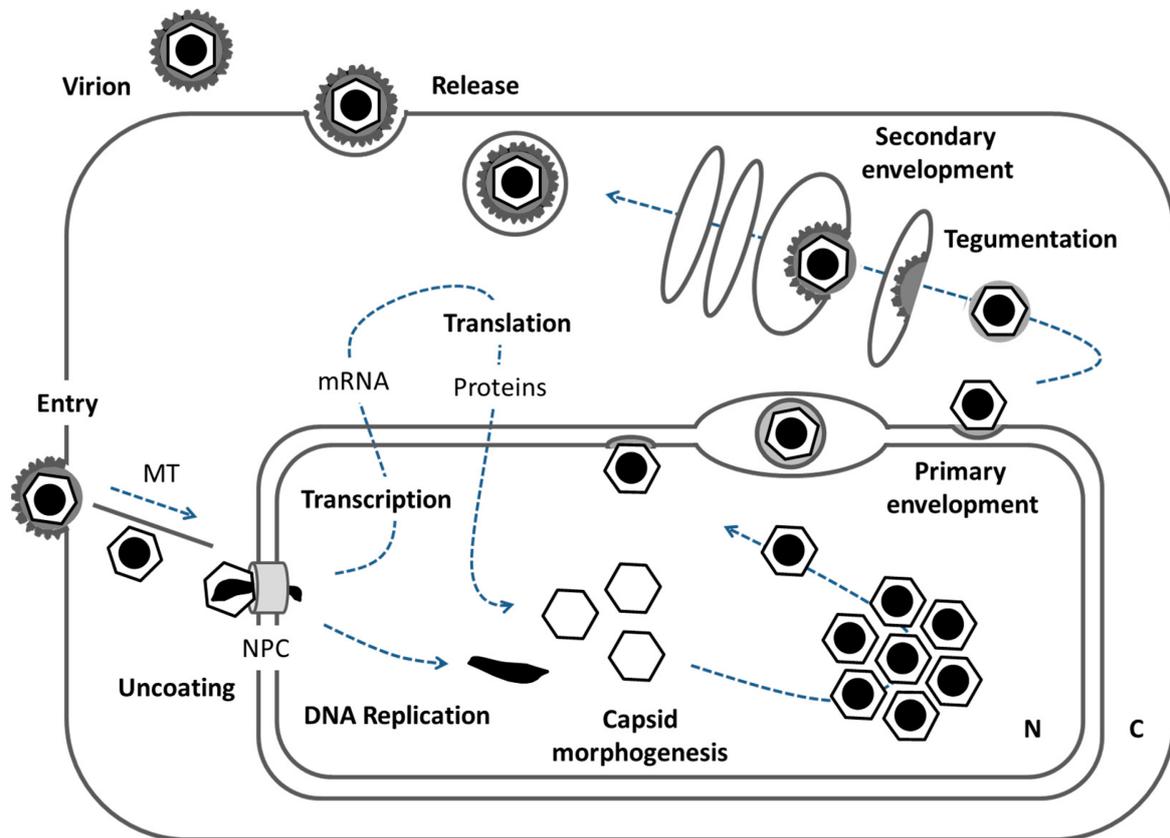
Figure 1: Replication cycle of the herpes virus. Reproduced under the Creative Commons Attribution license from Bailer. S. *Venture from the Interior—Herpesvirus pUL31 Escorts Capsids from Nucleoplasmic Replication Compartments to Sites of Primary Envelopment at the Inner Nuclear Membrane.*[232]

Herpes simplex is amongst the most pervasive of human viral infections with a high global prevalence for viral sub-types HSV-1 and HSV-2.[4,5] The most common clinical manifestations of the viruses are oro-labial and genital disease with HSV-1 traditionally associated with the former and HSV-2 the latter, however significant overlap exists.[6–8] Primary infection with HSV-1 or -2 occurs when the virus invades through injurious breaches of the epithelial, mucosal or corneal surfaces subsequent to contact between a seropositive and seronegative individual. This may be asymptomatic or lead to a diverse range of clinical manifestations such as recurrent HSL, gingivostomatitis, herpes gladiatorum, herpetic whitlow, eczema herpeticum and ocular infection (Figure 2).[9]
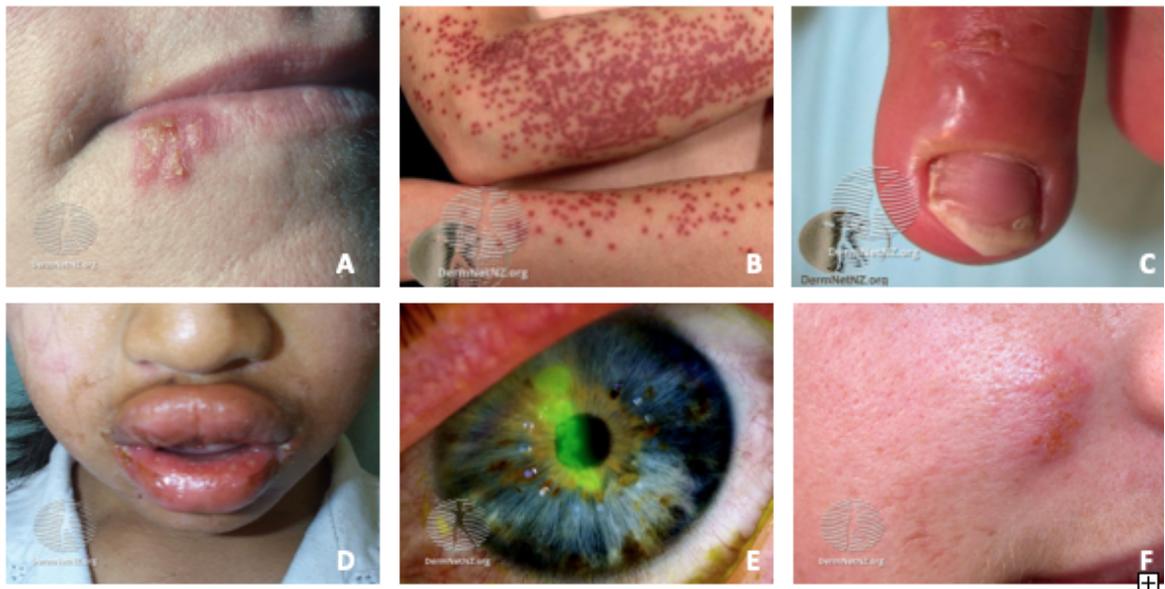
Figure 2: Various clinical manifestations of Herpes Simplex Virus. A: Herpes Simplex Labialis. B Eczema herpeticum. C: Herpetic Whitlow. D: Herpetic gingivostomatitis. E: Dendritic ulcer. F: Herpes gladitorum. Reproduced under the Creative Commons Attribution Licence from DermNet NZ.

Often the initial infection can include a prodromal phase of general malaise, anorexia, tender lymphadenopathy and fever. For oro-labial HSV, this is followed by painful vesicular eruption and subsequent ulceration of the mucosal surfaces, which may last for up to three weeks. In the very young, primary HSL can have potentially serious consequences for feeding and risk spreading to other anatomical areas leading to complications such as ocular herpes simplex.

From an immunological standpoint, the attack and defensive interplay between viral and host defences is multifactorial and complex. Host defences are triggered at various stages of infection, from the initial innate response to localised viral invasion, through to development of adaptive immunity.[10] On initial membrane contact or virion endocytosis, the capsid is transported through the cytoplasmic architecture and injects viral DNA into the nucleus. Cellular responses to this are mediated via production of anti-

viral cytokines such as interferon (IFN) alpha and beta, which act to inhibit mRNA translation and degrade viral RNA, thereby abrogating the viral replication process.[11] Apoptosis may also be triggered in an attempt to prevent viral synthesis, release and further infection of other cell units.
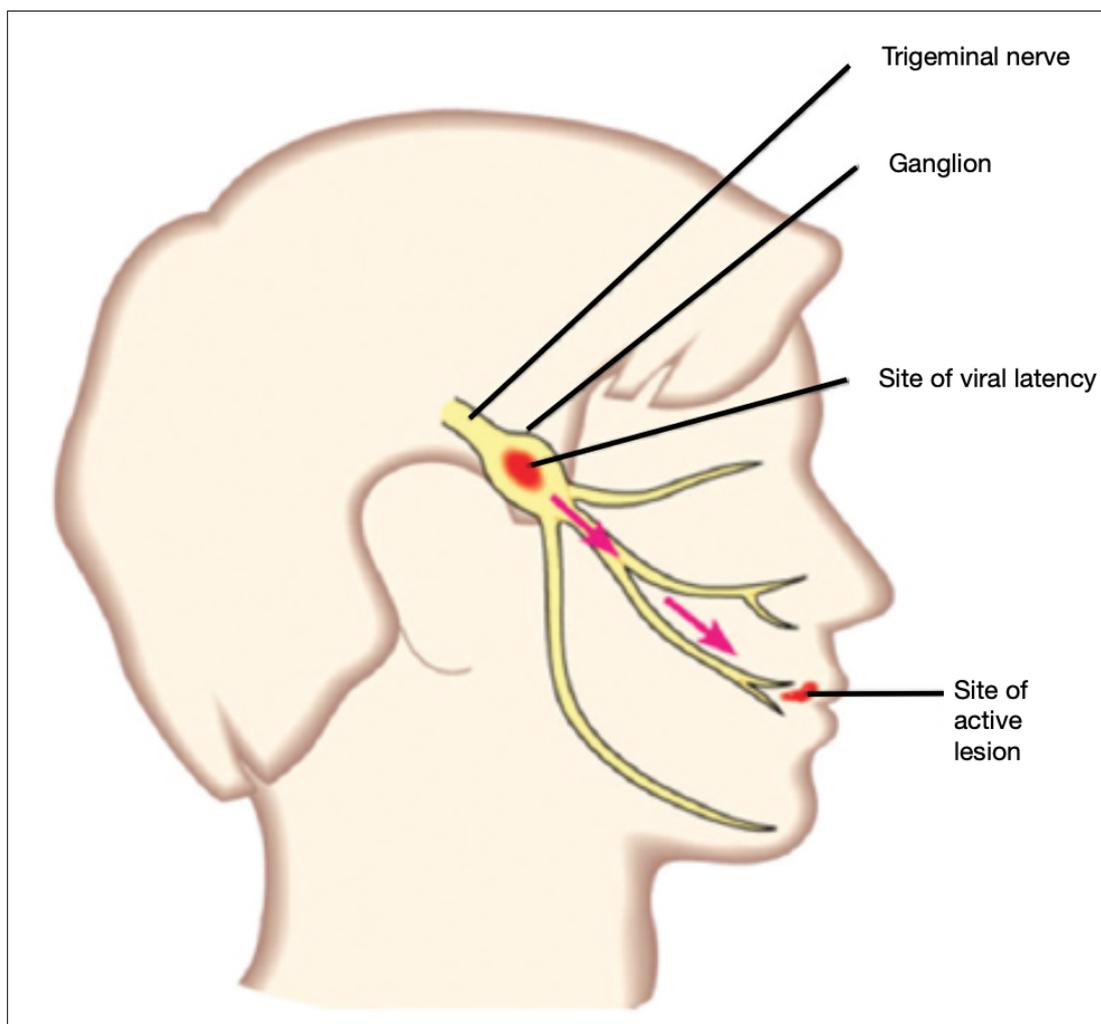


Figure 3: Anatomical sites of HSV dormancy and reactivation during a recurrent episode of HSL. Reproduced under the Creative Commons Attribution Licence 4.0 from Nagaraj et al.[233]

HSV is a lifelong infection due to its ability to establish a latent state within the nuclei of neuronal cells. During primary infection, the virion enters the terminal axon and moves to the nucleus via retrograde transport. Here, it triggers the transcription of a piece of virally coded RNA, the latency associated transcript (LAT), the products of which interfere with the normal host processes to combat intracellular infective agents, effectively hiding the virus in a dormant state. Periodically, HSV replication will reactivate and resultant virions undergo retrograde axonal transport to the peripheral cells innervated by infected neurones (Figure 3). During such a recurrent episode, clinical presentation may vary considerably from completely asymptomatic through to a range of severity including small, non-blistering lesions to large ulcerated wounds and secondary staphylococcal or streptococcal bacterial superinfection leading to impetigo or cellulitis. Reasons for reactivation are complex and varied between sufferers but include systemic events such as menstruation, fever, emotional and physical stress and fatigue or local insults to infected neurones by sunlight, thermal extremes or trauma.

During a recurrent episode of HSL, the cytopathic damage to skin epithelium and consequent accumulation of interstitial fluid leads to the characteristic blistering and associated inflammation. These symptoms resolve within a median of 10 days, on appropriate activation of innate and adaptive immune responses.[12] In some patients, inborn errors of antiviral defence, such as IFN defects, can result in life threatening manifestations such as herpes simplex encephalitis.[13] When this process occurs within the anatomical boundaries of the trigeminal nerve and its ganglion the defined disease process of HSL occurs. There is a spectrum of signs and symptoms for HSL with episodes variable between individuals, however a 'classical' natural history can be described within defined phases (Figure 4).

| | |
|---|---|
| **Prodromal (Stage 1)**<br> | The prodromal phase of a recurrent HSL episode is often signalled by a localised paraesthesia or 'tingling', pain and itch at the vermillion border of the lip. The prodrome commonly lasts for 24 hours or less, however may be prolonged in some sufferers. |
| **Erythema (Stage 2)**<br> | Erythema follows the prodromal phase of HSL as the inflammatory response to acute viral replication increases in intensity. Pain and itch persist. |
| **Vesicular (Stage 3)**<br> | The next stage is the development of fluid filled vesicles that tend to last for 48 hours. |
| **Ulcerative (Stage 4)**<br> | The vesicles rupture forming ulcers, the 'cold sore' that develops a characteristic crust which hardens over the first days. This is the longest phase of a recurrent HSL episode, persisting up to day eight or nine and presents a risk of secondary infection by common skin bacteria leading to complications such as impetigo and cellulitis. |
| **Healing - crust development (stage 5)**<br> | As healing progresses a protective crust develops over the HSL lesion. |

| Healing - loss of crust (Stage 6) | |
|---|---|
|  | As the immune response controls viral replication and wound healing occurs, the crust is lost leading to residual erythema. Whilst this visual evidence of the HSL episode remains for a number of days prior to the return to normal skin, the viral cycle is complete. |
| Healed (Stage 7) | |
|  | Complete resolution of the episode and a return to normal skin |

Figure 4: Phases of a recurrent episode of Herpes Simplex Labialis. Adapted from Semprini et al.[14]

### 1.2.1 MANAGING HERPES SIMPLEX LABIALIS

HSL is the most common herpetic disease process, presenting in an acute, but non-disabling, condition that can result in painful, unsightly and distressing facial lesions (Figure 2A) for which, sufferers commonly access over-the-counter topical and oral therapies from a high value commercial market.[15] In 2014, the United States sales for the only approved topical over-the-counter product Abreva (10% Docosanol) were $132 million and in the United Kingdom, £11 million for non-prescription Zovirax (5% aciclovir cream).[16,17] Overall, market value estimate for HSL products in 2018 were $2 billion in the United States market alone.[18] In addition to the pharmaceutical treatments available, there are many CAMs used for HSL, such as topical propolis, aloe vera, rhubarb, zinc, copper, calendula and hypericum perforatum. Kānuka honey is one such CAM that has a rationale for use in the management of HSL, through physicochemical properties common to all honeys and bioactive factors that may confer therapeutic benefit through anti-viral and wound healing mechanisms.[19-21]

## 1.3 Hypotheses

This thesis explores the hypotheses that a medicinal grade formulation of 90% kānuka honey and 10% glycerin is more effective in the topical treatment of HSL than standard 5% aciclovir cream, and that the assessment of this is feasible through the development of a PRN.

### 1.3.1 Complementary and alternative medicines

Complementary and alternative medicines (CAM) treatments are varied, encompass a range of modalities and are difficult to define, but broadly can be considered treatment approaches not part of standard medical care used either in addition to (complementary) or instead of (alternative) it.[22] Use of CAM is widespread with an estimated 70% of the world's population reliant on it for routine health related needs in 1993 and more recent, progressive surveying by the World Health Organisation (WHO) demonstrating a significant increase in the presence of CAM related national policy from 25 to 98 countries between 1999 and 2018 respectively (Figure 5).[23,24]
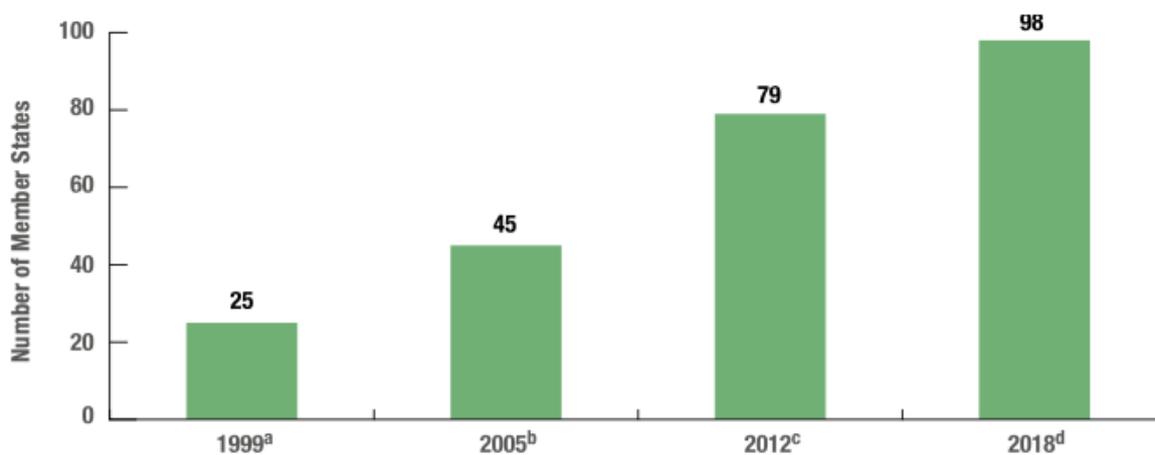


Figure 5: Growth in WHO member states with a national policy on traditional and complementary medicines, 1999 to 2018.23 Licence: CC BY-NC-SA 3.0 IGO.

Even in populations where highly regulated, evidence-based treatments are the standard of care, CAM therapy use is significant [25,26] and ensuring both the patient and clinician find common ground around appropriate treatments may present a significant challenge, particularly given the current paradigm of patient centred care. [27,28] This requires healthcare professionals to be open to patient beliefs and wishes regarding the use of CAM treatment approaches whilst promoting evidence-based standards. Some modalities, such as spiritual therapies and homeopathy, may offer no proven benefit and therefore not be clinically indicated, yet also do no harm and allow both patient and clinician to progress with a mutually accepted treatment strategy. [29,30] Some CAM products may be herbal medicines with known bioactivity, available over-the-counter and produced to a high standard with defined composition; however, many CAM products are not produced and packaged according to good manufacturing process (GMP) and lack the appropriate quality controls to allow confidence in composition required for safe integration into a treatment regimen. This is often not clear, presenting intrinsic difficulties for a clinician in facilitating the harmonisation of such treatments with mainstream clinical care to deliver a mutually acceptable consult with their patient. Indeed, there have been reports of substantive and dangerous contamination leading to calls for basic quality control and regulation. [31] Furthermore, there is a propensity for some patients to conceal their use of CAM therapies from their clinician, exacerbating their risk of adverse effects and interactions with concurrent medication. [32] There is also a clear need for establishing an effective evidence base and pharmacovigilance reporting system for CAM therapies to inform efficacy, adverse effects and interactions with standard treatment. The increased clinical demand for integrative medicine is reflected in the WHO 2019 Global Report on Traditional and Complementary Medicines showing a growth in member states with active regulation of herbal medicines from 65 in 1999 to 124 in 2018 (Figure 6). [24]
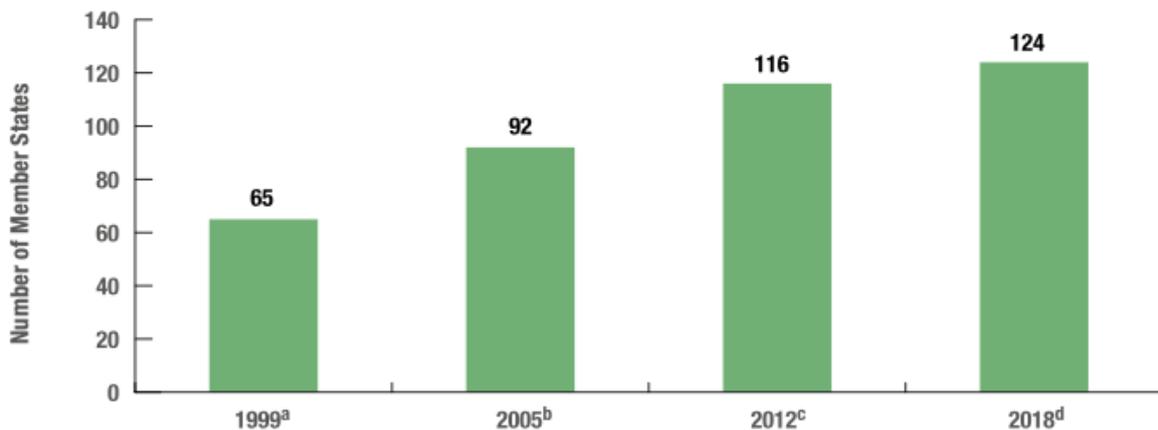
Figure 6: Growth in WHO member states with regulation of herbal medicines 2019.[24] Licence: CC BY-NC-SA 3.0 IGO.

### 1.3.2 IS KĀNUKA HONEY EFFECTIVE IN THE TREATMENT OF HSL?

The therapeutic value of honey has been purported for millennia with perhaps the most established and evidence-based indication as a topical aid to aid wound healing.[33] New Zealand has a strong apiculture industry with honey exports approaching 8,692 tonnes in 2016 representing a $348 NZD million national revenue.[34] Much of this is marketed as having therapeutic potential across a range of indications including wound and skin care, oral and digestive health and allergy. The kānuka (*Kunzea ericoides*) and mānuka (*Leptospermium scoparium*) tree are native to Australasia and part of the wider Myrtacea family. Honey derived from these species is considered high value due to a combination of preclinical evidence demonstrating unique bioactive constituents and highly effective marketing. High quality clinical data from adequately powered RCTs is, however, lacking. This is due to two preponderant issues, the cost of conducting robust clinical research and the negative perceptions in the context of pharmaceuticals around CAM by both clinical professionals and patients. Testing this rationale requires a robust randomised controlled trial able to recruit an adequate sample size achieving statistical power for a clinically relevant primary outcome variable.

### 1.3.3 Is an RCT feasible within a community-based PRN?

Treatments delivered within modern healthcare systems are evidence-based, that is, are required to be informed by robust scientific research providing both rationale and efficacy data for use in a particular therapeutic strategy. This approach, whilst undoubtably improving health related outcomes and patient access to quality treatments, has also made for conflict underpinned by stereotype, belief systems and commercial exploitation when considering the merits and appropriateness of CAM. For those CAM products that are manufactured to a high standard, widely marketed and available for purchase there is a potential to establish an evidence base for safety and efficacy in conditions for which use might be rationalised. This established pharmaceutical approach to product development is, however, rarely followed for CAM products, with a paucity of robust, high quality evidence to provide accurate and allowable therapeutic claims. Indeed, previous attempts to systematically review the use of CAM in the European Union failed due to the extraordinary heterogeneity in data and poor quality in methodology and reporting.[35] Reasons for this are many and include considerations of cost, regulatory requirement, patient access and commercial risk. The cost of a clinical trial is often many millions of dollars, with an average phase III dermatology study between 2004 and 2012 $11.5 million in the United States.[36] This is exclusive of the product development phases and production in an approved pharmaceutical GMP facility, the standard requirement in New Zealand in order to be able to make a medicinal claim.[37] Much of the cost of such trials is borne by the requirement for participants to visit a dedicated clinical trials unit with associated staff and overhead costs. For many CAM therapies that are available over the retail counter within existing markets this is not necessary and indeed, previous attempts by the MRINZ to pilot such products in traditional clinical trials models, such as our hospital-based clinic or general practice network, proved difficult to recruit for and not cost effective for significant sample sizes.[38–40] Despite the move to more stringent regulation of CAM therapies in many jurisdictions,[24] without an

appropriate mechanism to conduct robust clinical research and obtain safety and efficacy data this may simply exacerbate the aforementioned conflict with healthcare providers more likely to engage in an integrative approach.

The medicinal grade honey formulation used within the randomised trial reported in this thesis represents a product of quality and consistency appropriate to be subjected to the robust process of an RCT. Challenges of patient access, trial cost and recruitment form the basis of the methodology developed, namely the research infrastructure and trial design required to successfully answer the question of effectiveness. The creation and development of a novel, scalable PRN of 76 research trained community pharmacies is described alongside the evolution of the network, attraction of further stakeholders and studies and the expansion to an international clinical trials platform.

### 1.3.4 AIMS

#### 1.3.4.1    RCT AIMS:

1. To compare the efficacy of kānuka honey and 5% aciclovir cream for the topical management of HSL
2. To establish the tolerability and safety profile of kānuka honey for the management of HSL
3. To establish the acceptability of kānuka honey as a CAM therapeutic alternative for HSL

#### 1.3.4.2    PRN DEVELOPMENT AIMS:

1. To establish a regulatory precedent through ethics and Standing Committee On Therapeutic Trials (SCOTT) approval for the conduct of an interventional clinical trial in the community pharmacy setting.

2. To establish a trained network of pharmacist investigators.

3. To lay the operational foundations to improve PRN digital processes for study set up, data collection and reporting.

4. To fully recruit, complete data collection and publish the results within an international peer-reviewed journal for a PRN RCT.

5. To explore the advantages and disadvantages of the PRN in the context of traditional RCT models.

6. To formalise the PRN model as a relevant and accessible research capability with the potential for international expansion.

# 2 CHAPTER TWO - LITERATURE REVIEW

## 2.1 TREATMENTS FOR HSL

In the immunocompetent host, recurrent episodes of HSL are usually non-serious and self-limiting, although painful, unsightly and not without psychological impact on the sufferer.[15] There is, however, significant risk to uninfected, vulnerable groups such as the newborn and the immunocompromised who may develop serious clinical complications of primary infection. Overlap with HSV-2 genital infection from orogenital mucosal contact is also becoming more prevalent.[6] Consequently, there is much interest in the cure, prophylaxis and limitation of acute HSL episodes leading to the establishment of a highly lucrative therapeutic market.[16]

Historically, pharmaceutical treatment of herpes simplex infection developed from the early nucleoside analogues such as vidarabine used in ocular herpes infections[41]. These nucleoside analogues act to disrupt viral replication within the host cells. For example, aciclovir undergoes a series of conversions to aciclovir tri-phosphate which is a competitive inhibitor of viral DNA polymerase (Figure 7).[42,43]
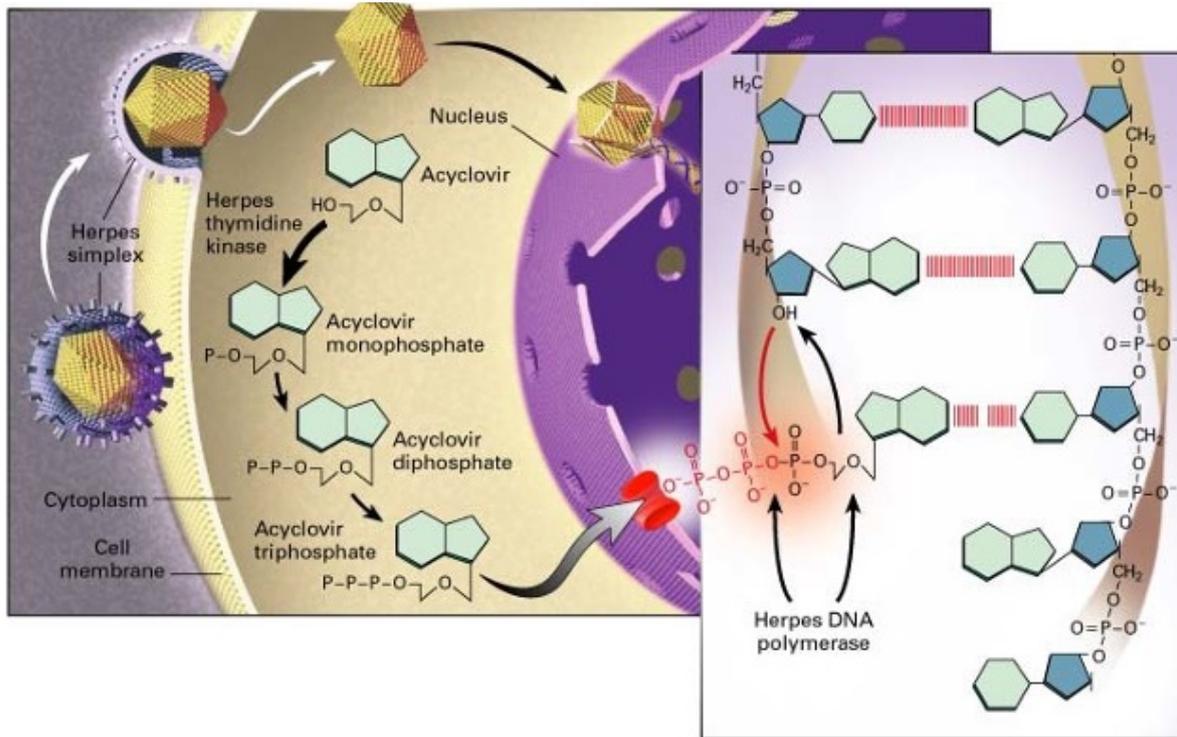
Figure 7: Inhibition of intracellular herpes simplex virus replication by aciclovir. Reproduced with permission from Balfour HH Jr. N Engl J Med 1999;340:1255-1268. Copyright Massachusetts Medical Society.

Currently there are a number of antiviral medications for a range of herpes simplex related indications. The dominant class, nucleoside analogues, can been delivered via both oral (aciclovir, famiciclovir and valacyclovir) and topical routes (aciclovir, penciclovir and triflurodine).

The literature on HSL intervention consistently reports a primary outcome of time to healing although the precise definition varies. Throughout the literature review for topical HSL treatments that follows, the respective stage used in the study, described in figure four, is added in parentheses where possible.

### 2.1.1 Literature review

A literature review was conducted within three individual searches, focused on topical treatment approaches only, inclusive of light therapies for the acute management of recurrent HSL. The first search focused on the general HSL clinical trial literature, the second and third on aciclovir and honey respectively. The high heterogeneity in study design, outcome variable definitions and investigational products rendered a complex body of evidence. Included papers were therefore required to report a time to healing in immunocompetent participants. Results were filtered for human, clinical trials. Abstracts were reviewed, followed by full text review and summary of relevant studies. All non-English language papers were broadly translated via an internet service, 'Google Translate', and reviewed for relevant outcomes. The primary treatment of interest is listed within each section, with active comparators commonplace in many interventional studies and therefore not duplicated in multiple sections.

#### 2.1.1.1 Literature review one: all interventions, excluding primary interventions of aciclovir and honey

A literature review was conducted to identify all studies of topical interventions in the treatment of HSL, excluding aciclovir as an isolated therapy which is reviewed specifically in section 2.1.1.2. The purpose of this was to review the types of treatment approaches to HSL previously investigated, including both pharmaceutical and CAM, and detail the study design, size and outcomes related to healing time.

The National Library of Medicine Pubmed database was searched on the 28th May 2020 with the following terms and filtered for human, clinic trials:

(((("herpes labialis"[MeSH Terms] OR ("herpes"[All Fields] AND "labialis"[All Fields])) OR "herpes labialis"[All Fields]) OR (("herpes"[All Fields] AND "simplex"[All Fields])

AND "labialis"[All Fields])) OR "herpes simplex labialis"[All Fields]) OR ((((("herpes la-
bialis"[MeSH Terms] OR ("herpes"[All Fields] AND "labialis"[All Fields])) OR "herpes
labialis"[All Fields]) OR ("cold"[All Fields] AND "sore"[All Fields])) OR "cold sore"[All
Fields])

232 results were returned, and all abstracts reviewed, with 88 remaining for full text
assessment. 47 relevant publications are summarised below, grouped by drug or inter-
vention type (Figure 8).
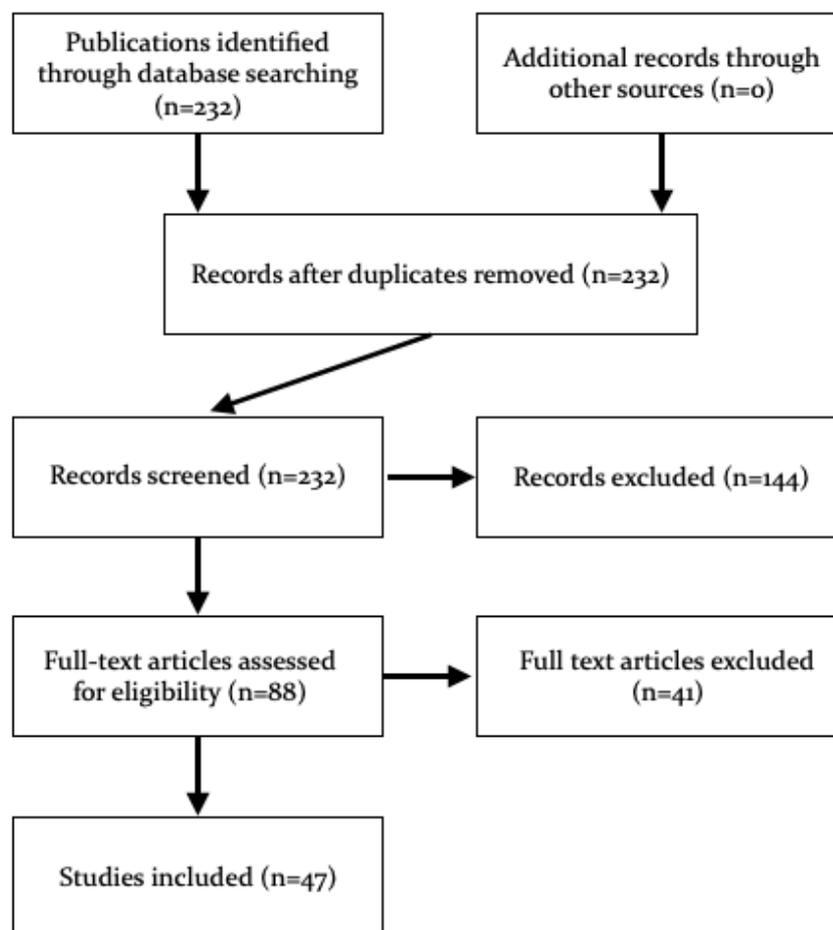


Figure 8: Adapted PRISMA flow diagram for literature review one.

Penciclovir is a guanosine analogue anti-viral drug available in both oral and topical forms.

A randomised, double-blind trial of 541 participants with ultra-violet induced HSL lesions reported a significantly reduced time to healing for topical penciclovir at median seven days compared to median eight days for aciclovir when applied upon onset of the papule stage (stage two to three), every two hours during waking hours for four days.[44] The setting was non-generalisable with HSL lesion induction by a single mechanism, ultra-violet (UV) light, and participants required to attend clinic for assessment daily. Limitations included use of a time to healing outcome not defined by lesion stage.

A small study of 20 adult HSL sufferers allocated either topical penciclovir or aciclovir showed a significantly faster time to healing defined as loss of crust (stage six, Figure 4), four days for penciclovir compared to six days for aciclovir.[45] Treatment was applied from the onset of the prodromal phase, every two hours during waking hours. Outcome assessment was clinician led, indicating potential bias. Limitations of the trial include inadequate justification for sample size, no apparent randomisation and an unclear assessment process.

A large definitive dataset from two randomised studies of 3,057 HSL episodes reported a significant reduction in participant reported time to healing defined by loss of crust (stage six) between 1% penciclovir at mean 4.6 days compared to placebo at 5.4 days.[46] Investigator led assessment to loss of crust was also reported as a significant difference, at mean five days for penciclovir and mean six days for placebo respectively.  Application of investigational product was directed to be applied within one hour of prodromal onset, a minimum of six times on day one, and every two hours during waking hours for four consecutive days. This well designed study offered high quality efficacy data for

penciclovir and a degree of geographic generalisability being conducted in both North America and Europe.

A randomised study of 248 HSL sufferers compared 1% penciclovir cream with 3% aciclovir and found no statistical difference in any outcome measures between treatment arms.[47] Total time to healing was reported as mean five days for both intervention groups for combined primary and recurrent HSL episodes but was not defined. 'Cure' was defined as re-epithelialisation with residual erythema allowable, equivalent to loss of crust (stage six) but not referred to subsequently in the manuscript. Confusingly, mean time to loss of crust was also reported, at six days for combined primary and recurrent episodes – longer than the outcome for total healing. Participants applied treatment within 24 hours of lesion onset, five times daily for up to seven days and were followed up by a clinician for assessment on days three, five and seven. Notably the 3% aciclovir used in this study is of a lower strength formulation than any other encountered in the literature, being most commonly 5%.

### 2.1.1.1.2 VIDARABINE

Vidarabine is an analogue of adenosine and as such competitively inhibits viral DNA polymerase.

In a double-blind, placebo controlled randomised study of 3% vidarabine in 76 participants mean time to healing not statistically different at 8.1 days for vidarabine and 8.7 days for placebo.[48] Healing was defined as loss of crust (stage six). Treatment was applied six times per day for seven days and lesions assess at two or three day intervals by a clinician.

Iontophoresis is a mechanism to enhance trans-dermal delivery of drug molecules through a charged 'push' through the skin.[49] A double-blind randomised study compar-

ing a single iontophoretic delivery of vidarabine, aciclovir and sodium chloride control showed a reduction in mean time to healing.[50] When defined as dry crust (stage five) mean healing time was significantly reduced for vidarabine at 2.4 days, compared to 5.2 for aciclovir and 4.8 for saline control. For time to complete healing (stage seven) there was no significant difference shown at 9.4 days for vidarabine, 11.7 days for aciclovir and 11.6 days for saline control. Subjects were required to present with a vesicular lesion (stage three) and yield a positive HSV culture for inclusion in the analysis. There are multiple limitations to the study with randomisation not statistician led allowing participants to select from a set of masked treatment vials, the lesion was actively disrupted at baseline using a scalpel for swabbing purposes and iontophoresis used a highly alkaline solution base promoting cellular entry of hydroxide over aciclovir.

### 2.1.1.1.3 ADENINE ARABINOSIDE 5' -MONOPHOSPHATE

Adenine arabinoside 5'-monophosphate is a nucleotide analogue and competitive inhibitor of HSV replication.

A double-blind randomised vehicle-controlled study of 233 participants demonstrated no topical efficacy for adenine arabinoside 5' -monophosphate.[51] The difference in mean time to complete healing (stage seven) was non-significant at 8.2 days in the active and eight in the placebo groups respectively. Lesion progress was assessed by clinicians. For mean time to loss of crust (stage six) this was 7.5 days for adenine arabinoside 5'-monophosphate and 7.3 days for control, also non-significant. Participants were not required to present within a particular time window from symptom onset, however 90% presented within 24 hours. Stage of HSL lesion for enrolment was also not mandated, with a range of presentations between no physical lesion (stage one) through to ulceration (stage four).

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#### 2.1.1.1.4 ISOPROPYL-2'-DEOXYURIDINE

A Hungarian study tested the use of a compound, 'Hevizos' - appearing to contain 5-isopropyl-2'-deoxyuridine, a nucleotide analogue, reporting within an abstract to be more effect than placebo. The study was described as double blind, with no-randomisation confirmed. Unfortunately, the full paper is unavailable, but highlights the international use of a variety of therapeutic approaches.[52]

#### 2.1.1.1.5 DOCOSANOL

Docosanol is a fatty alcohol thought to disrupt the fusion of the HSV envelope with the host cell plasma membrane, thereby preventing cellular entry of the virion, but with no inhibition of viral DNA replication.[53]

The combined results of two double-blind, randomised placebo-controlled trials assessing 743 participants demonstrated docosanol to significantly reduce time to healing, defined as loss of crust (stage seven) by a mean of 17.5 hours compared to placebo (4.08 and 4.8 days respectively).[54] Clinician assessment of lesions was twice daily for the first seven days and once a day from day eight to 10. Treatment was initiated within 12 hours of symptom onset, at the first clinical visit with all participants at a pre-vesicular stage. Whilst a high-quality controlled design, the studies undertook multiple sub analyses per group for a variety of efficacy related outcomes and therefore is likely subject to multiplicity related type I error inflation.

A prior study found a reduced time from early initiation of treatment to re-epithelialisation with residual erythema (stage six) with docosanol use at mean 2.5 days and mean 6.8 days with placebo, although numbers were too small to prove statistically meaningful.[55]

### 2.1.1.1.6      Clobetasol

Clobetasol proprionate is a topical corticosteroid cream widely used for topical inflammatory-based disorders.

A double-blind, randomised placebo-controlled pilot study of 42 HSL sufferers examined a combination regimen of topical 0.05% clobetasol gel and oral valacyclovir.[56] This reported a significant reduction in mean time to healing, defined as return to normal skin (stage seven) in the subgroup with classical lesions (n=10) of 5.8 vs 9.3 days. Participants were instructed to commence randomised treatment within one hour of onset of an episode of HSL and return for clinical assessment within 24 hours. Outcomes were clinician assessed every three days until return to normal skin. The time to healing secondary outcome was presented for classical lesions only, providing confidence in the efficacy for wound-based HSL stages but limiting generalisability in overall healing time for all lesion types. Further limiting interpretation, healing times were defined after consideration of the thrice daily participant reporting and every third day clinical assessment.

### 2.1.1.1.7      Propolis extract

Propolis is a bee product formed from bee saliva, wax and variable resin dependent on the surrounding flora which has a long history of traditional therapeutic use.[57]

A double-blind randomised, active controlled trial of propolis extract against 5% aciclovir in 397 randomised participants reporting a significant difference in median time to healing defined as crust formation (stage five) or complete epithelialisation (stage six), of three and four days respectively.[58] Participants were required to be at the vesicular stage (stage three) with no more than 30 hours passing since symptom onset and treatment was applied five times daily, no cessation point was defined. Assessments

of healing were performed by clinicians at enrolment, days one or two, and days three, four and five. For participants with unresolved lesions, further assessments were undertaken at days seven, nine and ten. The relatively late point of enrolment stage and the use of a contradictory definition of healing time which could be interpretable as crust formation or the following stage where the crust is lost revealing complete re-epithelialisation renders interpretation difficult in terms of the current body of evidence.

### 2.1.1.1.8 EPIGALLOCATECHIN-3-GALLATE

AverTeaX is a topical lipophilic epigallocatechin-3-gallate thought to interfere directly with viral and cellular proteins required for virion entry into cells.

A double-blind, randomised placebo controlled trial reported a reduction in time to healing, defined as loss of crust (stage six), of median 4.5 days for epigallocatechin-3-gallate, compared with nine days for placebo in 40 participants.[59] Healing was defined as loss of crust (stage six) with participants required to initiate treatment within 24 hours of symptom onset with application six to eight times daily with no cessation date defined. Daily clinic visits for lesion assessment were scheduled until the healing outcome was met. The reduction in time to healing in this study of 50% is extraordinarily optimistic in the context of the literature, and of note the nine-day time to loss of crust for placebo does not align with larger studies assessing an equivalent outcome. Furthermore, the study reported a 66% reduction in the blistering/ulcerative stage from three days in the placebo arm to one day in the active. Together these outcomes would make epigallocatechin-3-gallate amongst the most effective HSL treatment studied, however comparative studies against standard, established therapies such as 5% aciclovir are needed to corroborate this.

### 2.1.1.1.9      L<small>IGHT</small> T<small>HERAPY</small>

Near infrared light therapy has long been of interest in the management of various dermatological conditions, in particular HSV infection. Precise mechanisms are not established but suggested to be due to immune modulation.[60]

A double-blind randomised, trial of red light therapy compared to a placebo form was found to have no significant effect on the time to healing in all herpes simplex lesion types.[61] When defined as complete healing (stage seven) in the oro-labial subgroup (n=33) the mean was 7.5 days for light therapy and 7.0 days for placebo. For time to crusting (stage five) in the same oro-labial sub-group (n=39) the mean was 5.1 days for both red light and placebo light. Participants were required to initiate treatment within 48 hours of symptom onset and exposed to 15 minutes of light, which was repeated at four and 24 hours later. Clinician led outcome assessment was every third day until healing was complete. Interestingly red-light therapy appeared to have an adverse effect on the rate of recurrence at the sites treated for the oral lesions. The lack of reference to any participant diary implies a reliance on participant recall establishing any precision in time to healing outside of the assessment pattern, every third day.

A double-blind randomised, active controlled trial compared use of narrow waveband light to aciclovir in four groups comprising light alone (n=15), light plus placebo cream (n=15), aciclovir alone (n=15) and aciclovir plus placebo light (n=15). For the pooled results a significant reduction in time to healing was reported defined as loss of crust (stage six) at a mean of 4.3 days for light therapy compared to 8.5 days for aciclovir.[62] Light treatment was applied as a single dose over five minutes, compared to topical 5% aciclovir which was applied five times daily until resolved. Participants were required to initiate treatment within 36 hours of symptom onset and there appeared to be no stage dependent entry criteria. Participants recorded the time of healing, and were subsequently required to attend clinic for a nurse assessment, the mean times for

which were dramatically extended at mean 7.8 for pooled light therapy and 11.3 for pooled aciclovir groups respectively.

A double-blind randomised, placebo controlled study of 1072nm narrow-band light therapy in 32 participants demonstrated a reduction in self-reported mean time to re-epithelialisation (stage six) of 6.3 days for light treatment and 9.4 days for placebo device control.[63] Participants were required to attend for a treatment administration at a clinical visit within 36 hours of symptom onset. Device therapy was applied for three minutes, three times daily for two days in both groups. The outcome data was self-reported during telephone calls every two to three days, with a high degree of recall reliance.

A randomised, placebo controlled study of 1072nm light therapy was compared to placebo exposure in 87 participants in the initial 36 hours of a HSL recurrent episode. The active treatment group reported a significant median time to loss of crust (stage six) of 129 hours compared to 177 for placebo group.[64] Active or placebo device therapy was delivered three times daily for two days and required initiation with the first 36 hours of symptom onset. No specific reference to blinding control was described, however the identical nature of the devices was confirmed. There was no formal sample size calculation, based solely on feasible recruitment within the study duration. Participants reported outcomes at clinical visits held every two or three days until the healing outcome was met, in the absence of specific reference to participant reported outcomes within a more frequent diary this implies the possibility of recall bias.

### 2.1.1.1.10  MONOCAPRIN AND DOXYCYCLINE

Monocaprin is a compound with demonstrable activity against HSV and doxycycline an antibiotic with established anti-inflammatory effects.[65]

A double-blind randomised, placebo controlled study of monocaprin alone and mono-caprin in combination with doxycycline was conducted in 96 participants. Treatment groups were initially stratified into prodromal HSL presentations in whom healing defined as loss of crust (stage six) and vesicular presentations in whom healing was defined as return to normal skin (stage seven).[66] Participants were subsequently alloc-ated to sub groups which received either placebo alone, monocaprin alone or mono-caprin with doxycyline. For the prodromal group mean time to healing was 7.3 days for placebo, 7.6 days for monocaprin and 5.3 days for monocaprin/doxycycline. For the vesicular group mean time to healing in days was 7.4 for placebo, 6.4 for monocaprin and 5.5 for monocaprin/doxycycline. The reduced time to healing was significant in both monocaprin/doxycyline subgroups. The study has significant limitations. It was powered to detect a very large difference in healing outcome of 40%, in contrast to prior literature reports, was very complex with multiple analyses across multiple subgroups inflating type I error and ultimately stopped prematurely due to recruitment diffi-culties.

### 2.1.1.1.11    Nano emulsion

Nano emulsions are colloidal formulations that have potential to improve the delivery of topically applied pharmaceuticals.[67]

A deep penetrating nano emulsion formula, NB-001 containing soybean oil, poloxymer 20 and cetylpyridinium chloride (a quaternary ammonium cation with antimicrobial effects) demonstrated a reduced mean time to healing of 1.3 days compared to placebo in a 482 participant RCT.[68] Treatments were applied five times daily for four days. Only the paper abstract was available.

### 2.1.1.1.12 OXYGENATED GLYCEROL TRIESTERS

Oxygenated glycerol triesters have been used as oral lubricants to treat conditions such as xerostomia with clinical outcome data suggesting anti-inflammatory and wound healing effects.[69]

A topical formula containing oxygenated glycerol triesters, suggested to maintain optimal conditions for wound healing, was tested against both placebo and aciclovir in a randomised controlled trial of 106 participants.[70] The primary outcome focus was functional symptom relief, for which it proved superior; for time to loss of crust (stage six) or time to normal skin (stage seven) as a combined end point, assessed at day seven and 14 there was no difference between groups. Participants were enrolled with 36 hours of a new lesion onset and there was no stage requirement. Given the demonstrably broad time between these distinct stage of HSL healing, compounded by the assessment points being at weeks one and two, any inference around efficacy regards healing is limited.

### 2.1.1.1.13 SILICA GEL

Silica-base dressings enhance drug delivery to sites of wound healing and have been found to actively enhance the process.[71]

The efficacy of topical silica gel as compared to aciclovir cream was assessed in a 74 participant RCT.[72] Amongst secondary outcome variables similar proportions in each interventional group reported loss of crust (stage six) at seven days. Participants were recruited within 24 hours of symptom onset with no staging requirement, around 40% in each group presented within the prodromal stage (< stage four). There was no rationale or mechanism discussed for the use of silica bar clinical experience.

### 2.1.1.1.14    HYDROCOLLOID PATCH

Hydro colloid patches are physical barriers with the aim to optimise the wound healing environment.[73]

Compeed® is a hydrocolloid cold sore patch and has been tested against 5% aciclovir cream for the treatment of HSL.[74] The mean difference between groups for subject's global assessment of therapy (SGAT) was not statistically significant and non-inferiority was concluded. However, the study lacked the statistical power to rule out superiority due to the SGAT upper bound exceeding the nominated minimum clinically important difference, for which there was no justification. Median physician assessed time to healing, defined as return to normal skin (stage seven) was also similar between groups at 7.57 for the patch and 7.03 for aciclovir.

### 2.1.1.1.15    WISTERIA FLORIBUNDA, TRAPA NATANS, TERMINALIA CHEBULAE, COICIS LACHRYMA-JOBI, GANODERMA LUCIDUM AND ELFUINGA APPLANATA

A mushroom-based, herbal mixture, Wisteria floribunda, Trapa natans, Terminalia chebulae, Coicis lachryma-jobi, Ganoderma lucidum and Elfuinga applanata (WTTCGE) was assessed in 13 Japanese recurrent HSL sufferers, the mechanism is not elucidated but rationalised as involving increased natural killer cell activity.[75] The primary outcome was time to 'complete recovery' defined as an intact state, which likely indicated loss of crust (stage six) and for WTTCGE reported as mean 4.0 days compared to mean 7.8 days for the episode immediately prior to active treatment. The study was a single group non-randomised study, with no power calculation or associated sample size requirement defined.

### 2.1.1.1.16    DIOCTYL SODIUM SULFOSUCCINATE

Dioctyl sodium sulfosuccinate (DSS) is a sulphated surfactant with capacity to induce viral envelope disruption.

Traditionally used clinically as a laxative, DSS was assessed in a randomised feasibility study using participant reported outcomes and reported a total mean time to return to normal skin of 6.6 days for the active treatment group and 7.7 days in the placebo.[76] This study represented an early internet-based model of recruitment with participants directed to a screening form on a website, and if eligible print, sign and mail a consent form to the investigators who confirmed identity from medical records. Study product was sent out with instructions to apply immediately on the onset of an HSL recurrence and every two hours until healing. The HSL pictorial staging score was the basis of the system used in the thesis RCT. Interestingly a lack of generalisability was attributed to the selective availability of the internet at the time of the study (2004), with the reverse true for the kānuka honey trial presented.

### 2.1.1.1.17    ZINC DERIVATIVES

Zinc derivatives have been shown to inactivate HSV in various formulations in vitro.[77,78]

A Swiss study reports efficacy in terms of improved healing time for a zinc sulphate formulation 'Virudermin Gel', however the data set is unavailable.[79]

In a double-blind randomised placebo controlled study zinc oxide cream demonstrated a reduction in time to healing, defined as 'when only crusting remained' (stage five) from 6.5 days in the placebo arm to 5.0 days in the active treatment groups in a 46 participant RCT.[80] Participants were required to enrol within 24 hours of symptom onset and were followed up using daily diaries to assess healing with a final investigator led visit

on episode resolution. The study considered a reduction of one day in HSL lesion duration as clinically significant.

### 2.1.1.1.18 MELALEUCA ALTERNIFOLIA

The presence of compounds such as the monoterpenes has been suggested to confer anti-viral activity as seen in vitro for tea tree (*Melaleuca alternifolia*) complex extracts.[81]

A randomised, placebo controlled, single blind pilot study of a CAM therapy, Australian *melaleuca alternifolia* complex oil extract in 20 participants reported a median time to re-epithelialisation (stage six) of nine days in the active and 12.5 days in the control group.[82] Participants were identified and required to present to clinic as soon as possible from the onset of a new HSL recurrence. Eight participants in the active and six in the placebo groups presented at the vesicular stage or beyond. Participants were seen daily in the clinic for assessment. As a pilot study the trial was not powered to assess efficacy, although describes an ongoing full RCT, however review of the literature and clinical trial registries reveals no record for this.

### 2.1.1.1.19 RHUBARB AND SAGE EXTRACT

Rhubarb has been shown in vitro to inhibit the viral attachment, entry and replication phases of HSV; sage extracts have been shown to confer anti-herpetic activity in vitro likely through phenolic activity.[83–85]

A herbal CAM preparation containing rhubarb and sage extracts was examined in a three arm study of 149 participants and found the mean time to healing for rhubarb and sage in combination, aciclovir and sage alone at 6.7 and 6.5 and 7.6 days respectively with no significant differences.[86] This was corroborated by Kaplan-Meier survival analysis also finding no significance in healing times between treatments. Participants were required to present in the pre-ulcerative stage and commence treatment within

one day of onset, applying every two to four hours until day 10 to 14 or healing occurred. The definitions for healing relative to crust formation are confusing with the former occurring earlier than the latter by reported means. The post-hoc non-inferiority calculation within the results section was not defined *a priori* and as such is subject to type I error and inappropriate.

### 2.1.1.1.20 LOCAL ANAESTHETICS

Local anaesthetics offer clear benefit in managing the pain of HSL lesions and have been suggested in vitro to inhibit viral membrane fusion with cells.[87]

A topical anaesthetic cream containing 1.8% tetracaine demonstrated low quality evidence for a significantly reduced time to loss of crust at 5.1 days compared to placebo, 7.2 days.[88] Participants were required to present with 48 hours of symptom onset with no staging criteria and applied the treatment tow hourly up to six times daily. The rationale was derived from unpublished observations of HSV inhibition in vitro. The study reported no *a priori* sample size calculation with justification of a minimum clinically important effect, although the Kaplan-Meier survival analysis was appropriate.

A small study of seven participants with recurrent herpes simplex labialis reported in a letter to the editor, administered either lignocaine/prilocaine cream and placebo in a cross over trial treatment.[89] For the topical local anaesthetic was mean 2.6 days compared to 7.3 for placebo. Treatment was applied four times daily until healing, defined as disappearance of visible lesions (stage seven). Outcomes were participant reported daily with clinical visits on days three, seven and 14. No sample size calculation of justification of meaningful clinical differences was reported and although adequately powered through the significance in healing time reported, the difference seen to complete healing is atypically short in both the active and placebo groups.

### 2.1.1.1.21    MINT EXTRACT BALM

Mint extracts have been demonstrated in vitro to have a direct virudicial effect on HSV, presumed to involved direct disruption of viral envelope glycoproteins.[90]

A double-blind placebo controlled randomised study of mint balm was conducted in 66 participants receiving treatment to be commenced within four hours of symptom onset.[91] Participants were treated for five days applying product four times daily with data collection at clinic visits on days one, two, three and five only. The study was powered to detect a difference in symptomatic outcomes with the authors briefly stating a shortened duration of the healing period for the active group in the manuscript abstract but no detailed description in the text.

### 2.1.1.1.22    UNDECYLENIC ACID

Undecylenic acid is a monounsaturated fatty acid present in sweat, that disrupts viral envelope glycoproteins and has been shown to confer anti-microbial activity.[92]

In an RCT of 560 participants using a 15% topical formulation versus placebo time to healing, defined as complete re-epithelialisation (stage six), was 164.6 hours for undecylenic acid and 154.2 hours for placebo.[93] For time to crust formation (stage five), undecylenic acid reported 40.2 hours and placebo 42.5 hours. Neither outcome reached statistical significance. Participants were required to present within 30 hours of symptom onset and be at the papule stage or before (stage three) and applied treatment three hourly during waking hours until crust formation, followed by thrice daily until healed. Assessment was via daily diaries and clinic assessments. No sample size justification was presented, and the study was inadequately powered to assess all outcomes listed in the statistical analysis plan.

### 2.1.1.1.23  FOSCARNET

Foscarnet is a pyrophosphate analogue that inhibits the lengthening of DNA through the prevention of pyrophosphate exchange.[94]

3% foscarnet was assessed in a double-blind RCT of 143 treated participants showing a mean time to healing, defined as complete re-epithelialisation (stage six), of 6.1 and 7 days for foscarnet and placebo respectively with no significant difference.[95] Participants were required to be at a stage of HSL progression of no later that one hour after the formation of vesicles before enrolment, and applied treatment for five days, two hourly the first and every four hours for the remaining period. The assumed reduction in time to healing was 40% for the active group compared to placebo, a large clinical difference based on other therapeutic data for HSL.

A 302 participant randomised double blind study compared the use of 3% foscarnet cream and vehicle control in UV light induced HSL.[96] Time to healing, defined as vesicle appearance to loss of crust, was not reduced by foscarnet at 168 hours, compared to control at 173 hours, for all lesions. However, a significant reduction was seen for delayed classical lesion types, defined as those manifesting between 48 hours and seven days post UV irradiation of the lip. Treatment was applied two hourly immediately post UV exposure and continued for seven days, or longer if required to ensure any HSL lesions that developed were treated for at least four days. The exclusion of the prodromal period to ulceration renders interpretation of the time to healing data limited in the context of over all natural history.

### 2.1.1.1.24  ASCORBIC ACID

In vitro work found that ascorbic acid inhibited the replication of a number of viruses including HSV, suggesting free radical formation and direct binding to be responsible.[97]

A small, randomised double blind study of 46 participants examined the use of an ascorbic acid containing solution, Ascoxal, reporting a mean nurse assessed scab duration of 5.9 days for the active compared to 3.4 days for placebo, however as this appears to be the number of full days where the presence of a scab was recorded the full episode healing time is difficult to place in context with similar literature.[98] Treatment was applied for the first day only, through three, two minute applications of product soaked cotton wool to the lesion area 30 minutes apart. The design was intended as a highly complex randomised crossover study over two phases, one involving nurse led and one a participant led data collection. Failure of this process and lack of reported sample size calculation render interpretation highly limited. Furthermore, both placebo and active tablets contained cupric acid, a compound of current interest in HSL treatment that has been shown to bind to viral DNA and disrupt the replication cycle.[99]

### 2.1.1.1.25 DENOTIVIR

Denotivir is an isothiazide derivative of 5-amino-3-methylisothiazolocarboxylic acid, marketed as Vratizolin in Poland for the treatment of HSL. Although specific data are unavailable a 1993 abstract claims efficacy.[100] More recent preclinical study of isothiazide derivatives has not demonstrated any antiviral activity on HSV-1 however.[101]

### 2.1.1.1.26 IDOXURIDINE

Idoxuridine was the first marketed nucleoside analogue.

Initial efficacy in HSL was reported in 1966 at 3.5 days to loss of crust compared to placebo at 5.45 days for the vehicle control of dimethyl sulphoxide.[102] Participants reported with 24 hours of symptom onset and applied treatment thrice daily for three days. The study was not designed with an *a priori* sample size calculation or defined clinically important difference and did not report any measure of statistical significance. Con-

temporary in vitro work has also indicated that dimethyl sulphoxide may have independent activity against HSV-1 further limiting confidence in the isolated efficacy of idoxuridine.[103]

15% idoxuridine in dimethyl sulfoxide was subsequently tested in a double blind RCT of 301 participants with an acute recurrent HSL episode.[104] Idoxuridine demonstrated a significant reduction in mean healing time to loss of crust (stage six) compared to vehicle control at 6.3 and 8 days respectively. Time to complete healing was not significantly different between groups at mean seven and 7.5 days. Treatment was dispensed prospectively, commenced within one hour of self-diagnosis of an HSL episode in the pre-vesicular stage, and was applied six times daily for four days. Outcome progress was assessed daily in the study clinic until ulceration, then every other day until healing. The study definition of complete healing was confusing, allowing for residual erythema, which is the case for many HSL lesions at the loss of crust stage which tends to occur with complete re-epithelialisation. Furthermore, the initial time point used to calculate time to outcome variables was the papular stage which may represent abrogation of the overall estimate through excluding of the prodromal stages of infection (stage one). There was no reported sample size calculation or defined minimum clinically important effect.

A trial reported in a letter to the Editor of 10% idoxuridine gel compared to 5% aciclovir cream reported no difference in time to healing defined by loss of crust (stage six), however no numerical values and statistical rationale were reported.[105]

### 2.1.1.1.27    BETA-INTERFERON

Interferon production and signalling in vivo promotes viral clearance by host cells and addition of beta interferon to HSV cells in vitro has been shown to reduce viral production and protect trigeminal neurones.[106]

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Topical beta interferon was compared to placebo in double blind, randomised study of 14 cold sore suffers amongst a combined cohort of oro-labial and genital disease. A significant reduction in duration of episode was reported, the end point defined as disappearance of visible lesions (stage seven), from seven days using placebo to 4.7 days with active therapy.[107] Participants were preallocated treatment and instructed to apply it immediately from symptom onset through to disappearance of visible lesions; topical chloramphenicol was also applied, presumably to avoid superimposed bacterial infection. All episodes of HSL over a two year period were included. Upon onset of a new recurrence, or every three months participants were required to attend clinic. The method of data collection for healing was unclear, listed as subjective grading from one to three. No statistical rationale for sample size or meaningful effect was reported.

In a 30-month double blind, cross over study in 28 cold sore sufferers, topical beta-interferon was shown to significantly reduce the mean length of attack from 8.2 days prior to treatment use to 4.7 days with.[108] The article is accessible as an abstract in Hebrew and no specific outcome data comparing placebo with beta interferon is provided with no capacity to appraise the quality of design and reporting.

### 2.1.1.1.28    TROMANTADINE

An adamantane derivative, tromantadine shown to inhibit a late replication event in HSV replication,[109] is reported to have similar efficacy to aciclovir from two randomised trials, however the results of the studies were unobtainable.[110,111]

### 2.1.1.1.29    BUTYLATED HYDROXYTOLUENE

Butylated hydroxytoluene is an antioxidant known to inactivate lipid enveloped viruses.[112]

In a double-blind, randomised, placebo controlled pilot study, time to total healing (stage seven) was median 9.9 days for topical use of butylated hydroxytoluene, a compound shown to inactivate viruses through envelop membrane disruption, compared to 11 days for placebo, however the difference was non-significant.[113] Participants applied the treatment four times a day for five days and were required to present within the initial 24 hours of symptom onset. It is unclear whether the follow-up points on days one, two, four, six, then every three to four days until completely healed was face to face at clinic or remotely.

### 2.1.1.1.30    2-DEOXY-D-GLUCOSE

2-deoxy-D-glucose is a glucose analogue shown to inhibit sugar focused metabolic pathways and recently suggested in animal models to inhibit topical skin disease processes such as dermatitis.[114]

Early studies of 2-deoxy-D-glucose indicated efficacy against genital herpes,[115] through disruption of glycoprotein dependent cellular fusion and entry,[116,117] however two subsequent studies did not show clinical benefit for HSL.[118] The data were combined and reported in a short letter to the editor with no statistical rationale, small sample sizes and inadequately defined definitions of time to healing.

### 2.1.1.1.31    ETHER

The anti-HSV properties of ether have long been established, through the dissolution of viral lipid membranes.[119,120]

Topical ether was compared to placebo in a double blind, randomised trial finding no significant difference in outcomes between groups, including time to lesion healing reported for loss of crust (stage six) at mean 9.1 days for placebo and 8.2 days for ether.[121] For time to complete healing (stage seven) placebo was 9.1 days and ether 9.8 days. In-

terestingly, 75% of participants using ether and 77% using placebo reported subjective efficacy in terms of lesion severity and healing, highlighting the difficulties in assessment and apparent placebo effect.

### 2.1.1.1.32    CHLOROFORM

Chloroform is a lipid solvent with activity against enveloped viruses.[122]

In a randomised study of chloroform versus a camphor-in-oil placebo comparator, no significant difference in times to healing were reported in 50 participants.[123] For participants presenting within the initial 24 hours of HSL onset mean time to healing (undefined) was 5.7 days for chloroform and 6.1 days for placebo; for those presenting at 24 to 48 hours mean times were 5.8 and 6.2 days respectively. Participants were required to present within 48 hours of symptom onset and applied treatment via a swab for 10 minutes once daily for three days. Analysis was via photographs assessed by two observers. There was no a priori sample calculation and limited numbers indicated inadequate powering of the study.

### 2.1.1.1.33    5-CARBOXYMETHY-3-P-TOLY-THIAZOLIDINE-2,4-DIONE-2-ACETOPHENONEHYDRAZOLE

5-carboxymethy-3-p-toly-thiazolidine-2,4-dione-2-acetophenonehydrazole, is a treatment that has been shown to inhibit HSV replication.[124]

Data was pooled from two small studies reported as placebo controlled with no reference to randomisation or blinding. A reduction in attack duration for the active arm compared to placebo ointment is reported with the mean duration of 'attack' 7.3 days in the first study of 46 participants and 7.6 days in the second study of 17. Duration is given for a single group which is not specified as being the active or placebo, however the mean shortening of attack is reported as 3.4 days in the first group which commenced therapy between 12 and 24 hours after symptoms and 7.1 days in the group

commencing treatment between four and 10 hours after onset. No design and statistical rationale is provided rendering a highly limited interpretation.

### 2.1.1.1.34 SUMMARY

In summary, there is a diverse range of studied topical treatments for HSL, from complex traditional medicine formulations through to pharmaceutical grade products. For some, a specific mechanistic rationale is presented, for others justification is anecdotal. The quality of trial design and reporting is highly variable with many studies not defining a clinical meaningful effect for the primary outcome, omitting a formal sample size calculation or indeed any form of statistical analysis plan. Many studies are small and inadequately powered. A majority were placebo or vehicle controlled and potentially subject to unintended therapeutic effects of a comparator intended to be inert; a number incorporated active controls in established topical treatments such as aciclovir.

The preponderant clinical assessment of therapeutic efficacy in HSL trials appears to be a measure of time to healing, however this can be considered an extremely broad concept with significant heterogeneity in dependent parameters apparent, such as lesion stage, time from symptom onset at treatment application, clinician versus participant assessment of healing, wide spread of outcome data collection points, use of pictorial versus numerical severity scales for lesion staging and the definition of the primary healing end point itself. The inconsistency in the latter is the most important with studies reporting time to treatment success based on definitions as early as scab development, scab loss or return to normal skin, the outcomes of which differ dramatically. Very few studies were generalisable to real-world topical self-management of HSL in the community, with recruitment, treatment application or outcome assessment highly artificial incorporating strategies such as pre-allocation, daily clinician led assessment or targeted induction of HSL recurrence in highly selective populations. It

was evident that a generalisable study design in a representative population, reflective of the community-based, self-initiated treatment for an HSL recurrence was required.

### 2.1.1.2 LITERATURE REVIEW TWO – ACICLOVIR SPECIFIC

Aciclovir was the chosen comparator for the RCT of kānuka honey study given its established history of topical use for HSL in the New Zealand market since 1984, global recognition under household brands such as Zovirax, justified and proven mechanism of action with relative quality of evidence supporting its clinical use. The purpose of the second literature review was to provide a contemporary appraisal of the existing evidence for topical aciclovir use in HSL, including study methodology and clinical effect.

The National Library of Medicine Pubmed database was searched on the 28th May 2020 with the following terms and filtered for human, clinic trials:

(((("acyclovir"[MeSH Terms] OR "acyclovir"[All Fields]) OR "aciclovir"[All Fields]) OR (("acyclovir"[MeSH Terms] OR "acyclovir"[All Fields]) OR "aciclovir"[All Fields])) AND (((("herpes labialis"[MeSH Terms] OR ("herpes"[All Fields] AND "labialis"[All Fields])) OR "herpes labialis"[All Fields]) OR (("herpes"[All Fields] AND "simplex"[All Fields]) AND "labialis"[All Fields])) OR "herpes simplex labialis"[All Fields])

79 results were returned, and all abstracts reviewed with 19 undergoing full text assessment. 10 studies met the criteria with aciclovir alone as the primary intervention, as summarised (Figure 9).
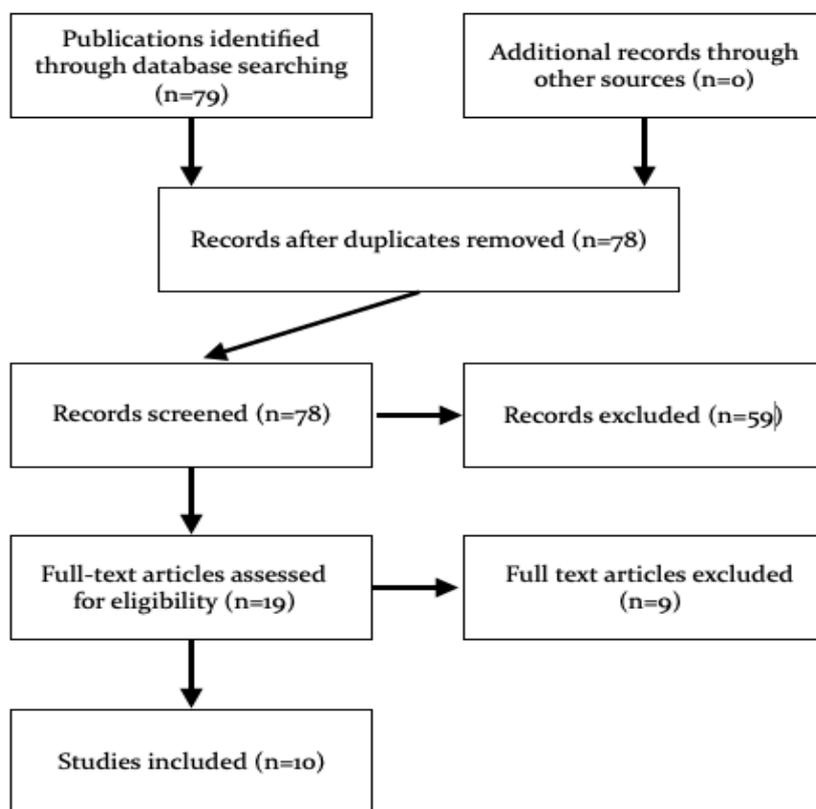
Figure 9: Adapted PRISMA flow diagram for literature search two

A double blind, three-arm randomised, cross-over study of 40 participants assessed 5%
aciclovir in a novel liposomal carrier, or ethosome, compared to vehicle control and
standard formulation 5% aciclovir. A significantly reduced mean time to crust forma-
tion (stage five) was reported at 1.6 days as compared to standard 5% aciclovir at 4.3
days and vehicle control at 4.8 days.[125] Participants were required to present to clinic
within 24 hours of symptom onset, be in the pre-vesicular prodromal stage and able to
be assessed for up to three HSL episodes during the trial period. Outcome assessment
was clinic focused each day for the first three, followed by every two to three days until
the lesion had healed. The paper defined the primary outcome as time to crust forma-
tion (stage five) but defined healing as time to loss of crust (stage six), only the former

reaching statistical significance. There was no *a priori* sample size calculation with a defined clinically meaningful difference between groups, although the authors acknowledged this recommending an adequately powered study to confirm the findings.

In a study of iontophoretically applied (to enhance penetration through charged molecule movement through the skin) 5% aciclovir cream in a placebo controlled randomised pilot study the median time to healing, defined from loss of crust (stage six) was significantly improved in the active group at 113 hours vs. 148 hours in the placebo.[126] Participants were required to be in the papule/oedema stage and assessed in clinic each day up to day 10. Use of Kaplan-Meier survival analysis was appropriate, although being a pilot no pre-defined clinically important difference or subsequent sample size rationale was defined.

In the two seminal randomised placebo-controlled studies of topical aciclovir mean time to healing, defined as loss of crust with permissible residual erythema (stage six) was 4.3 and 4.6 days for aciclovir compared to 4.8 and 5.2 days for vehicle control.[127] A total of 686 and 699 participants treated active lesions in the studies who had been pre-allocated randomised treatment with instructions to apply within one hour of HSL episode recurrence then five times daily for four days. Follow-up was daily at clinic until healing occurred in conjunction with participant completed diaries containing photographic stages of HSL lesions provided; the primary outcome of interest was the clinician assessed time to lesion healing. A clinically important difference of half a day was defined *a priori* resulting in a sample size requirement of 652 participants for each study based on an iterative simulation procedure, with over randomisation planned to account for the episodic nature of HSL. No justification was given for the clinical effect or standard deviation and it is stated that the study was continued until treatment target numbers were met with the implication of sample size based on treatment received over treatment assigned; this leads to potential bias in estimation of the difference

between groups. Whilst the primary outcome was a time to event variable, this was analysed inappropriately using a t-test rather than survival plots with Hazard Ratios (HR), although the latter was subsequently presented indicating a median duration of episode to stage six between 4.5 to five days.

A randomised, double-blind, vehicle controlled dual episode study of 5% aciclovir in a modified aqueous cream base reported no difference in mean time to complete healing (stage seven) between active (7.0 days, 7.1 days) and vehicle control (7.7 days, 8.1 days).[128] Treatment was required to be initiated within one hour of prodromal symptom onset and applied every four hours for five days or until clinician assessed healing was confirmed. Clinic-based assessments were daily for the first five days and every other day thereafter with daily participant diaries also completed. There was no pre-defined sample size calculation or use of a stated clinically important difference in time to healing between groups. Participants had all previously participated in a study of oral aciclovir for HSL, although a three month washout period was mandated to mitigate any risk of cross over effect.

From an abstract report of an 80 participant double-blind, placebo controlled randomised trial of 5% aciclovir ointment in a polyethylene glycol base, no difference in healing between groups was reported.[129] Participants were assessed for two HSL episodes each.

In a double-blind, placebo controlled, randomised study of 10% aciclovir cream in 69 participants a prolonged healing time of median 6.0 days in the active treatment group was reported compared to median 5.2 days in the placebo.[130] Healing was defined only as 'complete', likely stage six based on subsequent definitions by the same lead author. Participants were pre-allocated treatment and required to initiate treatment during the pre-vesicular stage only followed by application eight times daily for five days. Outcome assessment was clinic led daily for the first five days, followed by days seven,

10, 14, 18 and 22 if healing had not occurred. There was no sample size calculation based upon a meaningful clinical effect between groups. 352 HSL sufferers were prospectively dispensed randomised treatment, yielding only 69 eligible episodes. Median time to treatment application from symptom onset was two minutes for aciclovir and eight minutes for placebo, impressive but non-generalisable.

A double-blind, placebo controlled, randomised trial reported a significant reduction in mean time to loss of crust (stage six) between 5% aciclovir and placebo at 5.7 and 8.3 days respectively in the initial episodes of 30 participants.[131] The authors conducted a pooled analysis of all episodes in the study period including 19 re-entries, showing a non-significant difference of 5.4 days for aciclovir and 6.6 days for placebo. Participants were required to start treatment within 12 hours of HSL recurrence and apply five times daily for five days. Clinic led assessment was at least every other day or daily. The small study size and lack of *a priori* sample size calculation suggests inadequate powering of the study.

A small, 13 participant, double-blind, placebo controlled randomised study of 5% aciclovir reported a median time to healing at six days for aciclovir and eight days for control, however whether this was loss of crust or return to normal skin was not defined.[132] A total of 31 episodes across all participants were used in the analysis. Participants were pre-allocated treatment and instructed to apply it as soon as possible after prodromal symptom onset. For subsequent episodes, re-randomisation occurred but the authors report use of previous treatment allocations for these limiting data integrity. Whilst survival analysis was appropriately presented, no predefined sample size calculation with a stated clinically important difference between groups was reported.

In a double-blind, placebo controlled study of 5% aciclovir in 49 participants a faster time to 'complete healing' (either stage six or seven) was reported for aciclovir at me-

dian four days compared to median six days for placebo.[133] This was maintained for both the first episodes for each participant and subsequent one within the study period, with re-randomisation occurring after initial completion of treatment. Participants were required to attend clinic with 24 hours of initiating treatment and daily thereafter. No sample size calculation or clinically important effect was described.

A double-blind, placebo controlled, randomised trial of 5% aciclovir reported time to complete healing (stage seven) as mean 7.2 days for both aciclovir and placebo and for mean time to loss of crust (stage six), 6.4 days and 7.3 days respectively.[134] There was no requirement for stage at presentation however 98% of all participants were at the papular stage or beyond rendering a potential for bias toward shorter times to healing if more advanced HSL presentation formed a significant proportion of the group. The study subsequently focused on the sole statistically significant outcome, reduction in viral titre for the group treated with aciclovir. No minimally important clinical effect was pre-defined nor a sample size calculation reported. A duplicate publication derived from the presentation of the same dataset at an aciclovir symposium was not included in the literature review results, however interestingly reported different outcome values for healing to the manuscript, with those randomised to aciclovir reaching complete healing (stage seven) in mean 7.8 days compared to the placebo group at mean 7.3 days; for time to loss of crust (stage six) the mean time was 7.2 days and 7.3 days respectively.[135] The differences in time to healing were non-significant between treatment groups in both reports.

### 2.1.1.2.1 SUMMARY

In summary there is evidence that topical aciclovir is an effective and safe treatment of HSL. However, the issue of inconsistency in healing outcome definition, lack of *a priori* sample size calculation and omission of, or inadequately rationalised minimal clinically important difference persist in the body of literature for aciclovir, despite being the

standard over-the-counter topical treatment for HSL. This reinforces the need for an adequately powered, generalisable randomised trial that reflects the true performance of topical aciclovir in the community setting it is most often used. An attempt to meta-analyse the 10 topical aciclovir studies reported was rendered unfeasible due to four trials reporting median values only, two reporting mean values but no standard deviations and one lacking any data within the abstract version available. However, a 2017 systematic review and meta-analysis of all nucleoside antiviral drugs used for HSL provides insight as to time to healing, including aciclovir, compared to placebo.[136] A sub-group analysis of all topical nucleosides used for classical lesions, those progressing through the vesicular and ulcerative stages of HSL, reports a difference in time to healing of 0.8 days in favour of treatment (95% Confidence Interval (CI) -1.15 to -0.45). For aciclovir in both
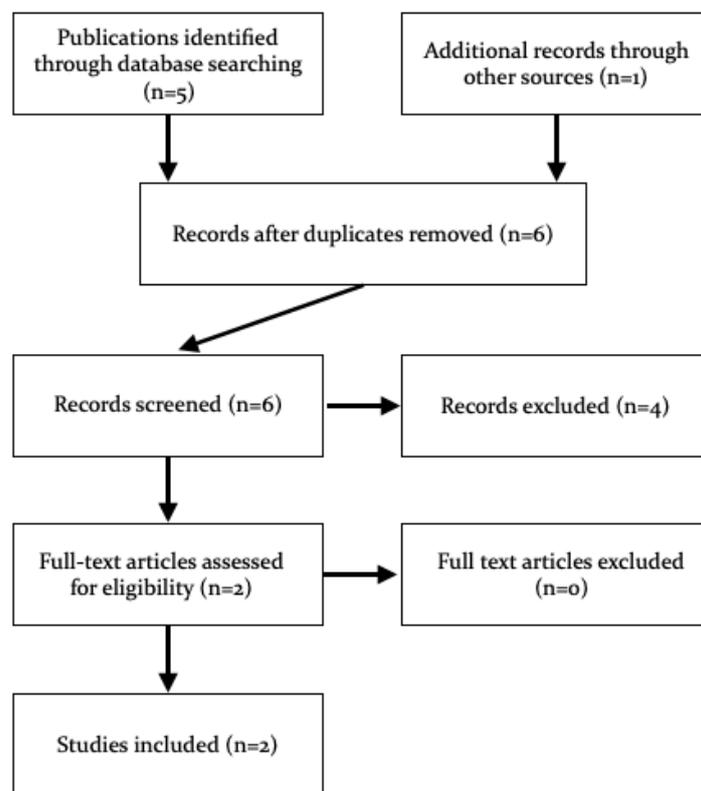


Figure 10: Adapted PRISMA flow diagram for literature review three.

topical and oral formulations the mean difference favours active therapy at -0.79 days (95% CI -1.12 to -0.46).

### 2.1.1.3    Literature review three-honey specific

The third literature review was focused on evidence for clinical use of honey in the treatment of HSL. The National Library of Medicine Pubmed database was searched on the 28th May 2020 with the following terms and filtered for human, clinic trials:

((("honey"[MeSH Terms] OR "honey"[All Fields]) OR "honeys"[All Fields]) OR "honey s"[All Fields]) AND (((("herpes labialis"[MeSH Terms] OR ("herpes"[All Fields] AND "labialis"[All Fields])) OR "herpes labialis"[All Fields]) OR (("herpes"[All Fields] AND "simplex"[All Fields]) AND "labialis"[All Fields])) OR "herpes simplex labialis"[All Fields])

The search yielded five results of which two were publications of the study included in this thesis, one was not accessible[137] and one was a commentary letter on the remaining randomised trial. One additional study was added through knowledge of the literature.[38] (Figure 10).

One study was a proof-of-concept clinical trial of a multi-floral honey from the United Arab Emirates, in which eight participants were assigned topical honey or 5% aciclovir for the first episode of HSL and crossed over for the next.[138] Randomisation was uncontrolled through simply requesting the participant to use the other treatment for the second episode and the study unblinded given the impossibility of masking honey with an aciclovir comparator. The mean time to healing was defined as return to normal skin (stage seven) and reported as significantly improved for honey at 2.57 days compared to aciclovir control at 5.86 days. Participants were required to initiate treat-

ment within one hour of symptom onset and report to clinic within 24 hours. Treatment with honey was through gauze soaked compress over 15 minutes, four times daily and aciclovir was applied six times daily, both until healing occurred. Assessment was clinician-led each day until resolution. There was no sample size calculation or *a priori* definition of meaningful clinical effect between groups and t-test analysis was performed instead of a more appropriate survival analysis.

The other study was the pilot study for this thesis, an open-label, randomised, cross-over study of kānuka honey (Honevo) conducted in New Zealand general practice, chosen due to an established network in place and the potential to conduct the study outside of one major centre.[38] Median time to healing, defined as return to normal skin (stage seven), was nine days for honey and 8.5 days for aciclovir with no significant difference between groups. Participants were required to present to clinic for randomisation via coin toss, to either honey for the current episode followed by aciclovir or the reverse, with treatment to be applied as soon as possible, five times daily until resolution. Participants self-reported in daily diaries. As a pilot, the study was inadequately powered and suffered from difficulties in recruitment. A clear methodological limitation of the pilot study was the use of the general practice setting, in terms of relevant participant access and compounded by a prospective recruitment strategy in that participants were required to enrol and wait for a recurrent HSL episode. The elapsed time from first enrolment to final data collection was 452 days, during which only 15 participants were recruited, of which nine suffered a recurrent episode of HSL.

### 2.1.1.3.1 SUMMARY

In summary, the clinical evidence for use of honey in HSL is limited due to the small size of both studies and other methodological issues. Both studies incorporated an active, aciclovir control versus placebo. The United Arab Emirates multi-floral honey study, whilst very small in the context of existing literature and suggesting inadequate

power, showed a statistically significant difference favouring honey, which reduced time to complete healing by 3.57 days compared with aciclovir, far superior to the 0.79 day reduction from the meta-analysis for topical nucleosides such as aciclovir.[136] Given the recurrent nature of HSL and significant, but not impairing or life-threatening clinical symptoms, it is unlikely that the majority of sufferers would present to general practice for treatment, as shown in the Fingleton et al study.[38] In New Zealand the cost of an appointment would be an additional barrier to success at this recruitment interface with patients more likely to visit a pharmacy to obtain one of the over-the-counter therapies available, either topical (e.g. 5% aciclovir) or tablet (Famivir) form.

## 2.2 HONEY AS A TOPICAL THERAPEUTIC

Honey is a natural substance produced by certain bees. Nectar is collected from the nectaries of flowering plants and stored in a specialised 'honey' stomach, or crop, for transport back to the hive. Here, the nectar is orally passed from one bee to another until the water content reduces from 80% to 20%, and, through the addition of enzymes, complex sugars have been converted into simpler forms. The resultant condensed and supersaturated solution is transferred to honeycombs where further evaporation occurs to produce the final honey product at around 18% water content.

The composition of honey is complex and highly variable, dependent on the particular species of bee, environmental influences and flowers nectar is sourced from. The primary constituents are monosaccharides, followed by disaccharides the specific sub-composition of which varies with fluctuations in temperature. The protein content of honey is also significant (up to 3%), mostly derived from pollens and honeybee salivary secretions, in the form of amino acids and more complex enzymes. Vitamins and minerals are also present in variable proportions, some of which confer dietary benefit and some such as heavy metals that are potentially harmful in excessive quantities. All honeys are mildly acidic due to the presence of organic acids, which, in combination

with the hypertonicity derived from the high osmolarity of honey, is responsible for its preservative effect. Another major component in honeys are the phenolic compounds, broadly divided into phenolic acids and flavonoids which have intrinsic bioactivity via antioxidant and other mechanisms. In preclinical assays, some isolated flavones such as chrysin have been shown to abrogate proliferation and induce apoptosis in cancer cell lines along with many other potentially beneficial physiological activities.[139–141]

Illustrating well the influence floral origin has on biochemical composition and resultant clinical manifestations is honey derived from the Ericaceae family of plants, colloquially known as 'mad honey'. This unique honey, common to Turkey, contains significant concentrations of grayanotoxins, compounds which bind to 'open' group two sites on voltage gated sodium channels, preventing inactivation and leading to persistent depolarisation. Clinical manifestations are variable but include significant cardiac arrhythmia, prolonged vagal stimulation with associated cholinergic symptoms, and altered mental status.[142,143]

The physicochemical properties common to all honeys, in combination with variable bioactivity dependent on location and floral origin, have promoted a long history of medicinal use referenced in four millennia old Chinese literature and evidence for harvesting of honey and associated products such as beeswax dating back over 8000 years in rock paintings.[144] In Ancient Greece, medical application of honey was widespread with Hippocrates employing its use for gastrointestinal, ocular, upper respiratory, skin and wound disorders.[145] In the recent era of evidence-based medical practice, honey has attracted increasing therapeutic interest for numerous clinical conditions with a significant base of literature exploring both preclinical rationale and efficacy in formal clinical trials.

### 2.2.1 Honey and wound healing

There are four distinct phases to the wound healing process, haemostasis, inflammation, granulation and remodelling.[146] A significant interest in honey as a treatment for wounds is reflected in the literature with systematic reviews for use in burns and ulcers reporting significant benefit in healing, although the quality of reporting was deemed limited.[147,148] Honey has a number of intrinsic physiochemical properties common to all forms, that may confer benefit to the wound healing process.

**1. Hypertonicity:** Being a supersaturated sugar solution, honey is hypertonic and highly viscous creating both a physical barrier and an osmotic gradient to draw out lymph from the injured tissue, thereby facilitating delivery of nutrients and cells and establishing an environment of autolytic debridement; this mechanism effectively is mirrored by the contemporary use of negative pressure wound dressings to achieve the same aim. These benefits are, however, dependent on maintaining the high osmolarity of honey and reduce with increased dilution by lymph.

**2. pH:** The acidity of honey promotes increased oxygen delivery to surrounding tissues and abrogates protease activity thereby stabilising the fibrin matrix necessary for effective granulation, during which new connective tissue and vasculature form on the surface of a wound.

**3. Hydrogen peroxide:** All honey contains hydrogen peroxide, a reactive oxygen species with diverse physiological effects. Hydrogen peroxide has effects on all stages of wound healing including platelet activation, recruitment of leucocytes, stimulating increased expression of pro- inflammatory, pro- tissue remodelling and angiogenic cytokines. Activity appears to be concentration dependent, with promotion of healing at lower concentrations and delayed healing at higher concentrations.[149]

Kānuka honey has been shown to confer significant anti-inflammatory effects in vitro, over that shown by mānuka, clover and a blend.[150] The mechanism was found to be pathway specific via Toll Like Receptors 1 and 2 implicating a downregulation of inter-leukin 1β (IL- 1β and nuclear factor kappa light chain enhance of activated B cells (NF-KB), mitigating the inflammatory response associated with wound healing and promoting optimal regulation of cytokine response. Kānuka honey was also shown to more potently stimulate tumour necrosis factor-α in vitro than mānuka and clover honeys.[151] The mechanism responsible for this pro-healing immunomodulatory effect was determined to be via arabinogalactan proteins present in the honey.

### 2.2.2 ANTIBACTERIAL PROPERTIES OF HONEY:

**1. Osmolarity:** The high osmolarity of honey creates a water trap to deny microorganisms sufficient water to multiply and actively dehydrates them. In addition, the typically acidic environment of honey further inhibits the growth of many bacterial species. Traditional clinical uses of hydrogen peroxide involve application of relatively high concentrations as an antiseptic acting through the destruction of bacterial cell walls.

**2. Methylglyoxal:** When diluted, honey loses the viscosity dependent physiochemical efficacy against micro-organisms, and the presence of compounds such as hydrogen peroxide and methylglyoxal (MGO) are responsible for sustained anti-bacterial effect.[152] MGO is an organic compound found in most honeys that induces non-specific damage to cellular DNA, RNA and other macromolecules; it is present in relatively high concentrations in mānuka derived honey which has recently been subject to aggressive marketing and efficacy claims based on this. However recent research is calling into question the reliability of anti-microbial effect that can be derived through quantification of MGO levels, also known as Unique Mānuka Factor (UMF).[153]

**3. Defensins:** In addition to the most commonly cited honey derived anti-microbial compounds, hydrogen peroxide and MGO, there are myriad other factors that contribute to the overall bioactivity of a particular honey type. Defensins are a heterogeneous family of peptides found throughout most species that confer immunomodulatory and anti-microbial function; bee defensin-1 is one such peptide demonstrated to have significant anti-bacterial influence in particular honeys, independently of MGO.[154,155]

**4. Inhibition of quorum sensing:** Quorum sensing is a process used by bacteria to communicate and promote pathogenicity based on population density through the controlled expression or down-regulation of specific genes.[156] Biofilms are formed by highly populated groups of bacteria, leading to a stable and complex structure that is much more difficult to combat compared to the same organisms in their planktonic state. Various honeys and other plant compounds have demonstrated inhibitory effects on both biofilm formation and quorum sensing of specific bacterial species in laboratory and human studies and are of interest as another much-needed potential therapeutic approach to the progressive issue of bacterial antibiotic resistance.[157,158]

### 2.2.3 HONEY AS AN ANTI-VIRAL

#### 2.2.3.1 PRECLINICAL

The preclinical evidence base for the anti-viral effect of honey is developing with activity demonstrated in preclinical studies.

The anti-influenza effect of a variety of honeys was demonstrated in vitro cell culture and plaque inhibition assays, with the strongest effect seen for mānuka.[159] Whilst a lower inhibitory effect was reported for honey alone compared to the neuraminidase inhibitors oseltamavir and zanamivir a synergistic effect was seen. These data were cor-

roborated for mānuka honey in a subsequent study reporting effective inhibition of the replication of a number of influenza virus strains and a synergistic effect with neuraminidase inhibitors.[160] The mechanism was reported to be due to the strongly virucidal activity of MGO, through disruption of viral surface proteins impacting interactions with host cells.

Both mānuka and clover honeys were reported to confer anti-viral activity against varicella zoster virus in vitro, another member of the herpesvirus family.[161] The study applied both honeys to cell cultures infected with varicella and showed dose dependent reduction in plaque size for both, with a marginally lower half maximal effective concentration for mānuka.

A Tunisian honey was tested against rubella virus in vitro, reporting anti-viral effect as measured by reduction in viral number.[162] Mechanisms were not established.

A potent anti-Human Immunodeficiency Virus 1 (HIV-1) effect was demonstrated in vitro for six out of eight Iranian mono-floral honeys. Healthy cells were cultured and infected with HIV-1 followed by treatment with honey solutions.[163] Antiviral effect was correlated with the level of MGO in each honey which was shown to act at a late stage of viral replication, post reverse transcription, likely inhibiting virion assembly.

Likely contributing to any demonstrable anti-viral action of honey is the flavonoid driven, up regulation of IFN synthesis, known to promote the cleavage of viral genetic material, inhibit viral protein translation and enhance physical resistance to viral entry by virions.[164] Together the existing literature suggests promise in potential direct and synergistic anti-viral effects for honey however supporting clinical evidence in humans is lacking.

### 2.2.3.2    CLINICAL

A genital herpes sub group of the United Arab Emirates trial of topical honey reported a significant reduction in mean time to healing from 6.28 days in those participants treated with aciclovir to 3.71 days in the group randomised to honey.[138]

In addition to the clinical trials using honey for HSL described in section 2.1.1.3, a randomised double-blind, active controlled study of 100 children with acute herpetic gingivostomatitis has been reported. Herpetic gingivostomatitis is a severe complication of HSV oral infection, resulting in widespread oro-mucosal lesions throughout the buccal surfaces, gums, tongue and lips (Figure 2D).[165] The study administered a combination suspension of honey from an undefined origin and aciclovir versus aciclovir alone. Significant median time to resolution of herpetic lesions was reported as three days in the combination group compared to six days for aciclovir.

An open-label, RCT of Tualang honey, administered orally to HIV positive Malaysian prisoners reported that honey at intermediate or high dose, presents a reduction in CD4 count compared to low dose and control groups.[166] The authors proposed a general immune boosting mechanism of action was responsible, with potential for direct anti-viral effects of bee products such as propolis and bee venom, which have been shown to confer anti-HIV effects in vitro.[167,168]

### 2.2.4 PAIN REDUCTION PROPERTIES OF HONEY

There are a number of potential mechanisms through which honey could provide anti-nociceptive benefit via both topical and oral use. Prostaglandin E2 and other arachidonic acid derivatives are a common target of non-steroidal anti-inflammatory drugs and involved in inflammatory related pain both centrally and peripherally.[169] Animal models have reported that direct infiltration of paw tissue with honey reduces

murine local pain response with an associated reduction in nitric oxide and prostaglandin E2.[170]

In a randomised, placebo controlled study 60 children were administered either amoxicillin-clavulonic acid, paracetamol plus placebo or amoxicillin-clavulonic acid paracetamol plus honey during the immediate recovery period post tonsillectomy. There was significant improvement in subjective assessment of pain over the initial two days, and significant reduction in analgesic use for the first eight days in favour of the honey treated group.[171] As a pilot study there was no predefined sample size of clinically meaningful effect, and no follow-up confirmatory study has been published.

Topical application of honey (Medihoney dressings, a proprietary blend) has also been tested within a descriptive, non-randomised feasibility trial of 40 patients with chronic, non-healing venous leg ulcers. 50% of participants reported a reduction in ulcer related pain over a 12 week period.[172] The precise compounds mechanistically responsible have yet to be elucidated, but likely involve both phenols and specific bee-derived peptides.[20]

In summary, these four properties of honey provide rationale for use of New Zealand kānuka honey for the topical treatment of HSL with outcome variables focused on assessment of time to resolution, time to healing of ulcerated episodes, degree and time to resolution of pain and associated adverse events.

## 2.3 SUMMARY

Overall, the literature base for therapeutic strategies in the management of recurrent HSL is diverse, in terms of study design, interventions and replication of results between studies. By far the most dominant strategy is still centred around use of nucleoside analogue drugs such as aciclovir, penciclovir and famiciclovir, both for acute and prophylactic use systemically with topical formulas readily accessible over-the-

counter in many countries. The overall quality of the studies was limited with missing sample size calculations, adequate rationale as to an important clinical effect, appropriate statistical analysis of time to event outcomes and numerous pilot studies reporting significant effect but no confirmatory study. The diversity of entry requirement for lesion stage and onset and the definition of the point at which a lesion is defined as healed requires caution in interpreting relative efficacy between studies.

The topical therapies investigated over the decades are numerous and target multiple points in the HSV replication cycle. A current (29th May 2020) search of the World Health Organisation International Clinical Trials Platform for 'Herpes Simplex Labialis' lists 87 trials since the start of the record in 2005, with 5 registered in the past 18 months alone. This perhaps either reflects the inadequacy of current treatment approaches to one of the most ubiquitous infectious diseases encountered by humans, the pharmaceutical market opportunity this extraordinary prevalence provides, or indeed a combination of the two.

The historical lack of generalisable data and reliance on an artificial clinical trials unit methodology for the topical management of HSL is striking, particularly considering the over-the-counter access to treatment for most patients. Given the preference for self-purchase of non-prescription medications for HSL it stands to reason that therapeutic performance should also be assessed in a real-world setting, subject to the delays in obtaining and applying treatment, routine stressors and formal assessment of convenience of use within day-to-day life.

The evidence underpinning the rationale for use of medicinal honey for acute HSL recurrence is compelling pre-clinically, and the preliminary clinical data warrants further investigation. The variability in honey composition must also be considered when drawing specific conclusions of efficacy. The progressive increase in use of CAM such

as honey for medicinal purposes urgently requires effective data collection methodologies to analyse claims for specific, quality products, through RCTs in order to establish safety and tolerability for informed use.

The RCT subsequently presented, conducted in the specifically developed PRN infrastructure, was designed to answer the following questions:

*Is medicinal grade, kānuka honey more effective in the treatment of recurrent HSL than the standard 5% aciclovir treatment available over-the-counter in New Zealand?*

*Is the conduct of a gold standard randomised controlled trial possible in the community setting relevant to the real-world clinical experience of HSL sufferers presenting with a recurrent episode?*

# 3 CHAPTER THREE: KĀNUKA HONEY VERSUS ACICLOVIR FOR THE TOPICAL TREATMENT OF HERPES SIMPLEX LABIALIS – STUDY METHODS

## 3.1 STUDY SUMMARY

In order to determine the safety and efficacy of topical 90% kānuka honey / 10% glycerin (Honevo) for the treatment of HSL a phase III, open label, parallel group, randomised controlled trial was conducted between 10th of September 2015 through to the 17th December 2017 (Appendix 1). This was conducted through a community-based network of research trained pharmacy investigators who recruited, consented, randomised and dispensed study investigational products to enrolled participants. 952 participants were randomised across 76 community pharmacy localities across the North and South islands of NZ.

## 3.2 STUDY HYPOTHESIS

That a topical formulation of 90% kānuka honey/10% glycerin is more effective than topical, 5% aciclovir cream for the acute treatment of recurrent HSL.

## 3.3 STUDY OBJECTIVES

The primary objective was to compare the effectiveness of topical Honevo with standard, over-the-counter topical 5% aciclovir for the treatment of HSL.

Secondary objectives were to establish the safety and tolerability of Honevo, further define differences in healing times for distinct phases of the HSL episode, determine any difference in maximal pain and time to pain resolution.

## 3.4 Study interventions

Participants were randomised to receive either:

1. Medicinal grade, 90% kānuka honey/10% glycerin (Honevo)

2. 5% aciclovir cream (Viraban)

### 3.4.1 Honevo

Honevo is a medicinal grade, 90% kānuka honey / 10% glycerine formulation produced under Cosmetic Toiletry and Fragrance Association (CTFA) certification by Zealand Health Manufacturing in Tauranga, New Zealand (Figure 11) (Appendix 2 and 3). All honey undergoes flash pasteurisation to



Figure 11: Honevo. 90% kānuka honey and 10% glycerin formulation.

>80°C prior to sterile storage at the apiary. The pasteurised kānuka honey is then tested to ensure it is within range for the following parameters: pH 3.4 to 3.8; measured in triplicate sub-sampling for each batch with 5g honey diluted with 20ml of distilled water prior to assessment with a calibrated digital pH monitor. Water content is determined by refractometry (Bellingham and Stanley standard model Abbe-type refractometer) and required to be <18%. Acceptable honey is processed in 20kg batches under certified wet-room conditions to a ratio of 90% kānuka honey and 10% glycerin, followed by immediate filling and sealing of sterile product packaging. Each batch of

honey is subjected to a 'chemical fingerprinting' assay which profiles specific markers proven to classify a honey as kānuka or not (Appendix 4). All batches were required to meet the standards set out in table 1.

| Compound | Required Concentration (mg/kg) |
|---|---|
| Leptosperin | ≤150 |
| 2'-methoxyacetophenone | ≤2 |
| Methyl syringate | ≥5 |
| 3-phenyllactic acid plus 4-methoxyphenyllactic acid | ≥500* |

**Table 1: Chemical markers used in the classification of kānuka honey**

Honevo units are required to be stored between 6 and 35°C and maybe gently warmed if crystallisation has occurred, in order to return the product to a fluid state. Potential adverse effects of the topical use of honey include pain/stinging due to mild acidity and allergic reaction which is due to the residual presence of pollens and bee body material. The risk of the latter is mitigated by the use of medicinal honey, which is pasteurised and filtered.

For trial purposes, participants were instructed to apply Honevo five times a day until skin had returned to normal, or 14 days, whichever was sooner.

### 3.4.2 Viraban

Viraban is a 5% aciclovir cream, manufactured by Pinewood Healthcare, Ballymacarbry, Clonmel, Ireland and distributed by AFT Pharmaceuticals, Auckland, New Zealand (Figure 12). The base cream is formulated from polyethylene-glyceryl stearate, dimeticone, acetyl alcohol, liquid paraffin, white soft paraffin and propylene glycol (Appendix 5). A white cream, it is applied to the HSL lesion as early as possible, five times a day. Potential adverse effects are mild and include pain, drying and flaking of the skin, itch and a rash. Viraban should be stored below 25°C. The choice of aciclovir as the active control comparator for a novel HSL intervention is supported by the relatively large proportion of reported studies providing evidence for it's use, well established mechanism of action, affordability and availability. Other topical nucleoside analogues such as penciclovir are not available over-the-counter in New Zealand and the other main alternative approved for over-the-counter use in the United States, docosanol (Abreva®), is not registered or approved for use.

Figure 12: Viraban cream. Topical 5% aciclovir in a polyethylene and propylene glycol base.

For trial purposes, participants were instructed to apply Viraban five times a day until skin had returned to normal, or 14 days, whichever was sooner.

### 3.5 Outcome variables

The natural history of a classical HSL lesion involves an uncomfortable prodrome followed by a vesicular eruption, open blister and subsequent wound granulation and healing.[12] To most adequately assess the effectiveness of an HSL product in the real-

world setting, time to event data was deemed most appropriate to assess differences in the objective and subjective symptomatic course of each episode.

### 3.5.1 PRIMARY OUTCOME VARIABLE

The primary outcome variable was healing time from randomisation to the return to normal skin.

There is marked heterogeneity in the definition of healing across the HSL literature, with common use of soft or hard crust formation or loss of crust and allowing for residual erythema.[73] This is likely based upon the rationale that at the point of lesion healing, the viral replication cycle has ceased. Given the more pragmatic focus on effectiveness of the investigational products within a real world clinical setting of community pharmacy, the typical treatment interface for most HSL sufferers, return to normal skin was chosen as the definition of healing, stage seven on the pictorial chart used in the study (Figure 4 and 15). Furthermore, the known aesthetic priority for treatment for many sufferers, in addition to managing the pain, itch and general discomfort of a classical HSL lesion, provided further rationale for this.

### 3.5.2 SECONDARY OUTCOME VARIABLES

A number of secondary outcomes were assessed to further define any difference in clinical course between the two interventions used.

### 3.5.2.1    SPECIFIC STAGES OF HEALING

Time from randomisation to stage four (open wound) and time from stage four to stage seven was specifically assessed in order to establish any stage of wound/healing specific benefits for each (Figure 4 and 15). It was hypothesised that Honevo would provide a faster progression from open wound/ulcer to return to normal skin, due to

known effects on wound healing. Similarly, topical aciclovir might be expected to provide earlier stage benefit when applied in the pre-ulcerative phases due to mechanistic effects on the HSV replication cycle.

### 3.5.2.2 PAIN

Pain is a significant symptom of HSL and aciclovir has demonstrable effects on pain reduction.[127] As such, and in lieu of the reported analgesic effects of honey in various cutaneous conditions,[171,172] time to pain resolution was assessed through comparison of reduction of pain intensity using an 11 point numerical rating scale from 0 (no pain) to 10 (severe pain). Highest pain severity was also assessed, defined as the maximal participant reported pain score during the study period.

### 3.5.2.3 PARTICIPANT ACCEPTABILITY

Acceptability of randomised treatment was reported by each participant at the end of the study period, using a scale from 1 (unacceptable) to 10 (acceptable). Generalisable data, collected in a real-world setting was a core objective of the Honevo trial and PRN development. Comparing the use of topical Honevo for the management of HSL versus the long established and widely used 5% aciclovir allows for a meaningful context in terms of participant acceptability and potential uptake in an over-the-counter setting.

### 3.5.3 POST HOC OUTCOME VARIABLES

In order to benchmark the presented study with other, high profile clinical trials of HSL, a post hoc analysis was performed for time from randomisation to stage six (residual erythema), (Figure 4 and 15) a criterion often reported in the current evidence base. Additionally, the proportion of aborted episodes that did not reach the blistering

stage was estimated, defined as those participants whose baseline stage was three or less, and who progressed to stage seven, without recording stages four, five or six.

## 3.6 PARTICIPANTS AND STUDY SETTING

### 3.6.1 PARTICIPANTS

Participants were required to be aged 16 years and over, with an acute episode of HSL and able to present to a study pharmacy within the first 72 hours. The rationale behind this was to ensure that the prodromal phase was captured, whilst allowing sufficient time for participants to attend their local study pharmacy for enrolment. Whilst other physician led, prospectively recruited studies initiated treatment within 24 hours, this was likely too restrictive for the generalisable design and recruitment interface and would have potentially excluded a large number of otherwise eligible participants.

#### 3.6.1.1 INCLUSION CRITERIA

1. Aged 16 years or over at the time of enrolment.
2. Presentation to a pharmacy for treatment of a cold sore.
3. First cold sore symptoms (including prodromal symptoms) within 72 hours.

#### 3.6.1.2 EXCLUSION CRITERIA

4. Pregnant or breastfeeding.
5. Known allergy or suspected allergy to honey, bees, aciclovir, and/or glycerin.
6. Any other condition which, at the investigators' discretion, it is believed may present a safety risk or impact the feasibility of the study or the study results.
7. Participant has used oral aciclovir or other antiviral medicine, or any topical treatment, medical or complementary, on the current HSL lesion.

8. Participants planning to take/use any concomitant medications, which in the opinion of the investigators, could affect the HSL lesion during the course of the trial. This includes any topical product, medical or complementary, on the HSL lesion, oral aciclovir or other antiviral medicine, oral complementary therapies for HSL, such as lysine, any other medications.

There was a broad scope for exclusion given to the pharmacy investigators in terms of other medications, conditions and factors that may influence a decision to proceed with enrolment. This was in part to reflect the real-world setting of access to over-the-counter medications via the pharmacy interface and in part to streamline the recruitment process on recognition of unique time and resource limitations in the context of a clinical trial.

### 3.6.2 STUDY LOCALITIES

The recruiting study sites were all community pharmacies located within NZ. There were no restrictions for involvement such as membership of an established corporate model or being an independent business. In total 76 pharmacy study sites met the requirements and undertook the registration and initiation process described in chapter four. Following approval from Medsafe and Health and Disability Ethics Committee (HDEC) to receive investigational product and commence recruitment, each pharmacy study site was visited in person by myself for formal site training and initiation. Overall there were 209 pharmacist investigators trained and registered to conduct the RCT.

## 3.7 Study procedures

Potentially eligible participants were identified either prospectively through social media (Figure 13), advertising placed within the pharmacy using posters and 'shelf-talkers' or opportunistically at the time of presentation to the pharmacy while seeking treatment for HSL.



Figure 13: A typical Facebook advert for the study that was delivered with a 30km radius of Waitara in the North Island of New Zealand

### 3.7.1 Visit one

#### 3.7.1.1 Screening

Upon first presentation to a pharmacy study site, each participant was read a general study description:

*'This is a trial for people over 16, who aren't pregnant, with a cold sore that has started in the last 72h for which no other treatments have been used. People must not be allergic to honey, bees or glycerin.'*

This was composed to specifically screen out ineligible or unwilling participants prior to the recording of any identifiable information and thus mitigating the need to obtain consent, imperative to avoid wasteful progression through the enrolment process prior to realising ineligibility. Due to the nature of the recruitment interface in the pharmacy, embedded within a busy hybrid clinical and retail environment, effective screening of all potentially eligible participants was limited for Consolidated Standards of Reporting Trials (CONSORT) reporting. Consequently, no data were collected for participants prior to the point of progression past the screening statement.

#### 3.7.1.2 Informed consent

Following participant agreement, the pharmacy investigator assigned the next sequential, unique study ID indicating the site code and participant number. This was taken from a pre-constructed participant pack, containing the participant information sheet and consent form (PISCF) (Appendix 6), back-up paper study diary (Appendix 7) and pre-paid and addressed return envelope. PISCF documentation was provided for read-

ing and discussion, prior to obtaining signed informed consent when the investigator was satisfied the trial related risks, benefits and procedures were understood.

### 3.7.1.3 BASELINE DATA COLLECTION

The pharmacy visit one work sheet (Appendix 8) was then completed with the participant, collecting baseline HSL episode scores for pain and stage, a brief narrative of medical history, current medications, demographic data, inclusion/exclusion assessment, ethnicity, average number of cold sores per year, time since last cold sore and contact information.

### 3.7.1.4 RANDOMISATION AND BLINDING

On completion, the participant was assigned their randomised study medication by the pharmacy investigator who opened a sealed, opaque envelope containing the allocation derived from a securely held master randomisation schedule generated by the study statistician. This was dispensed with written instruction to apply the topical product five times daily until skin had returned to normal (stage seven) or fourteen days elapsed, whichever occurred first. Finally, the participant was given the back-up paper diary and envelope and free to leave.

Unfortunately, the physical characteristics of honey precluded the blinding of study participants to treatment allocation. Pharmacist investigators were also privy to randomised treatment at the point of dispensation, however, central Medical Research Institute of New Zealand (MRINZ) investigators remained blinded with only the study physician (myself) unmasked in the event of an adverse event.

### 3.7.1.5 DATA TRANSFER FROM PHARMACY TO COORDINATING STUDY TEAM

Paper copies of the case report forms were certified on each individual page prior to being faxed by the recruiting pharmacy investigator to a centralised number. This number was derived from an internet cloud-based subscription service 'eFax', which receives traditional facsimile data and converts this into a portable document format (pdf) which is then emailed immediately to an address of choice. This system allowed notification and receipt of recruitment data immediately and to any location via mobile device thereby allowing review of appropriate inclusion, real time actioning of protocol deviations and violations and review of data integrity. Following this, all eFaxed data was transcribed into a digital case report form held in a commercial survey platform Wufoo.

### 3.7.2 PARTICIPANT REPORTED DATA COLLECTION PHASE

During the treatment application period participants were required to complete a daily diary to record compliance, pain and cold sore stage each day at 6pm. This was preferentially delivered digitally to facilitate live time data integrity checks and ensure that data entered was chronologically relevant, compared to paper diaries which are able to be completed at any point in the data collection phase. The interventional, participant reported data collection phase of the study used Wufoo, which allowed delivery of electronic diaries via a short messaging service 'SMS' service, 'BurstSMS'. This required manual scheduling of each day's text message containing an embedded hyperlink for the relevant day's diary to be delivered to each active participant (Figure 14 and 15).
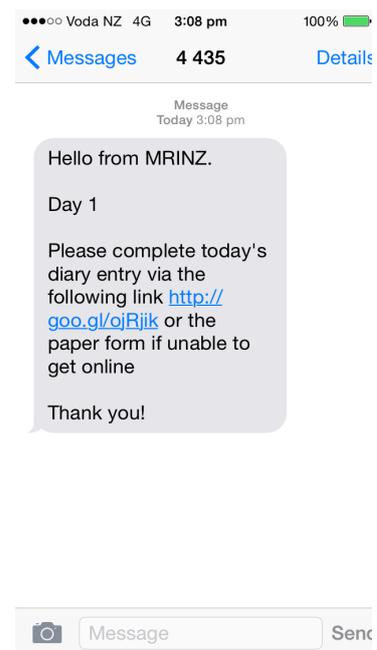


Figure 14: SMS reminder message for the electronic diary.

**MEDICAL RESEARCH INSTITUTE OF NEW ZEALAND**

## Cold Sore Study eDiary Day 2

Please enter your ID code *

[          ]

Enter a number between 1 and 870.

Please confirm which study day you are recording *

[          ]

Enter a number between 2 and 2.

How painful has your cold sore been today from 0 (no pain) - 10 (severe pain) *

[          ]

Enter a number between 0 and 10.

How many times have you applied the treatment today *

[          ]

What stage is your ulcer today from 1 - 7? *

[          ]

Enter a number between 1 and 7.

| Stage 1 Prodrome | Initial onset of tingling and itching. No sore is present and skin appears normal. Not everyone gets these symptoms. | |
| --- | --- | --- |
| Stage 2 Redness | Skin has some redness and swelling. No blister. May itch or feel tender. | |
| Stage 3 Small blister | Small blisters form in the affected area, clear at first turn yellow with time. Usually painful. | |
| Stage 4 Ulcer | Completely formed cold sore with open areas where blisters were. Sore and painful. | |
| Stage 5 Crust | After some days a flaky crust develops resembling a scab. | |
| Stage 6 Drying up & Healing | Crust flakes off with healing skin underneath. Cold sore cycle is complete, maybe some residual redness of skin for a few days. | |
| Stage 7 Healed—skin back to normal | Skin is entirely back to normal. | |

[ Submit ]

Figure 15: A study electronic diary as rendered on a mobile device web browser

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Due to the increasing logistical complexities of managing multiple and increasing simultaneous recruits, each requiring 16 individual SMS messages to be triggered, the approach evolved to use an application programming interface (API), 'Sequencer', which allowed a predefined schedule of SMS to be triggered upon enrolment and stopped on completion. Each participant was also supplied with a back-up, paper version diary, for use in the event of system or device failure or indeed for those participants with no internet connectivity or mobile phone access. Prepaid and addressed envelopes were also supplied to maximise return of used paper diaries. In case of dual input for a particular data point, the digital entry was used for analysis.

### 3.7.3 VISIT TWO

The final study contact for participants was the follow-up assessment, scheduled for the first working day after study day 14. This was initially attempted as a telephone call from a study nurse based at a clinical trials unit in Auckland. Adverse events, acceptability, concomitant medication use and narrative feedback were collected and a request for paper diary return made, if used (Appendix 9). In the event of contact failure, two further telephone calls were attempted by the study nurse, a second the following working day and a third at day 22 or the next working day. Failing this, the MRINZ central study team attempted further follow-up contact via telephone, email and/or SMS, up to study day 35. After this time point a participant was classified as lost to follow-up. There was no requirement for return of investigational product.

## 3.8    SAMPLE SIZE

A number of relevant past studies were reviewed in order to estimate the appropriate sample size for a definitive randomised controlled trial of Honevo compared to 5% aciclovir for the treatment of HSL, discussed in detail in chapter two.

The lack of definition of a minimally clinically important difference, or justification of the differences used across most studies was problematic. The reported outcomes tended to demonstrate an improvement between one half and one day in time to healing compared to control with the most efficacious treatments, currently used in clinical practice. In order to have statistical confidence in the power prior to commencement, a defined difference in effect was required to accurately calculate the sample size. The kānuka honey trial presented in this thesis assumed a median duration of lesion in the aciclovir control group of five days, aiming to achieve a median reduction in healing time of one day for the active kānuka honey treatment resulting in a HR of 1.25.

This decision was based on a number of factors. Three comparable studies of aciclovir reported mean times to healing of 4.3, 4.6 and 5.85 days respectively, leading to an estimated time to healing in the aciclovir control group of five days.[127,138] The clinically important difference in time to healing suggested in a 2017 meta-analysis of all published data on the use of nucleoside analogues compared to control in HSL was 0.8 days (95% CI -1.15 to -0.45) for all topical nucleoside analogue drugs.[136] For combined study data for aciclovir administered both topically and systemically the reduction was 0.79 days (95% CI -1.12 to 0.46). Al-Waili reported a difference in mean time to healing between honey and aciclovir of 3.28 days suggesting a difference well in excess of one day was achievable with kānuka honey[138]. Finally, the study team consensus was that a one day reduction in the time to healing would be a meaningful effect size for a non-pharmaceutical, natural product.

The Gehan method was used with an assumption of a reduction from 5 days to 4 days in the kānuka honey arm and median difference of one day, an HR of 1.25 and a total of 423 participants per group (846 in total) were required with 80% power and a 5% type I error rate to determine this difference. Incorporating an attrition rate of 10%, 950 participants were therefore required to be randomised between treatment groups.

## 3.9  STATISTICAL ANALYSIS

Analysis was by intention to treat. Those participants who provided time-to-event data but did not provide the primary end point were censored and those with no time-to-event data were excluded from the analysis. For all time to outcome variable analysis (healing and pain), Kaplan-Meier plots with associated product-limit based estimates of median time to event and HR, calculated using Cox proportional hazards were used with a random effect for participants to take into account the parallel design. Sensitivity analysis was performed for both primary and secondary outcome variables, considering important potential confounders such as age, time to presentation from reported onset of symptoms and stage of cold sore at presentation. Interaction analysis was performed for primary and secondary outcome variables, to assess potential differences in treatment effects due to baseline stage at randomisation. Maximal pain between groups was assessed using a paired $t$-test and for acceptability left skew required the use of the Mann-Whitney test with Hodges-Lehmann estimator of location.

It was also intended to undertake analysis by protocol principles. There were, however, very few protocol violations that would have distinguished these populations with five participants, one in the kānuka honey and four in the aciclovir group, receiving the incorrect treatment post randomisation. In lieu of the large sample size it was decided that a per-protocol analysis would not add to the assessment of bias within the intention to treat analysis.

SAS 9.4 was used for analysis.

## 3.10    Safety

### 3.10.1    Adverse events

The International Council for Harmonisation of Technical Requirements of Pharmaceuticals for Human Use (ICH) defines adverse events (AE) as *'any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment'* within their Good Clinical Practice (GCP) guidelines.[74] All occurrences meeting this definition were reported at the follow-up telephone call, via direct contact by the participant with a central MRINZ investigator or upon presentation to the study pharmacy. At each reporting point full details of the event were collected by the investigator for review by the study physician who directly followed up with the participants to assess severity and causality.

### 3.10.2    Serious adverse events

Serious adverse events (SAE) are defined by ICH as 'any untoward medical occurrence that at any dose results in death, is life-threatening, requires inpatient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability/incapacity or is a congenital anomaly/birth defect.[74] These events required reporting to the study coordinating centre at the MRINZ within 24 hours and to the approving regulatory bodies.

### 3.10.3    Data and safety monitoring board

A data and safety monitoring board (DSMB) was appointed as a condition of study approval to meet and review the first 100 participants data for AEs and SAEs. This com-

prised of two physicians independent of the study, with experience in clinical trial conduct and reporting (Dr Phillipa Shirtcliffe and Dr Kyle Perrin, Capital and Coast District Health Board).

## 3.11 REGULATORY

### 3.11.1 HEALTH AND DISABILITY ETHICS COMMITTEE AND STANDING COMMITTEE ON THERAPEUTIC TRIALS

A submission to the New Zealand Northern B Ethics Committee was made on the 21$^{st}$ of May 2015 and formally approved on 9$^{th}$ of June (Appendix 10). SCOTT approval was required and granted on 3$^{rd}$ of June 2015 (Appendix 11). Both committees deemed that the risk to participants was low and the setting appropriate. Locality authorisation was obtained for each individual site, prior to local recruitment commencing at a pharmacy. Participants were reimbursed at an approved rate of $25 via cheque, on completion of the study. Pharmacies were reimbursed at an approved rate of $40 per recruitment.

### 3.11.2 AUSTRALIAN NEW ZEALAND CLINICAL TRIALS REGISTRY

The study was registered at the Australian New Zealand Clinical Trials Registry (ANZCTR) on the 23$^{rd}$ June 2015 reference: ACTRN12615000648527.

## 3.12 FUNDING

The study was funded by Honeylab, Tauranga, New Zealand who had no involvement in the design, conduct, analysis or reporting of the study.

## 3.13    PROTOCOL AMENDMENTS

The following protocol amendments were made and approved by the regulatory bodies throughout the course of the study period.

**1. 15th July 2015:**
The PISCF was adjusted to clarify data storage on an offshore server, located in Sydney, Australia.

**2. 8th June 2016:**
An exclusion criterion for allergy to bees was added to the protocol and PISCF following the DSMB review of the first 100 participants. The block size for randomisation was updated to four to account for a required increase in allocation codes to compensate for high variability in recruitment rates between individual sites.

**12th June 2017:**
Three additional secondary outcomes were added to the protocol *a priori* to analyse specific stages of HSL episode time to healing:

- Time to stage four from randomisation
- Time to stage seven from stage four
- Acceptability of treatments

An additional sensitivity analysis was added to the statistical analysis plan to consider the important confounders: age, time to presentation from reported onset of symptoms and stage of cold sore at presentation. This was an interaction analysis for all primary and secondary outcome variables to assess potential differences in treatment effects based on these confounding factors.

# 4 Chapter four: data

## 4.1 The randomised controlled trial

### 4.1.1 Total study duration

Between the 10th of September 2015 and the 13th of December 2017, 952 participants, 475 randomised to aciclovir active control and 477 to kānuka honey, were recruited to the trial from 76 community pharmacy research localities.

### 4.1.2 CONSORT flow diagram

Of the 952 participants recruited, 91 were lost to follow-up (49 aciclovir and 42 honey) and nine withdrew due to adverse events (three aciclovir, six kānuka honey). The resultant 852 participants provide data for the primary survival analyses, with degree of censoring indicated below (Figure 16).

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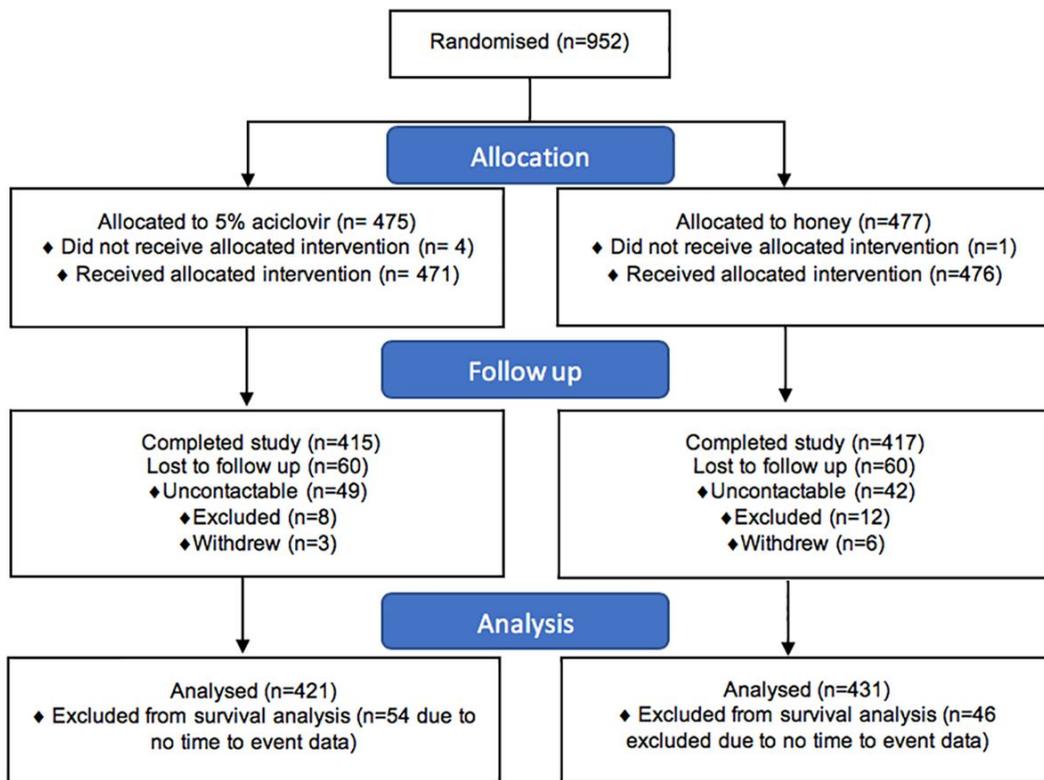
Figure 16: CONSORT study flow diagram. Reproduced from Semprini et al.[14] under the Creative Commons Attribution Non-Commercial (CC BY-NC 4.0) licence.

### 4.1.3 Participant characteristics

Participants were predominantly middle aged, female and well matched between treatment groups for all categorical descriptive variables (Table 1).

|  | Median (IQR) | |
| --- | --- | --- |
| **Continuous variables** | **Aciclovir n=475** | **Honey n=477** |
| Age (years) | 42 (30 to 54) | 43 (29 to 55) |
| Time since last HSL episode (months) | 3 (1 to 7) | 3 (1 to 6) |
| Episodes of HSL in previous year (N) | 3 (1 to 5) | 3 (2 to 5) |
| Pain score at baseline | 2 (1 to 4) | 2 (1 to 4) |
| Time from onset of symptoms to randomisation (days) | 1 (0 to 1) | 1 (0 to 1) |
| **Categorical variables** | **N/475 (%)** | **N/477 (%)** |
| Female sex | 364 (76.6) | 350 (73.5) |
| Ethnicity | | |
| • NZ European | 360 (75.8) | 354 (73.5) |
| • Māori | 62 (13.1) | 66 (13.8) |
| • Other | 53 (11.1) | 57 (11.9) |

**Table 2: Participant characteristics**

### 4.1.4 Primary outcome variable

There was no difference in time from randomisation to stage seven (return to normal skin) between aciclovir and kānuka honey. Kaplan-Meier based estimates (95% CI) were 8 (8 to 9) days for aciclovir (33 participants censored in that some data was provided for inclusion, however the primary end point was not met) and 9 (8 to 9) days for kānuka honey (44 censored); HR (95% CI) 1.06 (0.92 to 1.22), p = 0.56, (Figure 17). An interaction analysis was performed treating stage at randomisation as a continuous variable in order to highlight any potential relationship between this and randomised treatment. No interaction was seen, p = 0.49.
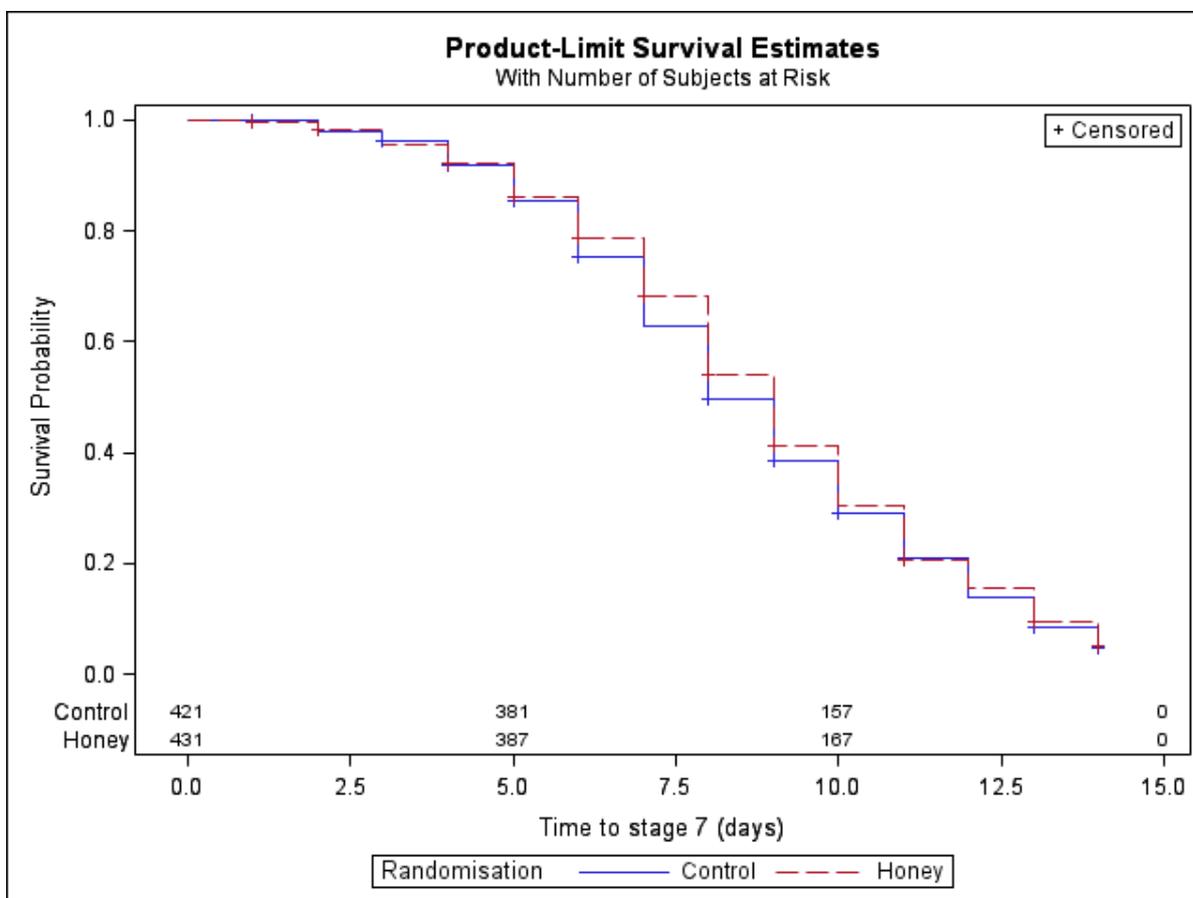


Figure 17: Kaplan-Meier survival plots for time for skin to return to normal, stage 7.

### 4.1.5 Secondary outcome variables

#### 4.1.5.1 Healing time

##### 4.1.5.1.1 Randomisation to stage four

There was no difference in the median estimate of healing time from randomisation to stage four (open wound) of 2 days for aciclovir (3 censored) and 2 days for kānuka honey (9 censored): HR (95% CI) 1.05 (0.92 to 1.20), p=0.46, (Figure 18).
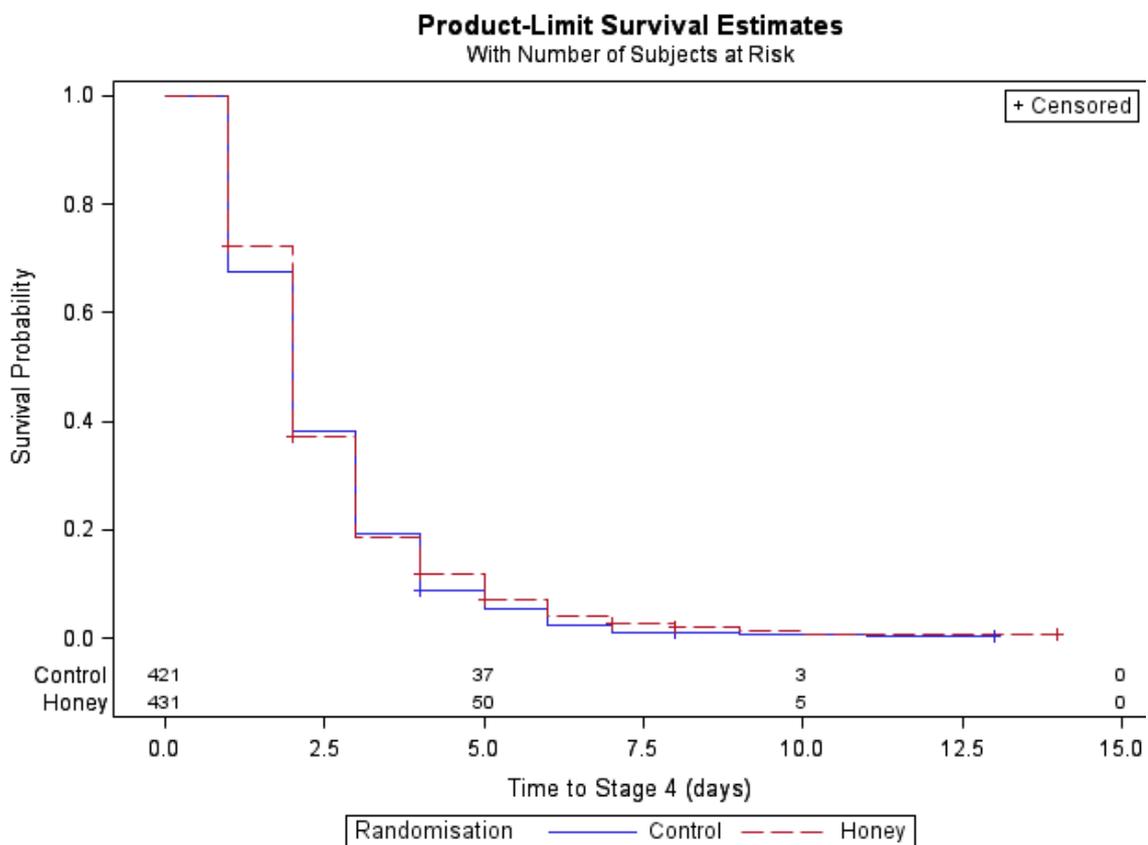


Figure 18: Kaplan-Meier survival plots for time to ulceration of HSL lesion, stage 4.

For time from stage four to stage seven, there was no difference between aciclovir, 7 days (35 censored) and kānuka honey 7 days (40 censored); HR (95% CI 1.03 (0.90 to 1.19), p = 0.66 (Figure 19).
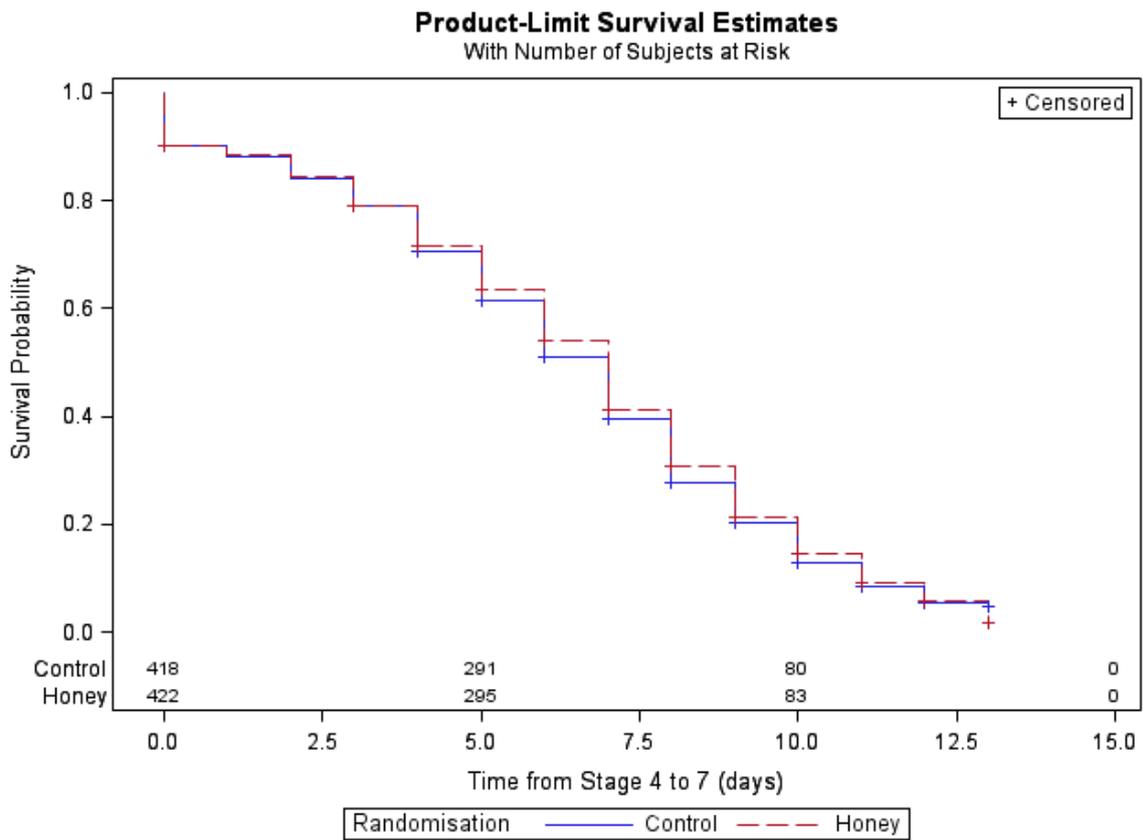


Figure 19: Kaplan-Meier survival plots for time from ulceration of HSL lesion to return to normal skin, stage 4 to stage 7.

#### 4.1.5.2 PAIN

##### 4.1.5.2.1 TIME TO PAIN RESOLUTION

All pain related outcome variables were similar between treatment groups. Estimated median time (95% CI) to resolution of pain was 9 (8 to 9) days for aciclovir (36 censored) and 9 (8 to 9) days for kānuka honey (42 censored); HR (95% CI) 1.04 (0.91 to 1.20), p=0.56 (Figure 20).
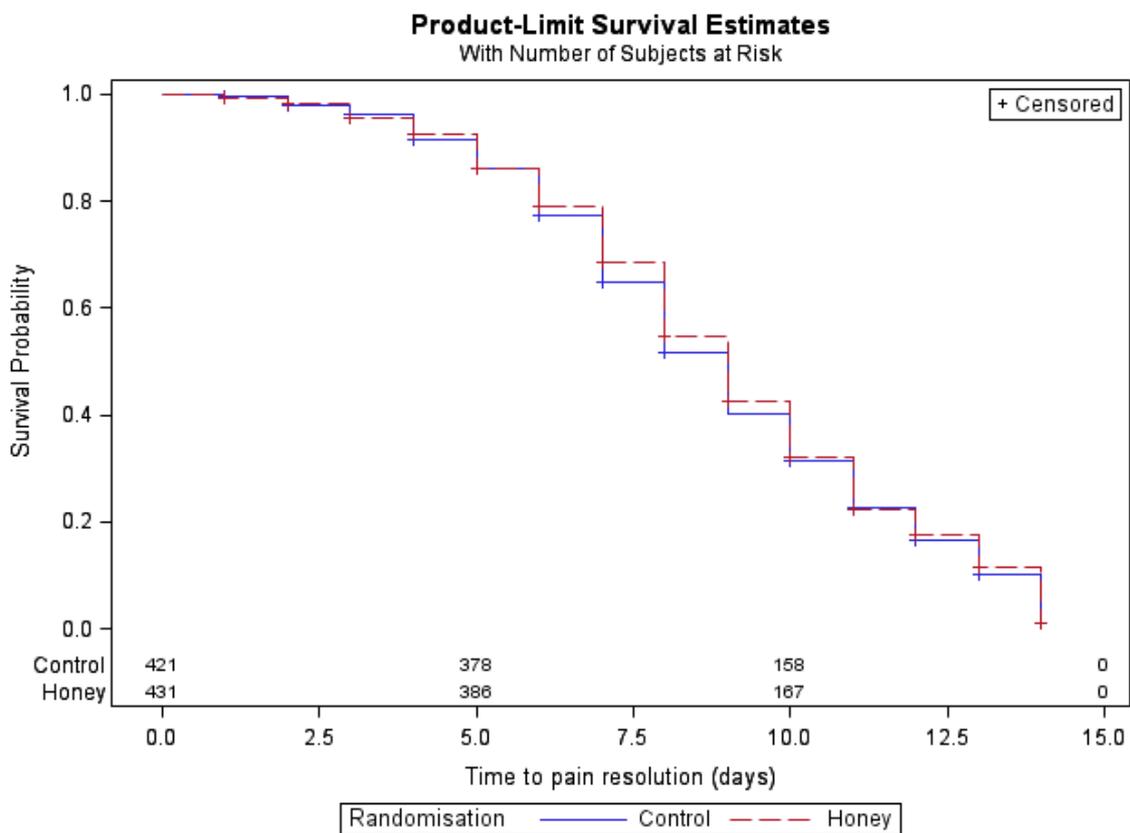


Figure 20: Kaplan-Meier survival plots for time to pain resolution.

The median (inter-quartile range, IQR) maximal pain for aciclovir was 3 (2 to 5) and for honey 3 (2 to 5), with a difference (95% CI) for aciclovir minus honey of -0.02 (-0.32 to 0.28), p = 0.90 (Figure 21).
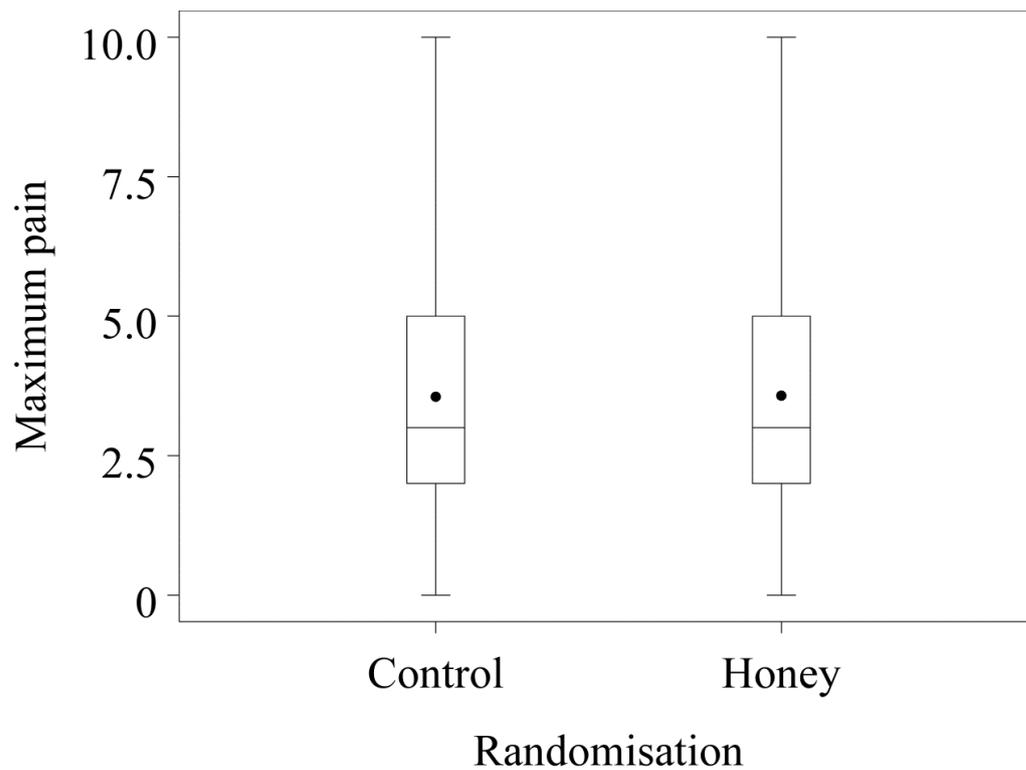


Figure 21: Box plots for maximal pain recorded during the study period.

#### 4.1.5.2.3 ACCEPTABILITY

There was no difference in participant reported acceptability between treatments (honey N=381; aciclovir N=380), with the median (IQR) score for aciclovir 9 (8 to 10) and honey 9 (8 to 10), p=0.12. (Figure 22).
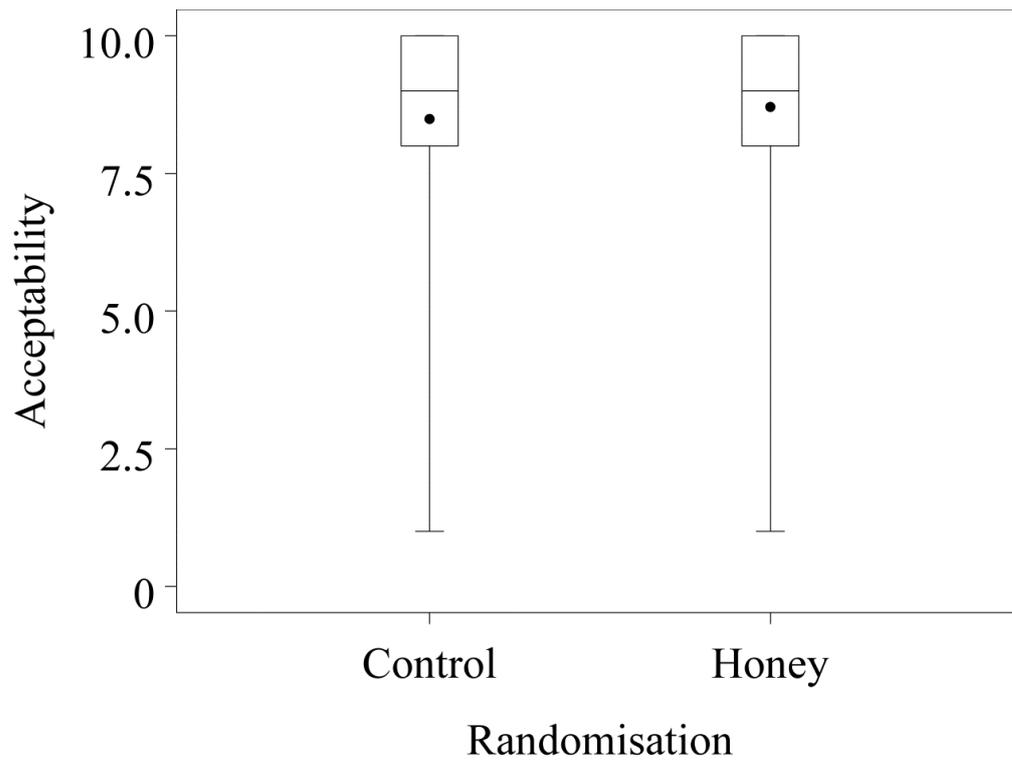


Figure 22: Box plot for treatment acceptability.

#### 4.1.5.3       POST HOC ANALYSES

##### 4.1.5.3.1      TIME TO STAGE SIX

A post hoc analyses was performed in order to benchmark the healing used in the major aciclovir studies, defined as loss of hard wound crust, with residual erythema permitted to the equivalent healing time reported as stage six. There was no difference between the aciclovir, 5 (5 to 6) days (7 censored) and honey treatments, 5 (5 to 6) days (17 censored); HR (95% CI) 1.06 (0.93 to 1.22), p = 0.39 (Figure 23).
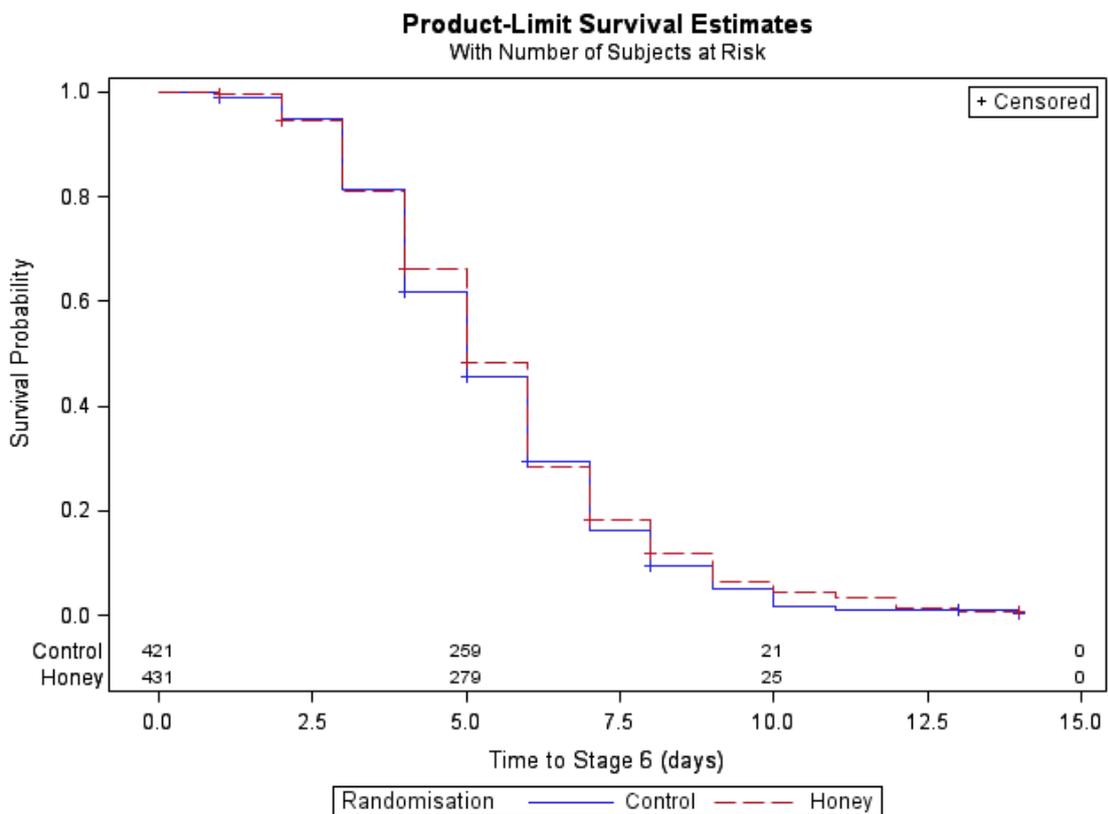


Figure 23: Kaplan-Meier survival plots for time to loss of hard crust, stage 6.

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### 4.1.5.4 PROPORTIONS OF ABORTED HSL EPISODES

43/421 (10.7%) met the definition of aborted HSL episode (baseline stage of three or less and reaching the primary endpoint with no recorded wound stages) in the aciclovir group and 45/421 (10.7%) in the kānuka honey group; relative risk (RR) (95% CI) 0.93 (0.63 to 1.39), p = 0.73.

### 4.1.5.5 SAFETY

Two SAEs were recorded, hospital admissions for atrial fibrillation and urinary retention, both deemed unrelated to the investigational product by the study physician.

There were 17 AEs reported that were classified as 'definitely, probably or possibly' related to the study treatments, six for aciclovir and 11 for kānuka honey (Table 2).

|  | Pain | Swelling | Worsening HSL |
|---|---|---|---|
| Aciclovir (n = 6) | 0 | 2 | 4 |
| Honey (n = 11) | 3 | 2 | 6 |

**Table 3: Adverse events for each treatment group.**

# 5 Chapter five: assessing the Pharmacy Research Network

Various metrics were explored in order to assess the effectiveness of the PRN as a clinical trials methodology. The following data describe and quantify the development of the PRN over the kānuka honey trial, although were not pre-defined outcomes and can be considered exploratory in nature.

## 5.1 The 'burn in' period

A pharmacy local to the MRINZ main office agreed to be the first locality (John Castle Chemist, Newtown Wellington, site A) providing an invaluable opportunity to test and hone study site initiation processes and respond in person to any initial complications with recruitment and study resources. Each site is required to have a regulatory approved locality PI responsible for all study conduct. In order to gain approval from SCOTT, an individual must provide an up to date curriculum vitae, evidence of GCP training and agree to the terms and details of the clinical trial. The vast majority of clinical sites activated throughout the clinical study had little or no research experience and consequently formal knowledge of GCP was lacking. Fortunately, a number of online modules are available for introduction to the principles of GCP and provide certification on completion. The Honevo study used a resource provided free of charge by Roche and approved by Transcelerate, a non-profit body that promotes the quality of clinical research through standardised training materials.[75] Site A was approved by SCOTT to receive investigational product on the 19th of June 2015 and, following delivery of investigational product, agreements and site initiation, the first recruitment took place on 10th of September the same year. Subsequent pharmacy sites were staggered as the study recruitment progressed and data management process evolved becoming established with a total of 76 study localities approved over the two-and-a-half-year data collection period for 952 participants (Figure 24).

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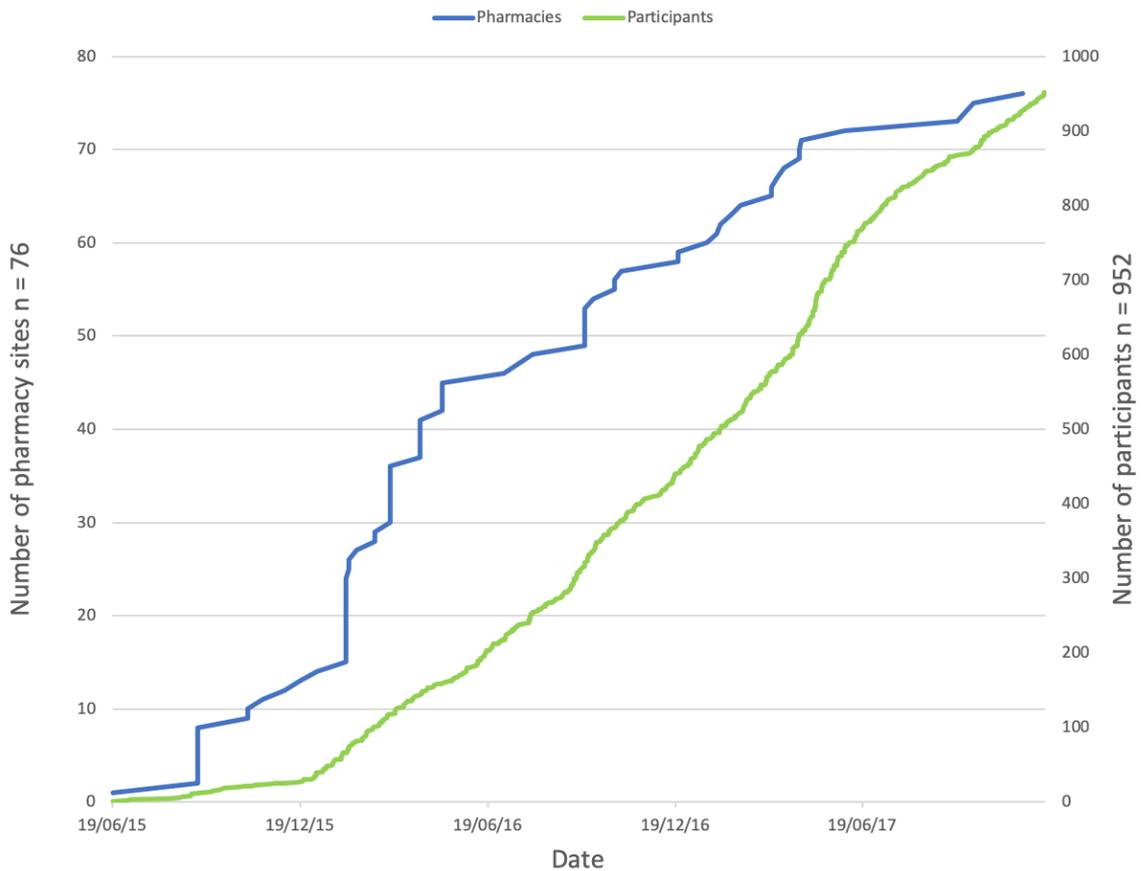
Figure 24: Pharmacy locality and participant recruitment over the study period

## 5.2 STUDY SCALE, LOGISTICS AND RECRUITMENT

### 5.2.1 SITE COORDINATION AND TRACKING

The sample size and number of trial sites required to complete the study presented complexities in organising the participant data and ensuring traceability to the individual investigator responsible for its collection. Each site was allocated a sequential ID, consisting of a capital letter that cycled through the alphabet prior to the addition of a second cycle, e.g. A, B, C... Z, AA, BB and so on. The final locality code used was XXX representing the 76th pharmacy based in Gisborne. This allowed a

nationwide recruitment net, further increasing the generalisability of results (Figure 25).
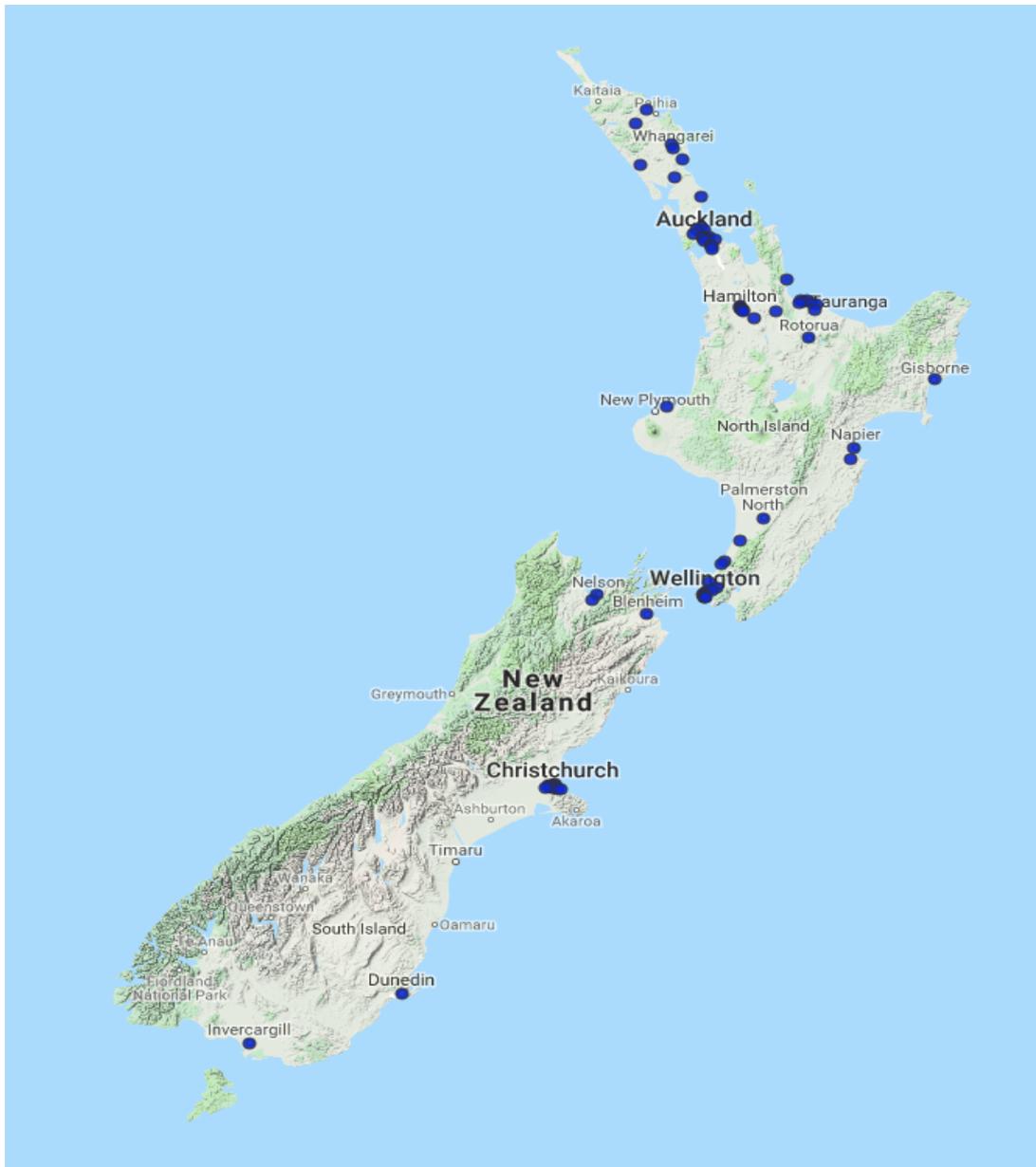


Figure 25: The location of all 76 Pharmacy Research Network study sites throughout NZ

## 5.2.2 INVESTIGATIONAL PRODUCT SUPPLY AND DEMAND

To mitigate the variance in recruitment rates between individual pharmacies and thus avoid the need for redistribution of a finite investigational product supply, a 1:1 randomisation schedule was chosen that generated 78 possible site codes, each having 30 codes to allocate, using a block size of four. For the nine sites that recruited the 30 allocated participants (Figure 27), a further set was provided by the blinded study biostatistician and couriered within sealed opaque envelopes to the pharmacy. Fortunately, there was large amount of investigational product stock available for the study, and redistribution was further avoided through the initial supply of a small amount of each allocation at site initiation, with further supplies being couriered on an as needed basis.

## 5.2.3 PHARMACY RETENTION

Retention of individual sites posed difficulties, with high staff turnovers apparent in the community pharmacy sector, many investigators leaving post, retiring, moving overseas or to another pharmacy. Often these investigators would implement the trial in their new site, and a new investigator would require training to maintain the study at the previous pharmacy. One investigator relocated three times over the course of the study but continued the trial in each new pharmacy under a new locality authorisation. In order to maximise the availability of study staff for recruitment, Medsafe approval allowed for appropriately trained pharmacy technicians and retail staff to conduct all study activities. For those pharmacies involved in the trial for longer periods there were clear signs of recruitment fatigue which was actively managed through targeted retraining visits by me, involvement in media interviews (section 6.2.2.5) and the introduction of study newsletters listing individual pharmacy recruitment rates to promote competitive edge.

## 5.2.4 RECRUITMENT

Recruitment rates were relatively consistent over the three-year period, with an overall rate of 1.1 participants per day from the first to last enrolment and a monthly high of 100 (Figure 26). This contrasts with the MRINZ general practice-based pilot study which recruited 0.03 participants per day over its respective study period.[38]
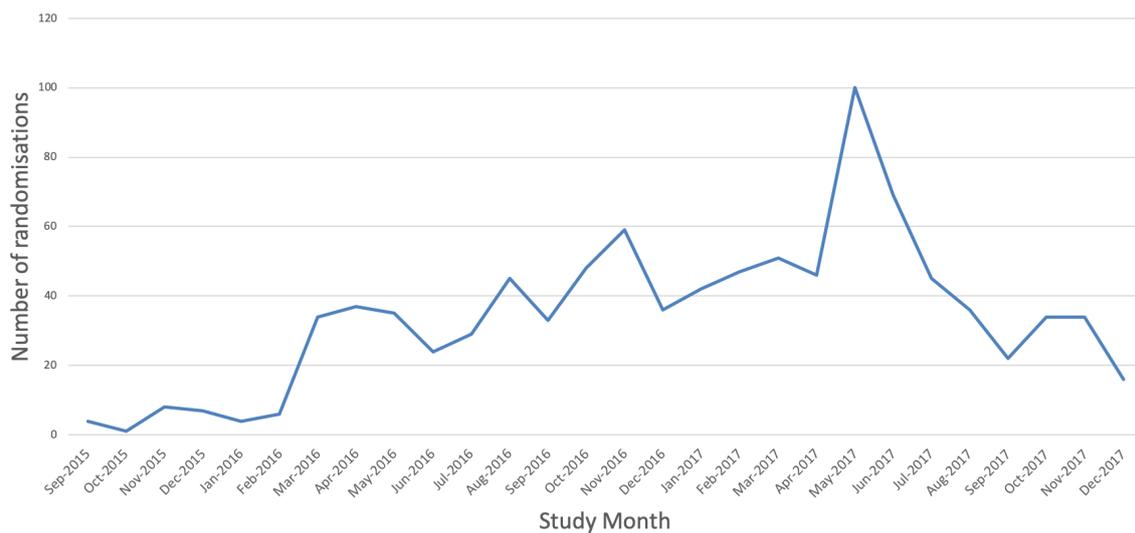


Figure 26: Overall monthly recruitment for the study period.

At the individual pharmacy level, recruitment was fluid throughout the trial period. New sites continued to be set up almost through to study completion with the 76th site initiated in Gisborne on the 24th November 2017 and the final participant enrolled on 13th December 2017. Recruitment statistics by site varied tremendously (Figure 27; Table 3), with a range of zero to 62 and median number of participants recruited per site nine.
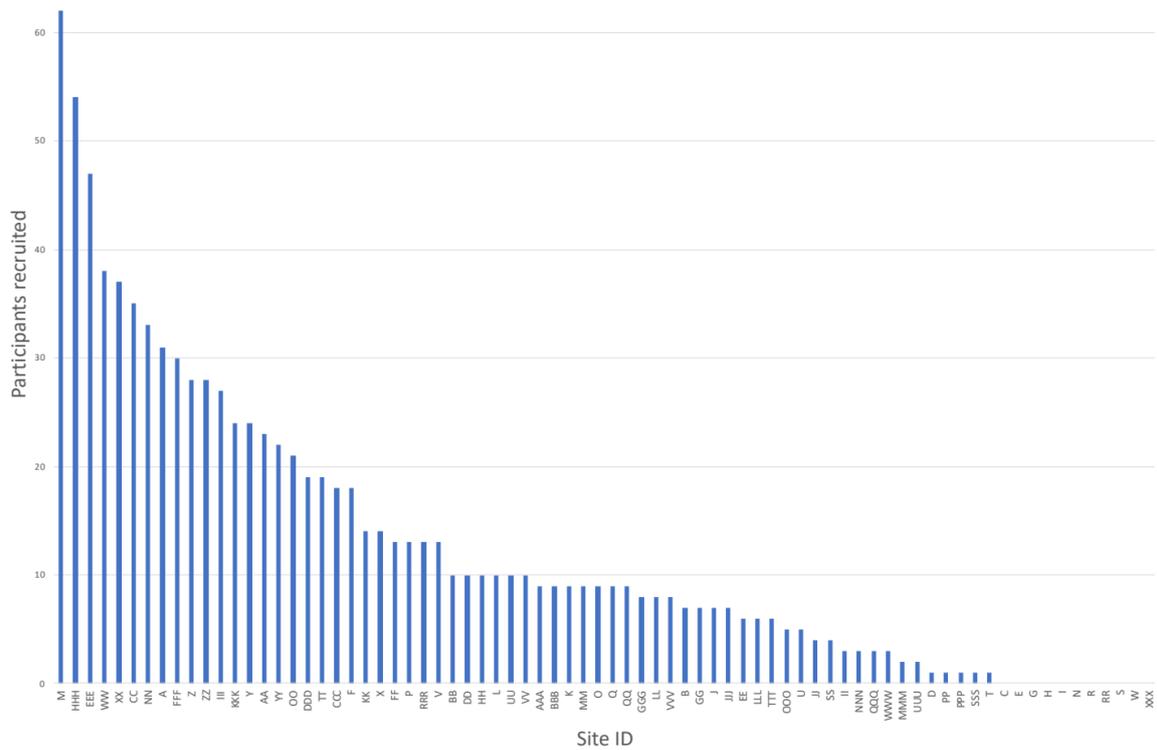
Figure 27: Total number of participants recruited by each pharmacy site.

| Overall Number of recruits | Number of pharmacy localities |
| --- | --- |
| 0 | 11 |
| 1 to 9 | 32 |
| 10 to 19 | 16 |
| 20 to 29 | 8 |
| 30 + | 9 |

**Table 4: Distribution of study pharmacies recruiting within specified overall milestones.**

The median (mean) time to first recruitment from SCOTT pharmacy approval across sites was 42 (67) days, ranging from six to 438. Many factors influenced this period, such as unforeseen staffing issues requiring the rebooking of training slots or sudden departure of the pharmacy PI and need to recruit a replacement. The strongest influence was likely to be site confidence in conducting the initial participant visit. Once the first participant had been successfully enrolled at a site the median (mean) number of days until the second recruit was 7.5 (23) days, (range 0 to 245), suggesting the key step in the study pharmacy development process was to encourage immediate engagement through early enrolment post initiation.

Of the 952 participants recruited overall, 120 (12.7%) were lost to follow-up of whom 91 (9.6%) were uncontactable, the rest either excluded for baseline ineligibility or withdrawing due to adverse effects or worsening symptoms. This was encouraging in the context of a recent systematic review which found a median (IQR) of 6% (2 to 14%) loss to follow-up in 191 analysed trials, taken from the top five medical journals by impact factor from 2005 through 2007.[76] A highly conservative estimate of maximal loss to follow-up was defined *a priori* within the sample size calculation for the Honevo trial at 10%, replicating the generally accepted upper limit of introducing a high risk of bias and impacting the validity of results.[77] This was based on established assumptions around participant attrition and aimed to account for the unknown recruitment mechanism, no benchmark for the quality of inclusion criteria assessment by the pharmacist investigators, potential barriers to participant retention within an entirely remote data collection capacity and no face to face follow-up. Importantly, the attrition rate and underlying reasons were consistent between randomised study arms, meaning any bias was likely to be equal between groups.

### 5.2.5 THE HIGH PERFORMING NETWORK

There was a striking variation in the rates and volume of recruitment between pharmacies as shown in figure 31, highlighting the need to also consider the rate of individual pharmacy recruitment when ranking performance. The first site initiated, A, illustrates this well, having recruited the eighth highest number of participants overall, but ranked $25^{th}$ over the time from each site's first recruitment to the formal conclusion of the study.

At a more granular level, it was apparent that interest in the concept of pharmacy-based research varied tremendously between both owners and the staff delegated to conduct the study. The most successful sites were of course those that had a drive from both, and these quickly sparked the concept of a high performing network (HPN). The initial definition of the HPN was total recruitment over the course of the study, highly advantaging the PRN member sites that had been active longest and not considering relative rate of recruitment as illustrated in figure 28.
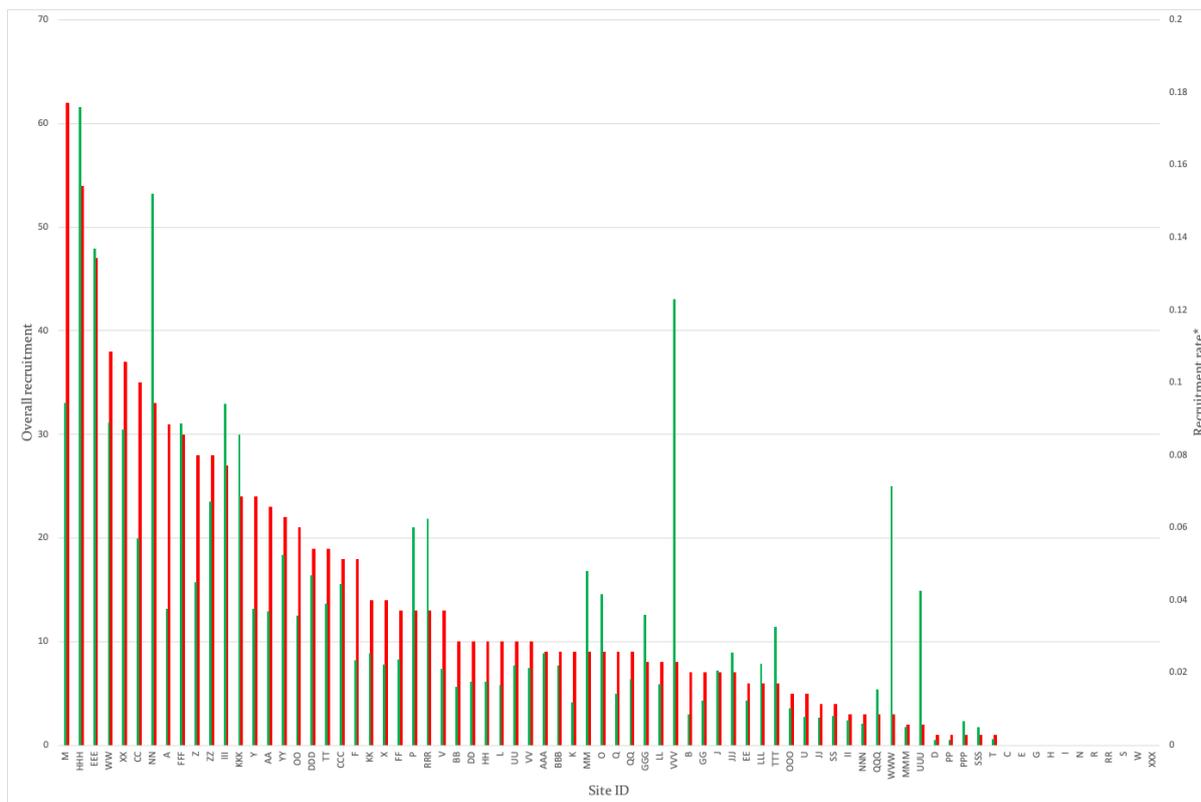
Figure 28: Overall recruitment (red) and rate of recruitment from first enrolment at a site until study conclusion (green).

Other factors also presented as potential confounding influences. For the kānuka honey study there was a statistically significant association between recruitment rate, season and region (Tables 4-5). Comparing all regions to Wellington and winter as a baseline, recruitment in Auckland, Tasman and Waikato was inferior and Manawatu-Wanganui, Northland, Southland and Taranaki superior and there was no overall seasonal difference in recruitment nationally. Whilst this analysis was exploratory and not predefined, it shows the value to a wide geographical recruitment net with quality in regional performance variable dependent on season.

| Region | Estimate | Standard Error | T Value | Pr > \|t\| |
|---|---|---|---|---|
| Intercept | 0.0500 | 0.0032 | 15.6300 | <0.0001 |
| **Auckland** | **-0.0300** | 0.0052 | **-5.9000** | **<0.0001** |
| Bay of Plenty | 0.0100 | 0.0056 | 1.8700 | 0.0600 |
| Canterbury | -0.0100 | 0.0059 | -1.6300 | 0.1000 |
| Hawke's Bay | -0.0100 | 0.0129 | -1.1600 | 0.2500 |
| Marlborough | -0.0100 | 0.0094 | -1.2500 | 0.2100 |
| **Manawatu-Wanganui** | **0.1000** | 0.0072 | **14.0900** | **<0.0001** |
| **Northland** | **0.0200** | 0.0066 | **3.1000** | **0.0020** |
| Otago | 0.0100 | 0.0099 | 1.1900 | 0.2300 |
| **Southland** | **0.0900** | 0.0085 | **10.1500** | **<0.0001** |
| **Taranaki** | **0.1300** | 0.0060 | **20.7900** | **<0.0001** |
| **Tasman** | **-0.0300** | 0.0107 | **-2.5300** | **0.0116** |
| **Waikato** | **-0.0300** | 0.0107 | **-3.0200** | **0.0026** |
| Wellington | 0.0000 | - | - | - |

**Table 5: Variation in recruitment by region. Wellington is the reference for region.**

| Season | Estimate | Standard Error | T Value | Pr > \|t\| |
|---|---|---|---|---|
| Autumn | 0.0034 | 0.0041 | 0.8300 | 0.4059 |
| Spring | -0.0058 | 0.0048 | -1.2100 | 0.2280 |
| Summer | 0.0023 | 0.0051 | 0.4500 | 0.6503 |
| Winter | 0.0000 | - | - | - |

**Table 6: Variation in recruitment by season. Winter is the reference for season.**

# 6 Chapter six - discussion

## 6.1 The randomised controlled trial

### 6.1.1 Summary of outcomes

There was no significant difference between 5% aciclovir cream and 90% kānuka honey/10% glycerine formulation for the topical management of HSL across any outcome variable tested. All measures of time to complete healing, onset of wound stage, healing from wound to return to normal skin, pain resolution, proportion of aborted episodes, maximal pain and participant acceptability were similar between the two treatment groups. The study was adequately powered to detect a one-day difference in time to healing between treatments. Importantly, the upper 95% confidence limit for the primary outcome variable HR was within the pre-specified superiority boundary of 1.25. allowing confidence in excluding a significant difference of this magnitude.

### 6.1.2 The addition of real-world evidence

This study represents one of the largest single clinical trials of HSL ever undertaken and provides evidence for the use of medicinal kānuka honey as a viable alternative treatment option to aciclovir for HSL.[173] The large sample size randomised within the novel research infrastructure provided adequate power, allowed recruitment of a representative, national cohort of New Zealanders, mitigating the jurisdiction bias usually encountered through the geographical limitations of traditional research models that require participants to attend specific, most often urban, clinical trials units.[178] Capturing the heterogeneity of presentation and progression of HSL manifestation in the community setting is a strong benefit to this approach and ensured outcome data was rep-

resentative of usual treatment access. Furthermore, the study avoided the need for highly inefficient and costly pre-randomisation to ensure treatment was commenced within the prodromal phase.

The use of the clear, written and photographic scale for HSL lesion assessment, delivered via smartphone removed the need for expensive and burdensome clinic visits with participants able to self-report standardised outcomes within their usual daily routines. This also allowed for real time data collection to ensure maximal recall accuracy, monitoring and immediate follow-up by the study investigator minimising missing data. This approach complements the traditional RCT approach essential to establish efficacy, safety and regulatory data for novel therapeutics within a controlled and supervised, but artificial environment. However, prior methodologies have been limited in their ability to provide external validity and definite measures of drug performance in highly diverse, real-world settings.[179] Importantly, for manufacturers of such classes of over-the-counter treatments that do not command the budget for cost-prohibitive, traditional drug development processes[36] the inaugural PRN study has demonstrated the feasibility of conducting a large scale, comparative RCT utilising a cost and time effective, compliant community-based infrastructure able to provide robust outcome data.

The finding that a medical grade, 90% kānuka honey/10% glycerin formulation might be considered as an acceptable therapeutic alternative to aciclovir is particularly valuable to more vulnerable, minority groups of HSL sufferers. It was anticipated that the over-the-counter availability of an evidenced treatment such as Honevo would be of interest to pregnant or breastfeeding women, given the lack of conclusive evidence for safe use of aciclovir and patients allergic or sensitive to it. Since the completion of the study and publication of the results, I have fielded a number of calls in this regard from PRN pharmacies.[14] There may also prove to be a case for use in immunocompromised individuals amongst whom a significant rising rate of aciclovir resistance has been

shown, from 3.8% to 15.7% in a nine year period, thought to be due to the increasing viability of allogenic stem cell transplants.[180] Importantly, the results have allowed pharmacists to directly draw on robust evidence in the recommendation of a CAM, a class of product which is too often purchased at a premium without evidence of efficacy.

### 6.1.3 TIME COURSE OF EFFECTS

#### 6.1.3.1 PRE-WOUND STAGE

Our expectation was that the potent anti-viral activity of aciclovir would result in an increased proportion of aborted episodes compared to kānuka honey, however this was not the case with the study showing no difference in the proportion of episodes not progressing beyond the vesicular stage to an open wound but returning to normal skin. This suggests that the magnitude of anti-viral effects of kānuka honey may be similar to aciclovir or alternatively that the clinical benefit of the antiviral effects of aciclovir may be attenuated by the time a person presents with an established coldsore.

The HSV replication cycle, once triggered in the centrally located dorsal ganglion, migrates to the skin epithelial surface where the process continues in conjunction with a host inflammatory response and associated symptoms (Figure 3). By the point at which an HSL sufferer is aware a recurrent episode has triggered, the infective cycle has often become established, with a viral load sufficient to precipitate the wound-based stages. Given the fast increase in viral load and the highly efficient acquired immune response to this, targeted anti-viral therapy such as topical aciclovir may have limited efficacy on increasing the rate of aborted lesions, as suggested in the literature.[181] Likewise, whilst the intended action of such antivirals might result in a reduction in viral replication and thus load, once the inflammatory response has been triggered there will be limited efficacy in abrogating this. Consequently, any anti-viral activity that may be present in kā-

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nuka honey, as suggested by various preclinical experiments with mānuka honey against influenza, varicella zoster and rubella viruses, would be subject to the same constraints of efficacy.[159,161,162]

### 6.1.3.2 WOUND STAGE

Considering the known positive effects of honey on wound healing processes such as angiogenesis, granulation, epithelialisation, reduction in oedema and debridement, our expectation was that the wound-based stage of HSL would resolve faster in the group randomised to the kānuka honey treatment compared to aciclovir. This was not the case, with no difference between the treatment groups demonstrated in the time from ulcer development at stage four to return to normal skin at stage seven.[19] It was considered whether variation in stage at enrolment influenced the time to healing between treatment groups, however an interaction analysis showed no evidence of such an effect.

Reasons for this may be that once the host inflammatory response has been triggered, the continued presence of HSV maintains this activation signal and cytopathic damage continues. The positive effects of honey on healing are therefore potentially attenuated until this replication phase is complete. Alternatively, any reduction in the length of the HSV viral replication phase induced by aciclovir may lead to a beneficial effect in late healing stages, similar to that observed with kānuka honey.

### 6.1.4 THE PRIMARY OUTCOME DEFINITION IN CONTEXT

The PRN is a highly generalisable clinical trial infrastructure with the capacity to recruit and collect both objective and subjective data from heterogeneous participants reflective of a broad population. Considering this unique capacity, the known aesthetic consequences and associated psychological stigma felt by many HSL sufferers, it was more

appropriate to report to participants a meaningful outcome of return to normal skin.[15] This was in contrast to the bulk of the published literature which tended to use a more virologically defined end point of loss of crust, used as a proxy for the end of the viral replication cycle, after which visual evidence of the HSL and symptoms can still remain.[46,54,182–185] A post-hoc analysis for time from randomisation to stage six was however performed in order to provide some context which demonstrated a reduction in healing time to this stage of median five days for both aciclovir and honey. Whilst this appears in line with the most highly cited, previously reported data,[127] it should be noted that the appropriate analysis for Kaplan-Meier derived time to event data is estimated medians, allowing for the accommodation of censored data and for the highly skewed nature of such datasets. Many of the previous studies report mean values which cannot be accurately compared to similar interventional study designs reporting medians.

### 6.1.4.1 A DEEPER REVIEW

A deeper review of the HSL interventional literature described in chapter two was undertaken, filtering out non comparable end points such as clinic reviews for healing stage over one day apart and studies recruiting non-equivalent participant groups to the kānuka honey such as paediatric patients or the immunocompromised. Each remaining study is summarised below for healing definition extrapolated to the pictorial stages used in the kānuka trial, times to healing for the active treatment and placebo are converted to days where required and highlighted as to whether this time to event end point was reported as a mean or median value (Table 6). Three studies have data points for both times to stage six and stage seven, and one for stages five and six which are included respectively.

| Study | Stage | Intervention | Outcome | Active (Days) | Placebo (Days) |
|---|---|---|---|---|---|
| Formica et al[76] | 5 | DSS | Mean | 3.5 | 4.9 |
| Godfrey et al[80] | 5 | Zinc oxide | Mean | 5 | 6.5 |
| Honarmand et al[184] | 6 | ACV | Mean | 3.4 | 4.3 |
| Hull et al[183] | 6 | ACV | Mean | 5.7 | 6.5 |
| Evans et al[182] | 6 | ME609 | Median | 5.4 | 6.8 |
| Raborn et al[186] | 6 | PCV | Median | 4.6 | 5.4 |
| Sacks et al[54] | 6 | Docosanol | Median | 4.08 | 4.8 |
| Kaminster et al[88] | 6 | Tetracaine | Mean | 5.1 | 7.2 |
| Shafran et al[93] | 6 | Undecylenic acid | Mean | 6.9 | 6.4 |
| Spruance et al[187] | 6 | PCV | Median | 4.8 | 5.5 |
| Habbema et al[55] | 6 | Docosanol | Mean | 4.7 | 8 |
| Lawee et al[95] | 6 | Foscarnet | Median | 6.1 | 7 |
| Shaw et al[188] | 6 | ACV | Median | 5 | 5 |
| Van Vloten et al[131] | 6 | ACV | Mean | 5.7 | 8.3 |
| Femiano et al[45] | 6 | ACV | Mean | 6 | 0 |
| Femiano et al[45] | 6 | ACV | Mean | 4 | 0 |
| Evans et al[182] | 7 | ME609 | Median | 9 | 10.1 |
| Shaw et al[188] | 7 | ACV | Median | 9 | 10 |
| Fiddian et al[133] | 7 | ACV | Median | 4 | 6 |
| Fiddian et al[133] | 7 | ACV | Median | 6 | 8 |
| Spruance et al[135] | 7 | ACV | Mean | 7.8 | 7.3 |
| Boon et al[44] | Not stated | PCV | Median | 7 | 8 |
| Spruance etc al[130] | Not stated | ACV | Median | 6 | 5.2 |

**Table 7: Variability in healing definitions for comparable studies of HSL treatments. ACV = aciclovir; PCV penciclovir; DDS = dioctyl sodium sulfosuccinate**

The variation in reported time to healing is marked between studies. By far the most commonly used definition is time to loss of crust (stage six), rationalised by representing the completion of the viral replication cycle and therefore the therapeutic window in which an active therapy can potentially provide an effect. For this group, time to heal-

ing for placebo ranges from 4.3 to 8.3 days with the afore mentioned caveat of variable use of mean and median, which renders precise comparison difficult due to the presence of normal and non-normally distributed data. The extreme values of this range, however, are both reported means and highly illustrative of the variability in study outcomes for HSL.

There is inconsistency in the healing times reported between studies conducted by the same investigators over time. An early study of aciclovir versus vehicle control found no statistical difference in time to loss of crust (stage six) between groups (7.2 and 7.3 days respectively,[135] yet two studies of the same active and comparator reported by members of the same group 20 years later demonstrated marked differences in healing times to the same end point (stage six) of 4.3 and 4.8 days for study one and 4.8 and 5.2 days for study two, respectively.[127] A point of difference appears to involve a shorter time to treatment initiation in the latter study of mean 0.6 hours, with the first reporting 58% of participants initiating therapy in the initial eight hours from onset and the remainder within nine to 25 hours. In addition, the base composition of aciclovir cream was variable between the first and latter studies, using polyethylene glycol and propylene glycol respectively, which may contribute to the discrepancy through differences in effective drug penetration through the skin barrier. Closer review of other similar studies yields longer times to the same healing end point, with a study of penciclovir reporting six days for its active comparator aciclovir, in propylene glycol base (Zovirax) with treatment initiation defined as from onset of prodrome.[45] For context within this thesis, the large kānuka honey study presented reported a median of five days to loss of crust, or stage six, for the 5% aciclovir comparator, Viraban, which contains both propylene glycol and poly-ethylene glycol (Figure 23).[14]

Another consideration in the interpretation of previous studies is the potential beneficial effect of 'placebo' treatments comprising the vehicle base of the active intervention. This issue was investigated in the relatively small and minimally cited study of 5%

aciclovir in propylene glycol compared to placebo (propylene glycol vehicle) and no treatment.[188] This trial highlighted that both the active and placebo interventional groups healed faster than no treatment at all with median time to complete healing (stage seven) of nine days for aciclovir, 10 days for placebo (propylene glycol vehicle) and 13 days to complete healing for no treatment. This suggests that the regular application of a topical substance comprising the vehicle control may enhance the healing process. Recognition of this property is the rationale for the Compeed® cold sore patch, which comprises a hydrocolloid dressing and is widely used as an over-the-counter treatment of HSL.[74] The potential for such 'placebo' topical controls to affect wound healing has been established in pre-clinical studies with demonstrable influence on specific aspects of physiological processes, such as DNA synthesis, angiogenesis and epithelial surface migration rates.[189]

The apparent superiority of multi-floral honey from the United Arab Emirates over aciclovir in reducing HSL healing time, reported by Al-Waili et al was not confirmed in the kānuka honey community pharmacy-based study and there are some clear explanatory differences between the two.[138] Firstly, the small sample size in this cross-over study of eight participants receiving aciclovir and multi-floral honey rendered the study vulnerable to type I error, the rejection of the null hypothesis when it is in fact true.[190] This possibility is supported by the magnitude of the reduction in mean healing time to normal skin reported for honey (2.57 days) compared to aciclovir (5.85 days) which is not consistent with the magnitude of effect reported for the wide range of pharmaceutical and CAM treatments studied to date.[136] Second, the study designs differ, with physician reported outcomes obtained in the Al-Waili study compared to patient reported in the PRN trial. Another difference is that the composition of specific honeys is likely to differ greatly, with the United Arab Emirates multi-floral honey potentially containing more potent anti-viral, pro-wound healing immunostimulatory and/or anti-inflammatory factors not present in kānuka honey, leading to the markedly different outcome in terms of time to healing to the kānuka study presented. Further research is required to

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both understand specific differences in composition between the honeys through laboratory analysis, and the conduct of an adequately powered RCT testing both for the treatment of HSL.

Such inconsistency in reported outcomes, likely influenced in part by design factors such as lack of vehicle control, physician versus patient reported assessment, variable use of healing definition and assumption of zero effect placebo creams highlight the importance of adequately powered, RCTs conducted in community, real-world settings. To date, the pharmacy-based study presented in this thesis is the sole clinical trial in the literature to offer generalisable data as to how therapeutic agents, in this case kānuka honey and aciclovir cream, perform in a more representative patient population, during their individual, heterogenous daily routines. The data reported for topical kānuka honey in a 10% glycerin base can be interpreted as offering no greater benefit in time to healing compared with aciclovir, and conversely no greater benefit is seen for aciclovir compared to kānuka honey.

### 6.1.5 THE PARTICIPANTS VIEW

The participant feedback from the 761 acceptability scores reported a median 9 (out of a maximum 10) for both aciclovir and honey, indicating that both topical treatments represent a positive treatment experience for HSL sufferers.

### 6.1.6 STUDY LIMITATIONS

#### 6.1.6.1 DATA SEMANTICS

There are a number of statistical considerations relevant to the interpretation of the data. The study was designed to determine if either treatment was superior to the other, and whilst tempting to interpret the results of the study to show equivalence, or

non-inferiority of effectiveness between topical kānuka honey and 5% aciclovir this was not the predefined analysis. Inclusion of a non-inferiority analysis in the *a priori* statistical plan would have allowed a valid additional analysis through the predefinition of a non-inferiority bound. A decision was made to not retrospectively undertake this analysis due to the inherent risk of hypothesis switching. However, there is some rationale for considering non-inferiority between kānuka honey and aciclovir is likely. For the primary endpoint, time to healing, the 95% CI for the difference between kānuka honey and aciclovir was very narrow at 0.92 to 1.22. The upper 95% CI limit of 1.22 was within the pre-specified superiority boundary of 1.25, allowing confidence in excluding a significant difference of this magnitude.

## 6.1.6.2   CHOICE OF CONTROL

The decision to use an active comparator was based on the previously demonstrated efficacy for aciclovir and other nucleoside analogue drugs compared to placebo in earlier studies.[127,136,173] In addition, the 2017 FDA guidance pertaining to clinical trials of recurrent HSL states 'i*n addition to placebo-controlled trials, sponsors could also consider superiority trials against an active contro*l'.[191] Certainly, the preferable study design for the study of kānuka honey discussed would have been a three way, vehicle controlled randomised trial incorporating a glycerin topical as the control group. This subsequently was highlighted in discussions with tier one pharmaceutical companies interested in the results of the trial with a view to licensing of the product. Whilst this limitation was recognised from the outset, and notwithstanding the complexities in calculating an adequately powered three arm sample size for time to event analysis, the sheer numbers required for this were not considered feasible or proportionate to the available study budget. Encouragingly it is now clear that such a study would be a realistic undertaking in the PRN, given the performance and scalability of the PRN demonstrated.

### 6.1.6.3 BLINDING HONEY

Masking a honey-based treatment at a concentration of 90% is impossible due to the physical characteristics such as viscosity, taste and smell which are vastly different to the white, scentless cream formulation of topical 5% aciclovir. Both participants and consenting pharmacy-based investigators were therefore privy to allocation, whilst the central MRINZ investigator remained blinded unless unmasking was required due to AE or SAE assessment and reporting. This is a clear potential source of selection bias although unavoidable. Interestingly, there is debate as to whether participants in clinical trials alter self-reported data dependent on knowledge of the treatments they have received, in oncology trials at least.[192]

### 6.1.6.4 A RELIANCE ON SUBJECTIVE OUTCOMES

A study reliant on a self-reported primary outcome is subject to the limitations of factors such as a social desirability bias, in which a participant might want to offer the response to trial questions that they perceive the investigators are seeking.[193] This can be further compounded by decreasing response bias over time, as the participant settles in to the trial process and becomes familiar with the study team. However, the large sample size with the fully automated and impersonal nature of self data entry likely mitigates this. Whilst the pharmacy investigators are provided a detailed, pictorial staging chart and trained to assess baseline HSL lesions, this is not equivalent to other studies that incorporate a specific dermatologist assessment and thus may be a source of variability. In addition, there was no objective visual corroboration of the participant reported return to normal skin, adding further potential for variance in primary outcome. Mitigation of these two limitations is currently being tested within a subsequent PRN study that incorporates clinical photography for remote, blinded assessment by an academic dermatologist in addition to the pharmacy investigators with a view to intraclass correlation analysis.

## 6.2    THE PHARMACY RESEARCH NETWORK

The successful completion of a RCT within a network of 76 pharmacies demonstrated the feasibility of the approach and has created a facility to produce high quality evidence for therapies used outside of traditional clinical trial infrastructure.

### 6.2.1 THE NEED FOR A PHARMACY RESEARCH NETWORK

The modern paradigm of the RCT is still recent with the first reported study enacted by the United Kingdom's Medical Research Council in 1943 for use of streptomycin for pulmonary tuberculosis.[194] A number of interesting historical approaches paved the way to this contemporary standard, dating back as far as the biblical reign of King Nebuchadnezzar who compared the impact of two diets, meat and wine versus vegetables and water, finding that the latter group were in better health after 10 days. Two millennia later the famed physician James Lind assigned various dietary treatment approaches to scurvy patients in a rudimentary, parallel arm study in which he found the group allocated a citrus component returned to full health.[195] Today, necessary and stringent regulatory requirements have resulted in a high quality and effective clinical trials industry allowing evidence-based healthcare systems to flourish and provide ever increasing standards of care. Models of clinical research thus naturally evolved into well-funded major units, often based within, or close to hospital premises resulting in a resourced but relatively high cost and geographically limited capacity to conduct formal randomised controlled trials. Globalisation of clinical research programmes has increased with a new standard of multi-centre, international studies often expected and a requirement to be conducted in line with harmonised regulatory processes and methodologies. This process is, as previously discussed in chapter one, extraordinarily expensive and consequently reserved for pharmaceutical companies who focus investment on potentially high value drug development with a view to licensed, prescription products or over-the-counter therapies suited to a global market, highly likely to offer return on the signific-

ant capital required to develop them. Topical treatments for HSL are a good example of this, with a number of major, high cost studies conducted in the traditional clinical trials unit setting the claims from which underpin a substantial global market value for products such as Abreva (docanosol) and Zovirax (aciclovir).[54,127] However, these are the exception to the rule, which sees the vast majority of accessible, over-the-counter treatments marketed to patients unable to provide robust efficacy claims due, in part, to limited or no access to the appropriate research infrastructure necessary to achieve this. Developing an evidence base for CAM therapies therefore represents a challenge for a number of reasons.

## 6.2.1.1 COST

Recent analysis attempts to quantify the average costs for clinical trials have estimated a randomised trial incorporating active or placebo comparators costs a median of $35.1 million USD.[196] The paucity of clinical evidence directly funded by smaller pharmaceutical and CAM manufacturing companies is therefore unsurprising. Clinical research organisations around the world with the expertise to conduct informative clinical trials consequently focus on high value study contracts, needing to develop and maintain the operational capacity to meet standards required for regulatory submission of data to bodies such as the FDA and European Medicines Agency (EMA). Many CAM products are produced by small, independent manufacturers that do not have the financial means to fund such research and development programmes, although investment opportunities are on the rise for such companies recognising the potential for 'out-licensing' or on-sale of their products. However, comprehensive clinical and marketing data is usually required and costly attendance at relevant business partnering forums essential to gain an audience with prospective investors and high tier pharmaceutical company representatives. This has resulted in small organisations seeking out clinical trial capacity to provide pre-pivotal study data as the basis of negotiations, an area where a capacity such as the PRN is proving attractive.

## 6.2.1.2 REGULATORY BARRIERS

Production standards for CAM products vary in New Zealand with some produced under food and safety certification in line with the 1985 Dietary Supplements Regulations,[197] some under cosmetic GMP and fewer yet under pharmaceutical GMP, the latter usually by manufacturers of both prescription medicines and consumer brands. Consequently, medicinal or therapeutic claims are not permitted for the majority of CAM products in NZ, even if they have undergone a suitable clinical trials process. Clinical trial applications to the New Zealand regulatory committee, SCOTT, for products such as Honevo that do not meet the pharmaceutical GMP standard, but are demonstrably produced in line with equivalent guidelines, have been approved on a case by case basis, the data from which has allowed discussions for registration pathways to international markets and a degree of confidence around efficacy.

As the number of enquiries pertaining to clinical research of CAM products continues to rise, the lack of a formal process to assess appropriate therapeutic claims for them in New Zealand requires urgent resolution. In the absence of this, opportunities to gain robust evidence to inform the many consumers that purchase these products continue to be lost, due to the high and prolonged expense of RCTs. Despite a legislation prohibiting manufacturers from making therapeutic claims, the reality of consumer use is often with a view to a specific therapeutic effect, despite what is stated on the label. Furthermore, inadequate enforcement of the current regulations is an issue, as illustrated by high profile cases of the promotion and sale of unevidenced and clinically dangerous 'cures' in NZ. One recent example containing sodium chlorite which has been promoted as a cure for many ailments, including COVID19 infection.[198] In Australia the Therapeutic Goods Administration (TGA) have a pathway for assessment complementary therapies, under which the products are specifically assessed for safety, quality and efficacy, a sensible approach that should be considered in the New Zealand context.[199]

### 6.2.1.3  ACADEMIC CAPACITY AND COLLABORATION

In addition to the financial, regulatory and commercial barriers there is a relative scarcity in academic interest and capacity to research CAM therapies. This situation persists despite the formation of dedicated agencies such as the United States National Centre for Complementary and Alternative Medicine in 1998, the Australian National Institute for Complementary Therapies in 2007 and the British Foundation of Integrative Medicine in 1997. Reasons for this are complex and multi-faceted but can perhaps be distilled to two primary barriers: funding and cultural.

Research funding is a contentious issue, hugely variable between countries and highly dependent on the incumbent priorities of government and peer review. Funding systems prioritise conditions that are associated with high morbidity and mortality and impact on wider healthcare infrastructure. Consequently, a focus on therapeutic potential of products such as CAMs to relieve non-life-threatening symptoms tends to be under resourced, with a cascade effect on educational opportunities, career pathways and development of field expertise. Many sources of support are thus outside of the traditional competitive funding rounds of national research programmes, usually through direct funding from manufacturing companies, as per the kānuka honey HSL study, many of whom must balance the risk of negative results and benefits of positive outcomes from a commercial perspective.

A robust clinical trials process is highly dependent on a multidisciplinary team encompassing regulatory, ethical, statistical, methodological, pre-clinical and clinical expertise relevant to the area of interest. There exists a clear cultural barrier between 'mainstream' clinicians, most often medically qualified physicians, and practitioners of more traditional therapeutic approaches, exacerbating the polar debate and non-holistic parallel approach to many patients management.[200] Formal assessments have

shown that clinicians are highly aware of CAM therapy use amongst their patients and have an interest in formal training the field, however current inclusion into university and medical school curricula is minimal if at all, although on the rise.[201] Ideally research priorities and subsequent funding allocations should encompass both the acute and long term clinical needs of a population whilst considering patterns of self-management in order to constantly enhance the scientific evidence base and maximise overall health care delivery. It is hoped that formalising a research infrastructure such as the PRN will support this aim.

Despite the widespread and increasing use of CAM therapies, the actual prevalence of clinical research for them is near impossible to quantify, firstly complicated by the lack of a universal definition as to what constitutes a CAM and in part due to such research taking place in a wide range of countries and reported in a multitude of languages. A 2014 review of all historical randomised trials into CAM therapies from Korea alone yielded 360 studies but also found systematic omission from formal internationally recognised meta-analyses, leading to a high probability of bias, particularly considering that the preponderance of CAM therapies originate from non-Western, non-English speaking countries.[202] In this review poor study quality was often highlighted, in terms of non-adherence to the essential components of RCT reporting, detailed in standards such as CONSORT.[203]

Even if this array of barriers was overcome, the issue of research infrastructure capacity and appropriate methodological approaches remain. Traditional models and clinical interfaces are not suited to the effective recruitment of a representative population in which to assess the generalisable efficacy of CAM therapies, as evidenced in our group's pilot study of Honevo for cold sores in general practice.[38] This is fundamental to understanding the evidence for potential use with, not instead of, mainstream clinical treatments, a conundrum ongoing since the dawn of evidence-based medicine. And so,

the concept for a community pharmacy-based approach to the RCT was developed, leveraging the experience and capabilities of an internationally recognised clinical research institution with a track record of generating mainstream, level one evidence and the unique structure, accessibility and collegiality of the New Zealand healthcare system.

## 6.2.2 Developing the Pharmacy Research Network

Development of the PRN began early 2015 in preparation for regulatory approvals and trial initiation. There were a multitude of pre-defined considerations requiring a paradigm shift in traditional trial design, such as approaches to informed consent, recruitment strategy, case report form delivery and outcome reporting. It was also recognised that there would be many unknowns, requiring the project to have broad oversight and hands on supervision from the coordinating centre at the MRINZ facility located within Wellington Regional Hospital, NZ.

### 6.2.2.1 THE PHARMACY LOCALITY

#### 6.2.2.1.1 PHARMACY RECRUITMENT AND REIMBURSEMENT

The first stages of development involved canvassing pharmacies for interest in research and establishing appropriate financial reimbursement for involvement on a per recruitment basis. Interest was high from pharmacies that were successfully contacted, although often difficult to get to that point of engagement due to the sheer volume of marketing and sales requests each facility receives. Interestingly, once the study was established with recruitment momentum and parallel media exposure this issue of engagement eased with pharmacies actively seeking out the study team and applying to join the network of sites.

It was realised that an essential component of successful pharmacist engagement and retention was adequate and early consideration of the underlying community pharmacy business model. Whilst pharmacy investigators were not salaried research staff, their time and expertise required appropriate reimbursement and a small group of pharmacy owners was canvassed by the study Sponsor Honeylab prior to commencement. The general feedback was that $40 NZD per participant recruited was adequate to cover lost profit from a missed sale of HSL treatment and staff time spent conducting the enrolment study visit. In subsequent PRN studies this was realised to be a drastic underestimate when the resourcing requirements for set up, site-initiation, GCP training, monitoring and study close out were fully understood.

### 6.2.2.1.2 THE PHARMACY ENVIRONMENT

Gaining an in-depth knowledge and experience of the community pharmacy environment was essential to ensure research sites met privacy, security and investigational product storage requirements. A clear benefit in terms of seeking approval from Medsafe and SCOTT was the ability for all pharmacies to demonstrate an appropriate environment for the receipt, handling and dispensing of medicines. Interestingly, the presence of an appropriate consultation room in which to conduct confidential study visits was variable with a number of study pharmacies using a private office and some unable to meet the requirements at all. The mapping of suitable pharmacy localities actioned during the kānuka honey study has proven essential to the planning of subsequent PRN trial designs that are incorporating clinical photography capacity potentially involving intimate areas of skin.

### 6.2.2.1.3 PHARMACY LOCATION

Initially the location of study sites was considered to be ideal if situated within a high foot traffic area, such as a mall or central business district and major cities were thus initially targeted for pharmacy recruitment. Generally, the opposite was true, with the highest performing sites located outside of the three largest New Zealand cities of Wellington, Auckland and Christchurch. In addition, the initial pharmacy recruitment strategy involved the targeting of multi-pharmacy groups such as the Countdown supermarket chain, Green Cross (Unichem and Life pharmacy store brands) and other private conglomerates overseeing a number of corporate and franchised stores. Although partially successful, the highest performing stores tended to be privately owned, with the owner heavily involved in the clinical aspects of operations and the HSL trial itself. As reported in the exploratory analysis of recruitment rate by region and season in chapter five, there appear to be a number of influencing variables,

including location by season, that need to be considered when selecting pharmacy sites for specific study designs.

## 6.2.2.2 REGULATORY APPROVALS AND REGISTRATIONS

### 6.2.2.2.1 ETHICS

The administrative burden of clinical trials is high, particularly combined with the traditional paper-based requirements for data collection and study documentation. It was correctly anticipated that in a community-based, multitude of satellite research localities this would be significantly exacerbated. The traditional consent process incorporates an extensive PISCF, as demonstrated by a recent programme exploring a potential biomarker of asthma, periostin, comprising a number of clinical trial unit based, traditional studies. Each of these randomised trials were approved to use a lengthy PIS with word counts of 2254, 2556, 2477, 2728 and 2519 respectively and initial study visits scheduled for around one hour.[204-208] Given the time constraints on both pharmacy staff and potential participants, a consolidated version was developed (Appendix 6) containing 1296 words. This was subsequently approved after extensive deliberation at the HDEC which recognised both the lower risk nature of the study and the unique demands of the research environment.

### 6.2.2.2.2 STANDING COMMITTEE ON THERAPEUTIC TRIALS

As Honevo contains honey, a foodstuff that is routinely consumed, specific approval from SCOTT was required, being considered a new medicine under Part 11, Section 2.4 of the Medsafe guideline on the regulation of therapeutic products in New Zealand.[37] It is now realised that the SCOTT approvals for the HSL and prior Honevo pilot studies set an interesting precedent for low-risk, over-the-counter products. Section 5.3 of the guideline on the regulation of therapeutic products in New Zealand sets an expectation

for all investigational products to be manufactured in accordance with applicable GMP standards, which, in the case of SCOTT approved investigational products are classified as medicines as per the 1981 medicines act.[37] The pharmaceutical GMP certification required for a medicine is expensive, difficult to achieve and rare in over-the-counter product manufacturers, for whom the requirement does not apply from a commercial perspective, often already permitted to sell their product in settings such as community pharmacy. Honevo is produced in a 'nutraceutical' environment under GMP as assessed and certified by the CTFA of New Zealand (Appendix 2) and therefore distinct from a Medsafe approved drug, defined under the code of GMP for manufacture and distribution of therapeutic goods. Strictly, this precludes Honevo and many other highly utilised over-the-counter and alternative medicine products produced under similar certified standards from engaging in the New Zealand clinical trials process, severely impacting the evolution of a much needed, high quality evidence base. Indeed, subsequent applications to SCOTT for investigational products to be tested within the PRN have been scrutinised for lacking pharmaceutical GMP certification. This has required extensive rebuttal and provision of detailed documentation to the committee around the production standard operating procedures. To date all have been approved by SCOTT with special dispensation from Medsafe, but with the condition that no medicinal claims can be made. This highlights significant barriers to promoting the evidenced use of CAM therapies and risk of losing potential research investment to New Zealand from manufacturers who, if wanting to make medicinal claims for products, must seek an isolated small batch manufacture from one of 16 Medsafe approved sites, many of which do not have the production capacity for a specific formulation or charge a prohibitive premium. This is despite all products seeking approval for assessment in a PRN study being produced to a standard in line with an equivalent GMP deemed appropriate for commercialisation and already widely used over-the-counter for various ailments. The PRN infrastructure has highlighted the issue and created the opportunity to engage directly with Medsafe to address it.

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To further complicate matters, Honevo was subsequently argued to be a medical device under current New Zealand legislation based on the acceptance of a non-specific, physicochemical therapeutic mode of action. In turn, this has promoted the exploration of conducting systemic or topical clinical research on similar 'devices' without SCOTT review, potentially risking an undermining of the regulatory process for clinical trials that fundamentally exist to keep participants and future consumers informed and safe. This adds further urgency to the review of regulations around approval of clinical trials for CAM products to allow this research to be conducted in New Zealand within a robust and transparent system that maintains an emphasis on appropriate quality standards relative to the risk of the product under scrutiny. With the evolving demand for evidenced, non-prescription therapeutics growing internationally, New Zealand also risks losing the opportunity to attract associated funding pipelines.

### 6.2.2.2.3    Māori research review

A further requirement for appropriate registration of the kānuka honey HSL trial was obtaining a review of the research proposal from a Māori perspective, a crucial stage in all clinical trial development in NZ. For a typical single centre RCT, the local Wellington Hospital Research Advisory Group Māori (RAG-M) offer a facility for protocol and associated study documentation review, providing recommendations around cultural issues that arise. For the Honevo study in HSL two specific considerations were clear around the head being considered tapu (sacred) with the requirement for the investigational product to be delivered to it and the use of kānuka derived honey, a traditional medicine forming part of Rongoā Māori. At the time of application RAG-M refused review given the volume of intended clinical trial sites in community pharmacies throughout NZ, recommending that locality approval would be required from equivalent bodies within each Iwi jurisdiction involved. This was

logistically unfeasible, compounded by a paucity of such bodies that were actually able to provide review and so the HDEC application proceeded with individual review from a Māori researcher and General Practitioner, Dr Matire Harwood (Ngāpuhi). This was rejected by the Māori ethics committee representative who requested a central organisation as a contact point for Māori support. This was not a capacity that existed within New Zealand at that time and led to significant delay in study approval. Fortunately, a charitable health organisation, Tui Ora agreed to act in this capacity on a case by case basis, however, none of the 952 recruited participants accessed this service, including the 13.5% of participants that self-identified as Māori.

### 6.2.2.2.4    REGULATORY REPORTING

An unforeseen barrier to study administration were the logistics of registering and obtaining authorisation for individual investigators and sites to commence the trial. The online systems used in New Zealand are functional but not optimal for conduct of a clinical study incorporating 76 localities, all of which required specific authorisation by the regulator. Medsafe have confirmed that the number of participating sites was greater than in any previous clinical trial in NZ. In addition, the Principal Investigator (PI) at each study site was required to register online and seek approval within the system prior to being initiated as a recruiting centre. Interestingly, the usual profile of a PI representing a study locality in human clinical trials is a practicing medical Doctor, but key to the success of the PRN model was the ability for a pharmacist to assume this role. Fortunately, Medsafe were satisfied with the presence of multiple Pharmacist PIs, given the entire trials process was overseen by a medical doctor (myself) based at the MRINZ, an approach which has been successfully replicated in the Australian Pharmacy Research Network.

The regulatory reporting requirements proved complex due to the size and number of study sites involved. The biannual and annual reports for SCOTT and HDEC

respectively require levels of detail for per site recruitment encompassing the total number of participants enrolled, loss to follow-ups, number of withdrawals due to adverse events, safety data and all deviations and violation in a tabular form, a process which became challenging for 76 sites recruiting up to 100 participants a month combined.

### 6.2.2.3    REPRESENTATIVE PARTICIPATION

Under-representation of ethnic minority patient groups in clinical research is a long established issue.[209] In this study 13.5% Māori were recruited to the kānuka study of HSL (Table 1) close to the 2018 census complied shortly after study completion which reported 18.5% of New Zealanders identified as being of Māori ethnicity, across all ages. This is an important finding in the consideration of optimal approaches to reducing inequities in research participation with an overarching aim of representative participant groups for all studies conducted within NZ. Reasons for this are unclear but may be due to the increased geographic recruitment scope of the PRN infrastructure in contrast to the highly localised, traditional clinical trials units across the country. Furthermore, the community pharmacy represents a familiar and trusted interface that, for most, is accessed more frequently than general practice or hospital settings and also offers a more convenient, walk in, opportunity for participation in research. The relatively high use of social media also likely influenced representative recruitment though both increased outreach and the unique ability to hold conversations with potentially eligible participants using a 'safe', semi-anonymous line of communication with opportunities to confer directly with family and friends in a non-time pressured setting.

### 6.2.2.4    Resource requirements

The overall cost per participant for the study was a fraction of an equivalent within a traditional study construct. As the study was established in part to assess the efficacy of medicinal honey and in part to test the feasibility of a PRN these study costings are not precisely indicative of the established model, which has since added significant value in reducing human error and increasing data precision through use of custom developed, highly efficient digital capabilities. The inaugural trial has been highly informative for subsequent budgeting templates for the next generation of PRN trials, which are able to be conducted at considerably lower rates per participant than centre-based studies.

#### 6.2.2.4.1    Site training

There was significant time and financial investment in the set-up phase of the study. As the lead MRINZ investigator, the burden in terms of travel was 124 separate domestic flights over the active study period to initiate sites, train/retrain staff and conduct study close out visits. Personal attendance to each of the 76 sites for an initiation visit was critical to the PRN development success in order to ensure the suitability of facilities, deliver study materials, investigational product and most importantly forge a professional relationship with the pharmacy team through the training. The locality PI was able to train and authorise sub-investigators however, the 'train the trainer' model has since been removed for subsequent studies in order to demonstrate a more robust process for regulatory submissions. Commonly, a group of pharmacy staff were trained in staggered groups to allow full staffing of the dispensary and maintain usual business activities. On central investigator attendance, a general review of the pharmacy infrastructure was undertaken to ensure an area was available to provide adequate privacy for consultation and that investigational product storage facilities were sufficient and accessible. Training was delivered to all available pharmacy investigators face to face, recapping the general study design and outcomes, the need to apply GCP during all

study related activities and maintain sufficient documentation on site. A full enrolment role play was then undertaken, allowing time at each stage to discuss the clinical rationale and highlight any potential or indeed previously encountered pitfalls. On completion of the initiation visit, the training and delegation log was signed, investigational product reconciled and logged and approved advertising placed in the HSL product areas. All site master file documentation was paper-based and stored within a dedicated folder that was kept in a secure pharmacy location. The pharmacy was then deemed active and authorised to begin recruitment.

### 6.2.2.4.2 PARTICIPANT MANAGEMENT

Ongoing trial management was solely undertaken by me until a critical point, around 300 recruits, when up to 40 concurrent participants were submitting data to the online system. At this point a research assistant was employed, to assist with the daily monitoring of participant responses, investigational product stock management, maintenance of study documentation within the hybrid digital and paper structure, data entry for returned paper study diaries and reimbursement of participants on study completion via posted cheque. Each participant's progress through the study was tracked within an excel spread sheet, listing the data points required each day and allowing the monitoring of missing data from the Wufoo digital database and immediate follow-up. This was the primary benefit of digital participant reported outcome measures via the smart phone system, promoting highly accurate and chronologically relevant data. The system also ensured that both the overall loss to follow-up rate was minimised and non-continuance for reasons of adverse events or technological failure were established and rectified immediately.

### 6.2.2.4.3 GENERAL STUDY ADMINISTRATION

The hybrid paper/digital approach to the trial was novel to the MRINZ and developed to meet the relatively unique needs of the PRN. Traditional study conduct to that point

in time had been entirely paper-based, in line with the contemporary industry standard and centred around a hard copy folder per participant with a large, complex trial master file located in a secure location within the MRINZ and any external collaborating clinical trials units nationally or around the world. The challenge in maintaining such documentation according to the principles of ICH GCP, across such a large number of external study sites was significant and a number of strategies evolved for the kānuka trial. These included use of pre-collated participant packs provided in batches of 20 to each pharmacy, approaches to prevent unnecessary use of screening documentation through the generic statement read out to all potential participants and conduct of all subsequent study visit points through a remote data collection mechanism.

These small, but cumulative efficiencies were critical to the successful conduct of the PRN setting, however were not without consequences such as precluding the collection of proportionate data related to screen fails and a very large courier focused undertaking to replace updated protocol versions and study packs for the bee allergy exclusion amendment. In this regard study close out required specific consideration, with the need for in person visits to each locality to scan source documentation onto a secure, digital memory stick along with all generic study documentation contained within the master site file. This was left on site to meet the GCP requirement for investigator retention and access to source data over the subsequent required period of 15 years. This was a relatively costly endeavour given the number and spread of pharmacies throughout NZ however further supported a move to the entirely digital PRN platform, allowing an entirely paperless and mostly remote approach to all of these aspects.

### 6.2.2.5 MAINSTREAM MEDIA

The media was well utilised as a tool to promote recruitment of participants and pharmacies and introduce the concept for clinical trials methodology (Figure 29).[210-215]
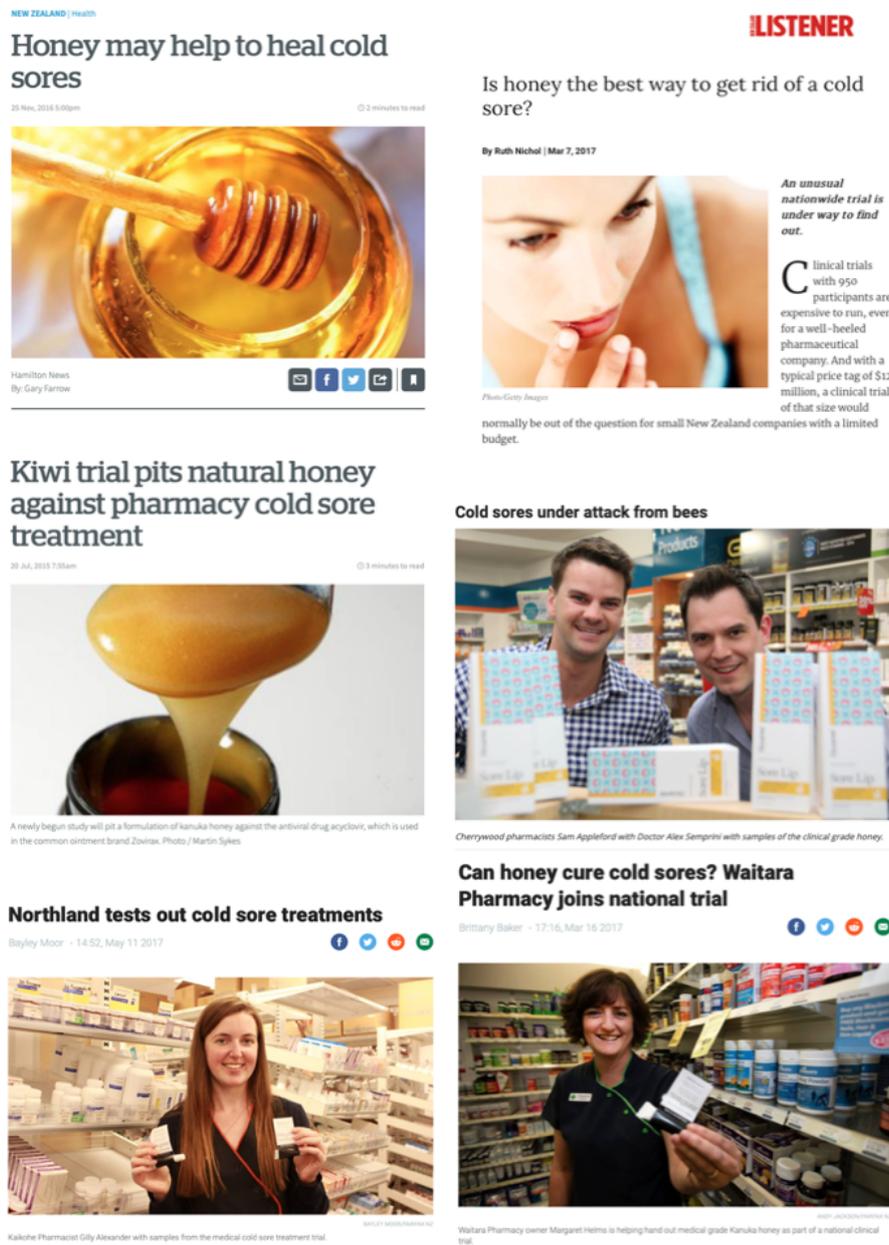


Figure 29: A selection of media coverage during the study.

Dr Shaun Holt, founder of Honeylab gave a 2016 TEDx talk in Wellington that gave further coverage to the ongoing study.[216] In order to enhance the validity of the PRN within the community pharmacy sector and attract new sites, a series of articles was run in the New Zealand Pharmacy Today magazine.

A similar approach was adopted to disseminate the results through the media, important for coverage of the PRN and to promote the capacity for high quality evidence to be collected for commonly used over-the-counter products.[217-222] Some international reporting also resulted with a British newspaper, the Daily Mail, and the United Kingdom National Health Service 'Choices' service publishing on the study results.[223,224]

## 6.2.2.6 SOCIAL MEDIA

Use of social media, in particular Facebook, was critical to the successful recruitment of the kānuka honey study. A relatively unused strategy for the MRINZ, there was a significant input into wider Institutional promotion to provide a base validity for study advertising using the platform. A major drive to increase the number of MRINZ page 'likes' was undertaken in parallel with regionally based adverts that listed the local study pharmacies able to recruit into the kānuka honey trial. At peak recruitment this strategy generated many hundreds of leads per week from direct messages, referrals, shares and post comments, allowing potentially eligible participants to be informed and ready to present to their local study pharmacy for a subsequent HSL episode. This also provided valuable insights into potential future regions for new pharmacy localities. On study commencement in 2015 the MRINZ Facebook page was followed by around 60 individuals, many of which represented employees and close contacts, in 2017 this had increased to 5,200 and currently in 2020 sits at over 7,800. The process that evolved for the PRN HSL study formed the basis of many other MRINZ studies and is now a highly effective standard recruitment mechanism and dissemination of results

and publications, staffed by a dedicated resource including an in-house medical illustrator to further enhance visual content (Figure 30).



Figure 30: A medical illustration used to disseminate the kānuka HSL study results. Reproduced with permission, credit to Cilein Kearns - Artibiotics

## 6.3 Towards an established model for community pharmacy research

As the Honevo trial progressed the potential was realised for a formal, embedded trials network in community pharmacy that could stack multiple randomised trials and act as a data collection conduit for qualitative research, device piloting and quality and improvement. The initial randomised trial of kānuka honey with a sample size of 952 and 76 study sites proved an excellent opportunity to both develop and demonstrate

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the robust capability of the PRN, from protocol to publication and highlight post-study marketing opportunities for potential study sponsors.

The data from the first PRN RCT presented in this thesis indicated that the most productive pharmacies appear to be based outside of the major urban centres, in more rural areas with a strong sense of local community engagement. For the next three randomised PRN trials in eczema, HSL and acne, all but one of the recruiting pharmacy localities are outside of the three most populous cities in NZ. Further assessment of similar regional performance patterns within these next studies will be insightful to gauge if there is varied prevalence of specific diseases and consequent implications for targeted recruitment. This is important, as for a condition such as HSL, prevalence in a large city such as Auckland (population 1.6 million) is likely to be at least equal if not considerably higher than a more rural setting such as Waitara (population 7000) and would present an obvious choice in focusing recruitment. However a Waitara pharmacy was the top rural performer, second highest recruiter overall and recorded the highest recruitment rate between first participant and study closure at 0.18 participants per day (Figure 28). This highlights the importance of community engagement, with specific feedback around the apparent lack of interest and time paucity of potentially eligible HSL sufferers declining the study in Auckland in particular. Furthermore the indicative variance in recruitment by season and location, described in chapter five, may prove informative in targeting specific pharmacies when required study sample sizes are small and expected to complete over one or two seasonal periods.

As the PRN transitioned into the next generation of studies, the HPN assisted greatly in defining a formal operating procedure for the network and novel areas of development to support educational and professional development in the sector. For each future study, a HPN PI is listed as a senior study team member in order to assist in developing the optimal approach to study design, ensure appropriate pharmacy reim-

bursement and to act as a named co-author for the publication output. Also supporting this steering committee approach, is the ongoing support and engagement of the Pharmacy Guild of New Zealand who provide invaluable introductions to innovative and engaged pharmacy business owners to further expand PRN membership. Ring fencing opportunities for non HPN pharmacies to join the PRN ensures an element of competition for study selection, casts a net for undiscovered research talent and most importantly increases access to research for the wider community.

### 6.3.1 EMBEDDING A RESEARCH PHILOSOPHY WITHIN COMMUNITY PHARMACY

The sample size for the Honevo study mandated a large number of pharmacies were initiated as study sites across New Zealand and subsequent insights into the various traits that appeared to influence research success have been critical to establishing the model. As an industry subject to the pressures of business management and profitability, certain criteria needed to be met in order to create a viable process for trial recruitment and develop an overarching priority to continue to engage in research. Central to this were considerations of data collection logistics, efficiencies in use of digital systems, time management, finance and investment in longer term skill sets to support a PRN pipeline.

#### 6.3.1.1 TRAINING, EFFICIENCY AND THE PAPER PARADIGM

Adopting a successful community-based approach to the randomised trial must begin with a pragmatic assessment of logistics and efficiency. Previous trial constructs in busy front line clinical settings such as general practice have failed due, in part, to a lack of consideration of basic strategies to counter the paucity of time available for opportunistic consent, governance considerations and investigator attrition.[225] Listening to both the frustrations, commendations and recommendations of the community study team was critical in addressing these and ensuring the establishment of a longer term PRN

strategy. During the kānuka honey study the process for research site set up and regis-tration was based on the traditional research initiation paradigm used by dedicated clinical trial units. This generated a wealth of feedback which has formed the basis of the next PRN methodological iteration.

Pre-trial initiation requirements involve the collation of an appropriate CV, current annual practicing certificate (APC) for site PIs, contract review, signing and counter-signing by the central institution and Sponsor representative, protocol acceptance and demonstration of GCP training. Perhaps the most widely problematic for the PRN was the ambiguous process for each PI in signing digital authorisations for HDEC and SCOTT locality approval; more than one pharmacy investigator reported this as a deal breaker for participation, requiring this to be undertaken face to face at the start of the site visit. This highlighted the fundamental importance of logistical efficiency and re-tention at the investigator level, not just that of the participant, and that an accumula-tion of inconveniences presents significant risk to the conduct of non-traditional em-bedded clinical research. Most pharmacy PIs responded positively to an acknowledge-ment of limitations and iterative approach to overcoming them. Investigator sign on, for example, was immediately consolidated into a pictorial, stepped guide and specific feedback provided to the Ministry of Health responsible for the online system.

For the kānuka honey study presented, the vast majority of the administrative, non-participant facing process, bar the electronic signature authorisations from HDEC and SCOTT, was entirely paper-based, requiring couriering or in person exchanges of doc-uments followed by complex filing systems and duplication between the Master and individual site study folders. A formidable knock on effect of this inefficient standard was made apparent when the DSMB required the addition of a specific exclusion cri-teria for those with bee allergies. This substantive amendment was quickly approved by the HDEC committee, allowing the newly versioned protocol to be implemented

and actioned for the study, however, all 50 or so of the pharmacies recruiting across New Zealand at that time required delivery of a hard copy new protocol, new participant packs containing the updated PISCF and the locality PI to sign and return an updated protocol acceptance form. This was progressively achieved at a significant administrative resource, and led to multiple protocol deviations with the inevitable, erroneous use of previous study document versions for recruitment during the transition phase. The contemporary, fully paperless PRN has been able to action similar amendments within 24 hours using a centralised update to the online study document portal and provision of digital surveys for signed acceptance.

The study required a large, heavy and complicated physical master study folder to be stored at each pharmacy site which contained all relevant superseded and current study paraphernalia such as advertising, contracts, protocols, regulatory approval documentation and any other associated material. In addition, pre-collated recruitment packs required storage in an accessible location with the opaque sealed envelopes containing the randomised allocations for each participant enrolled. A relatively common deviation encountered was the disordered use of randomisation codes with sites picking a random number or mistakenly starting at the last available identifier code. A move to electronic randomisation has removed this source of human error and further minimised problems of access.

The collection of participant reported data relied heavily on parallel use of the Wufoo survey platform and burst SMS/sequencer with a central investigator reviewing and reconciling each data entry point to ensure accuracy and timeliness with the ability to stop unnecessary diary deliveries once the primary outcome was met. Whilst not optimal in terms of a singular integrated approach, the impact of the digital methods trialled in the kānuka study was apparent, with all of the human error deviations related to completion of the paper forms during visit one and encompassing missed signa-

tures, erroneous dates, unchecked boxes for recruitment criteria and others. The majority of these were benign and consequently easily remedied, however errors of inclusion through missed assessments and delay in central review of documentation requiring manual faxing can, and did, lead to ineligible recruitment. This introduced a risk to study data integrity and an increase in administrative burden through the additional requirement to appropriately document and report the event. It was also learnt that green ink, the colour of choice for pharmacy, is not transcribed during faxing!

Collectively the issues encountered in the kānuka honey PRN trial highlighted the general deficiencies in paper-based study management, particularly in a remote research infrastructure reliant on a clinical workforce for whom research would form only a minor proportion of daily activity. A priority for PRN evolution has thus been to develop a fully paperless approach, facilitating a more defined and guided process ensuring all aspects of study data collection and administration are sufficiently addressed to guarantee quality in study conduct, effectively eliminate many protocol deviations and violations due to human or systems error and minimise the risk of investigator attrition due to complexity in system use and study participation. This aligns with a general but slow migration to the digital interface by the research sector, that increasingly recognises the benefits in terms of quality, participant diversity and expediency.[226]

Consequently, a second-generation PRN has been established, utilising a dedicated custom-built research portal delivered through digital, internet connected tablets provided to each study pharmacy. The associated benefits of this novel, customised system mitigate all the deficiencies in process discussed previously and create a highly robust and auditable methodology for the community-based conduct of randomised trials. Each research pharmacy is assigned a perpetual, specific identifier used across all trials they participate in and investigators registered to their specific site with relevant documentation either created directly within or uploaded to the system. Associated materi-

als previously required to be stored at each site within the physical master file are now centrally managed and delivered digitally to the investigators via the study portal, removing any need for manual replacement of updated versions. Signatures for training logs, documents and study eCRFs are all digitally actioned. All paper-based, site related case report forms have been transcribed to a digital format, allowing automated source data verification in each individual field (for example, mandating numbers in a mobile phone field or preventing recording of a follow-up visit date that is before the first) and all study diaries are emailed to participants automatically on completion of the digital enrolment forms. To facilitate senior oversight by the PI, a capacity to feed live study data into an automated 'dash' enables prompt review of adverse events, concomitant medications, up to date reporting to sponsors and promotes the timeliness of regulatory reporting. Currently this novel data capture system is being adapted to meet international regulatory submission standards such as the Clinical Data Interchange Standards Consortium (CDISC), a terminology standard that requires data to be transformed into a format suitable for formal applications to agencies such as at the FDA and EMA, an end research outcome intended by many CAM therapy manufacturers.

Additional features in development include a dedicated PRN app from which all reminders, notifications and data collection forms/diaries are delivered within a controlled centralised research portal directly to participants. Clinical photography has been developed, embedded within the portal capacity allowing PRN investigators to take and provide images for remote, blinded assessment by dermatologists. Ensuring transparency in the investigational product supply chain and matching the participant ID with the randomised treatment is crucial yet a common source of error using manual methods reliant on visual corroboration by an investigator. Matrix bar codes are pattern images that encode limited amounts of information that can be read by a scanning device such as dedicated handheld equipment linked to a computer or via

the camera function of a tablet device. Leveraging the utility of the PRN portal tablet in reading such a 'QR' code has potential to streamline the matching of randomised study stock to data collected in study visits. In addition, a QR code can ensure use of the correct participant ID for follow-up visits linking to a participant study card provided on enrolment.

## 6.3.1.2    FINANCIAL ASPECTS

Core to the founding of the PRN was the financial barrier to conducting quality research on the efficacy of over-the-counter products, widely sold for therapeutic purposes. Ensuring a sustainable model that met the operating costs of the coordinating centre and pharmacy research sites whilst enabling a viable programme for potential sponsors was therefore a high priority.

The core operating costs for the Honevo trial were not reflective of future budgets, being an intentional loss leader to build and test infrastructure and processes. However, key personnel and budgetary line items became apparent that allowed for more accurate costings of future protocol designs. From a coordinating centre perspective, the overheads and staffing required to run a 76 site, near 1000 participant randomised trial were minimal compared to a fully resourced clinical trials unit-based approach requiring multiple physicians, research coordinators and assistants to conduct study visits onsite. Whilst the 'back room' roles of monitoring, data management, statistics, accounting and senior programme oversight remained unchanged in delivering the PRN approach they were also subject to efficiencies derived from the use of direct digitally entered source data from the electronic diaries. This allowed the monitoring of data points in live time compared to awaiting the traditional process of double data entry transcription from paper case report forms.  The statistical analysis was able to be prepared in a matter of hours from the direct export of formatted data points from the Wufoo system, requiring only minimal data cleaning. The most significant impact,

however, was the reduction in core study staff required to initiate and manage the study of 952 requiring only a medical monitor who was also the coordinating investigator (myself) and a research assistant mid-way through.

For the peripheral pharmacy sites however, it was apparent that the reimbursement structure needed substantive review to recognise the significant, unforeseen resource required to register investigators, organise and attend in person training and host the monitoring and close out visits. One of the most successful approaches to site initiation and investigator training involved a formal presentation to all staff prior to pharmacy opening hours, followed by staggered small group training throughout the day. This approach was optimal for staff engagement however required them to attend an overview presentation outside of their contracted hours, followed by specific rostering of face to face training in order to maintain retail and dispensary cover. The initial PRN reimbursement survey did not consider this significant time commitment, which many pharmacies paid staff additional time for. It was therefore determined that an exclusive per participant fee was not an adequate approach to study site reimbursement and a defined recompense for site set up, initiation and training was required for future studies. Reflecting this, all PRN studies now begin with a specific discussion with a nominated PRN locality PI to establish a benchmark value for all other study input required from a pharmacy perspective. Promoting an inclusive, ongoing assessment of the financial structure will be essential to maintaining the pharmacy investment in the PRN.

The need to ensure appropriate recompense is proving particularly true in light of the recent New Zealand market entry of discount branded chains from the Australian market, able to undercut the costs of medicines and retail products such as toiletries. This sector disruption is forcing an unprecedented evolution of the traditional community pharmacy model in New Zealand and the proactive seeking of additional health related services able to be offered. It is encouraging that the work of the PRN is considered one

such avenue of service delivery, with pharmacies in affected areas such as Auckland increasingly approaching the MRINZ for study participation. The creation of a viable source of research income and surety in pipeline for pharmacy sites in turn helps ensure engagement and quality of output. This re-energised focus on community pharmacy service delivery has also formed the foundation for discussions with the Pharmacy Guild with a view to actively supporting various diagnostic screening programmes, strategies to collect qualitative data and the development of quality and improvement projects. There is an exciting opportunity for the non-commercial application of the PRN methodology to contribute directly to population health.

To support and coordinate the development of a research trained community pharmacist role and ensure longevity in capacity and implementation of evidence-based outcomes a 'PRN Axis' concept has been developed. The Axis will place geographically targeted research pharmacies across New Zealand with each providing a PRN PI that has undertaken a deeper clinical research training programme and receives additional full time equivalent research funding. These anchor points of the network will undertake regional responsibility for non Axis site coordination, sit on the PRN steering committee, aid in the development and recruitment for all studies and have an active role in the dissemination of study results.

## 6.3.2 AN INTERNATIONAL MULTI-CENTRE PROGRAMME

The publication of the kānuka honey HSL study methods in the British Medical Journal Open in 2017 led to communications from researchers at a CAM focused institute in Sydney, Australia who were facing similar logistical challenges to conducting a similar HSL study of an over-the-counter topical therapy.[227,228] The team requested more insights into the feasibility of setting up a PRN research model and the progress in terms of recruitment at that time for the Honevo study. The labour intensive experience of establishing the network and its maintenance so clearly reliant on forging relationships

with community pharmacy and associated stakeholders was protection enough of the 'intellectual property' represented by the PRN process, furthermore it is highly unlikely that a more traditional university-based organisation would justify the loss leader required to pilot the concept for the purposes of a solitary randomised trial. In the interests of the core mission of the PRN to enhance the evidence base for over-the-counter and CAM products, it was essential to collaborate with like-minded researchers and organisations in order to develop an international capacity with associated pipelines. The immediate expansion of the PRN was thus explored and soon formalised its Sydney-based, Australian counterpart in 2018 under a collaborative study of a second HSL CAM product.



Figure 31: The Australian and New Zealand PRN logo

Since the publication of the kānuka honey study for HSL, exploratory discussions have been undertaken with major Clinical Research Organisations around the Australia and New Zealand PRN capacity to support pharmaceutical RCTs and generalisable data generation for traditional medicines undergoing a mainstream drug development process.

Recently, overseas industry sponsors have registered interest in the development of a United States network.

### 6.3.3 BUILDING A PIPELINE

As the PRN established through the kānuka honey study and associated media promotion, industry interest into the novel capacity gained momentum. Regular requests for discussion are received around potential programmes for both small start-up companies through to established larger brands looking for a marketing edge via demonstrable clinical efficacy. Assumptions held by the smaller manufacturers with no or limited research and development experience around the cost of conducting clinical research were surprising with budgets of $20,000 NZD commonly allocated for a definitive study. There was a poor knowledge of specific, supportive funding mechanisms in New Zealand such as Callaghan Innovation project grants.[229] Indirect exposure to these commercial research and development opportunities by the PRN has built a wealth of knowledge that enhances initial consultation around clinical research possibilities for companies, further building the reputation and track record of the network as a capability of the MRINZ.

In 2017 the PRN concept was taken to Bio Europe, one of two major biotechnology research expositions held annually with 4,000 attendees and 24,000 partnering meetings. This was a scoping opportunity to understand any equivalent international capacity and to gauge interest around bringing research programmes to the New Zealand PRN. Delivering broad paraphernalia around the network (Appendix 13) and a number of partnering meetings both the novelty of the methodology, its affordability and ability to recruit from traditionally hard to reach groups was attractive to industry. However, it was also apparent from discussions with higher tier pharmaceutical companies that a multinational approach to PRN data, ideally using replica studies in either the United States or Europe, would likely be required to support out licensing negotiations.

In turn this has led to a gap analysis of the overall MRINZ capacity to meet the regulatory standards required for direct submission to agencies such as the FDA and EMA with a view to implementing a digitally based system specific to the PRN that conforms to these. This programme is now well advanced with clear benefits to all studies being supported by standard operating procedure documentation and specific adoption of the European Clinical Research Infrastructure Network (ECRIN) and CDISC standards that help demonstrate this level of compliance.[230] Subsequent attendance at Bio meetings in the United States as part of the New Zealand pavilion has resulted in wider networking nationally, with the MRINZ formally being invited to join Biotech New Zealand and the Wellington Health Tech Network based, in part, on achievements from the CAM and PRN programmes of research.

### 6.3.4    CONTINUING MEDICAL EDUCATION

From the outset the wider potential for a formal community pharmacy member-based network were considered and investigators canvased as to their priorities in being part of this initiative. A common theme involved meeting requirements for continuing professional development (CPD) via the New Zealand ENHANCE programme, administered via the Pharmaceutical Society of New Zealand. For the kānuka honey HSL trial this learning was conducted independently by the participating pharmacists due to resource constraints, although the site initiation training delivered by a physician encompassed detailed teaching around HSL from a mechanistic and clinical perspective. The potential to formally map a PRN study to online educational modules and journal papers that would meet defined CPD points was often discussed and has been successfully implemented for the subsequent HSL PRN study with positive feedback.

# 7 CHAPTER SEVEN - CONCLUSIONS

Herpes simplex labialis is a highly prevalent, recurrent and incurable condition which, for a third of those infected inflicts a lifetime of episodic physical and psychological discomfort. The severity of clinical manifestation between individual sufferers varies enormously and has resulted in a multitude of acute therapeutic strategies from licensed pharmaceuticals through to entirely un-evidenced CAM approaches. Review of the current literature for such strategies has highlighted a diverse range of reported efficacy in the topical management of HSL with limited data directly generalisable to the patient using it, at their own expense, in the community. The high prevalence of CAM use globally without a supportive body of high-quality evidence highlights the need for robust and accessible mechanisms to conduct research for these products. However, traditional models of randomised clinical trials are prohibitively expensive and often incapable of establishing evidence with high external validity.

## 7.1 IS KĀNUKA HONEY MORE EFFECTIVE THAN ACICLOVIR IN THE TOPICAL TREATMENT OF HSL?

This thesis centred on an RCT of Honevo, a topical CAM comprising 90% medicinal grade kānuka honey and 10% glycerin, for the treatment of acute HSL compared to an active comparator 5% aciclovir in 952 participants. The study showed no superior effectiveness, for either topical treatment, for any outcome variables including time to healing, pain resolution and proportion of aborted episodes between treatment groups. Both treatments were considered highly acceptable by participants.

For the primary outcome variable of time to complete healing (stage seven) there was no difference between the randomised treatments, with a HR of 1.06. The upper 95% confidence limit of 1.22 was within the predefined bound of superiority of 1.25.

Kānuka honey did not reduce the healing time in HSL lesions from stage 4 (ulceration) compared to aciclovir. This was unexpected, given the known positive effects of honey on wound healing processes such as angiogenesis, granulation, epithelialisation, reduction in oedema and debridement.[19] It was also hypothesised that aciclovir may provide greater early benefit through inhibiting the viral replication process to abort the natural history of the HSL episode. This was not supported by our finding that the proportions of lesions between groups that did not progress to the ulcerative stage and beyond (stage four) were similar. Interaction analysis of lesion stage at study baseline provided no evidence that the effect of randomised treatments were dependent on this clinical characteristic. There was no difference in pain severity between treatments.

There are a number of statistical considerations. The outcome variables were of a non-normal distribution and thus underwent non-parametric analysis with medians reported. For time to event outcome variables, data from ongoing episodes were censored and incorporated into the survival analysis. The definition of time to healing used was time to return to normal skin (stage seven) to allow a more complete data set, where as previous studies have defined this as time to loss of crust, allowing for residual erythema (stage six). For reasons of consistency and context, a post-hoc analysis for time from randomisation to stage six was performed demonstrating a reduction in healing time to this stage similar to the evidence base, with a median five days for both aciclovir and honey. There was no ability to mask the treatment allocations, potentially resulting in a degree of response bias.

The kānuka honey study presents a number of additional strengths in adding to the current evidence base for both topical use of aciclovir and honey for HSL. This is one of the largest single studies of HSL undertaken and the only adequately powered real-world trial providing sufficient power to conclude that there is no difference between aciclovir and honey in healing time. This large sample size together with the novel

study methodology allowed recruitment of a representative New Zealand cohort from 76 pharmacies both within and outside of traditional, usually urban, research centres, mitigating the jurisdiction effect of traditional research models and capturing the heterogeneity for HSL manifestation and progression in the community.[178] Using smart phone-based diaries containing photographic standards of lesion progression, it was possible to both facilitate study participation within usual daily routine versus costly and burdensome clinic visits and maximise chronologically accurate data capture. Overall this allowed the definitive assessment of kānuka honey and aciclovir in a highly diverse real world setting, representing the only randomised, real-world assessment of HSL management that can be directly extrapolated to the community pharmacy setting through which HSL treatments are predominantly accessed.

These findings suggest that medical grade kānuka honey may be employed as a therapeutic alternative to aciclovir cream, for the treatment of HSL. This alternative therapeutic approach is important not only for those who prefer CAM, but also in view of the emergent issues of drug resistance and the needs of particular patient groups unable to use current pharmacological therapies due to allergy and lack of safety data in pregnancy and breastfeeding.

The priority now is to investigate whether an aciclovir/honey combination cream in HSL might have greater efficacy than individual components, and whether medical grade honey might have similar efficacy and acceptability in paediatric HSL, a group from which we received much guardian led interest during the study period. Investigation of the efficacy of medical grade honey in other herpetic indications such as herpes zoster is also warranted.

## 7.2 Is the Pharmacy Research Network a viable infrastructure to conduct a randomised interventional trial?

The feasibility in conducting a large scale, comparative RCT utilising a cost effective, regulatory compliant and time efficient community-based infrastructure, whilst maintaining robust outcome data was demonstrated through the PRN. This provides a significant, previously unmet capacity to enhance the evidence base for non-prescribed medications such as CAM by overcoming the limitations of cost-prohibitive traditional models.

Previous research incorporating community pharmacists has been focused around qualitative surveying or recruitment into a wider traditional clinical research unit-based study. The kānuka study required pharmacy investigators to meet the regulatory standard for both a study investigator and locality setting prior to undertaking all aspects of a complex interventional trial. This directly resulted in the establishment of a globally unique capacity in the PRN, to conduct randomised, interventional studies for therapeutic products otherwise unable to access traditional models of research due to barriers of cost and difficult to recruit patient populations.

Subsequent momentum in PRN study throughput has allowed evolution of the system to incorporate entirely digital approaches, further enhancing efficiency and allowing the use of specialist data standards traditionally such as a CDISC, usually reserved for internal pharmaceutical data management teams, global clinical research organisations or specialist ad hoc data transformation providers. Since completion of its inaugural RCT the PRN has initiated three further fully funded studies, established an Australian arm in collaboration with a specialist institute in CAM therapies and has formalised an embedded education programme for research pharmacists with the support

of the Pharmacy Guild of NZ. Furthermore, the study Sponsor Honeylab has publicly reported the out-licensing of the kānuka honey product to a global pharmaceutical company based on the supportive clinical evidence provide by the RCT, for the treatment of HSL in the United States, Canada and Israel.[231]

The PRN methodology conceived, developed and tested within this thesis contributes to a much-needed paradigm shift in the approach to and capacity for level I evidence generation for CAMs and over-the-counter therapeutic products. Uniquely embedded within the community setting, the PRN allows representative recruitment of hard to reach patient populations to provide robust, generalisable data with high external validity. Through the continued development of track record through subsequent trials and active promotion of the network as a reliable, affordable and quality resource to conduct clinical research, there is exciting opportunity to establish this methodology as a viable resource for all tiers of industry research and development.

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# 9 APPENDICES

## 9.1 APPENDIX 1: STUDY PROTOCOL



# STUDY PROTOCOL KH10

### UTN: **U1111-1170-1537**

### ANZCTR no: **ACTRN12615000648527**

### **5% Aciclovir or Honevo™ as a treatment for cold sores**

### **12 June 2017**

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## Background

Herpes Simplex Labialis (HSL) is endemic worldwide causing characteristic blisters on the lips: 'cold sores'.[1] Recurrent episodes cause considerable pain and discomfort and risk auto-inoculation to different sites of the body and transmission to other people. Topical antiviral treatments such as aciclovir can reduce illness duration,[3] although the therapeutic benefit is modest, reducing the time to healing by about half a day.[4] Honey is a potential topical treatment for HSL as it has antimicrobial, anti-inflammatory, and immunomodulatory effects.[5,6] A small randomised controlled trial (RCT) from Dubai in 16 adult patients with recurrent attacks of herpetic lesions found topical application of honey was more effective than 5% aciclovir cream for HSL and genital herpes.[7] A recent feasibility study from our group identified that topical pharmaceutical grade kānuka honey for the treatment of HSL was highly acceptable to patients for the treatment of HSL. However, our experience suggests that future RCTs of honey versus aciclovir require a parallel group rather than crossover design, alternative sites of recruitment such as pharmacies rather than medical centres, less restrictive inclusion criteria, and both closer ongoing supervision of treatment and documentation of outcomes than that utilised in our study.

## Objective

To investigate the effectiveness of 5% aciclovir versus a Honevo™ in the topical treatment of cold sores.

## Design

Open label, randomised, 2-way active comparator, parallel group trial.

## Subjects

950 participants, aged 16 years or over, presenting to a pharmacy for treatment of a cold sore.

**Inclusion criteria**

- Aged 16 years or over at the time of enrolment
- Presentation to a pharmacy for treatment of a cold sore
- First cold sore symptoms (including prodromal symptoms e.g. tingling/ pain) within 72 hours

**Exclusion criteria**

- Pregnant or breastfeeding
- Known or suspected allergy to honey, bees, aciclovir (Viraban) and/or glycerin
- Any other condition which, at the investigators' discretion, it is believed may present a safety risk or impact the feasibility of the study or the study results.
- Patient has used oral aciclovir or other antiviral medicine, or any topical treatment, medical or complementary, on the current sore
- Patients planning to take/use any concomitant medications, which in the opinion of the investigator, could affect the cold sore during the course of the trial. This includes any topical product, medical or complementary, on the cold sore, oral aciclovir or other antiviral medicine, oral complementary medicines for cold sores, eg. lysine supplements, any other medication

**Study Procedures**

**Visit One**

**Recruitment and consent**

Potentially eligible patients will be identified at the time of presentation to their pharmacy for treatment of a cold sore. Participants who potentially meet inclusion and exclusion criteria will be offered the opportunity of taking part in the study.

The study will be explained to the subject and written information, approved by the relevant Ethics Committee, will be provided. If the participant chooses to take part in the study, he/she will sign the consent form prior to any study procedures, having had an opportunity to ask the pharmacist any questions.

Participants may withdraw consent at any time, without having to provide a reason. If a participant wishes to withdraw from treatment they may do so at any time. If a participant wishes to withdraw from the Day 15 follow-up phone call (Visit Two) they may contact the study Investigator to end their participation.

**Randomisation and treatment**

Participants will be randomised 1:1 to topical treatment with either:

- 5% aciclovir (Viraban) or
- Honevo™

The randomization list will consist of letter groups representing recruitment sites, with each letter preceding sequential three digit numbers (A001, A002 etc), which will correspond to one of two treatment groups, of which there will be an equal number. Allocation will be concealed by sealed envelopes to be opened in sequence at the time of randomization. Randomisation will be blocked by numbers of 4. Each study participant will be allocated a three digit participant code (randomisation code) preceded by the recruitment site's letter. The digit code will be used on the CRF and the patient diary for the duration of the study and will be obtained from the randomisation envelope at the time the participant is randomised to receive study treatment. Subject screening and randomisation information will be collected by study sites. Data will be collected on the number of potential participants who are screened but do not meet the entry criteria, or who meet entry criteria but decline to take part after discussion.

**Visit One Data Collection**

The participants and pharmacist will complete a worksheet on Visit 1 to collect information including demographic data, history of cold sores and duration of current episode. This worksheet will also ask for the exact time of onset of symptoms (to the nearest hour) and the current state of the cold sore ie. prodrome/ tingle; erythema; blister; ulcer; crusting; healing, healed (see appendix 2).

**Duration of Intervention**

Participants will be instructed to apply their respective treatment creams five times per day for 14 days, or until the lesion completely resolves, whichever occurs first. Participants should not use any other topical or systemic treatment for cold sores as part of the trial (See Concomitant Medication).


**Intervention Period**

During the treatment period, participants will complete a diary recording compliance, pain symptoms and cold sore stage each day (see appendix 3). The diary should be completed at the same time of day if possible, near the end of the day, with the final application of treatment. Participants with internet access will be asked to enter the information each day online via an e-diary. People without internet access will complete a paper diary only. All participants will receive a paper diary and for those using the e-diary, the paper diary will constitute a back-up, should the system fail or they do not have access to the internet at the time they wish to complete the diary. Return envelopes for the diaries will also be provided.

Participants with a mobile phone will receive a daily text message to remind them to complete the study diary, with a link to the e-diary for those able to complete it.

Participants will be given the phone number of the Pharmacist/ Investigator and a study Doctor/ Investigator at the Co-ordinating Centre in case they have any questions about the study, or diary card entry.

Diary data will be combined as necessary by the Co-ordinating Centre and according to prescribed data management guidelines.

**Visit Two**

Participants will be contacted by phone by a study nurse from the Follow-Up Centre or MRINZ on the first working day following Day 14 (the final day of diary recording) and asked that their paper diary be returned to MRINZ by post (a paid, self-addressed envelope will be supplied). If a participant does not answer a second call will be made the following working day and then a third attempt at day 22 (or the following working day if a weekend or holiday). Further attempts to contact a participant may be made after this by MRINZ staff via text, email or telephone call within three weeks of day 14. Follow-up data may be collected by any of these media.

Participants will also be asked about Adverse Events (which will be recorded and reported as per the 'Safety Monitoring' section), concomitant medication use and narrative feedback will also be recorded in relation to the study treatment (see appendix 4). If a participant has withdrawn from their study treatment they will be asked for a reason for their withdrawal, though they do not have to provide one.

Once the study is completed and the subject has completed the follow-up call, they will not be required to return their allocated study treatment. Study treatment accountability will be performed at a dispensing level and by subject reported application as per the study diary.

**Study Event Schedule**

| Visit No. | Visit 1 PHARMACY | Treatment Period (until resolution of cold sore) | Visit 2 PHONE CALL |
|---|---|---|---|
| Day No. | 0 | 1-14 | 15 (+ further attempts as de- |

|  |  |  | tailed above) |
|---|---|---|---|
| Informed consent | X |  |  |
| Inclusion/exclusion criteria check | X |  |  |
| Randomisation | X |  |  |
| Dispense study medication | X |  |  |
| Dispense patient diary | X |  |  |
| Demographics / question-naire | X |  |  |
| Commence patient diary (electronic and/ or paper) |  | X |  |
| Text message diary reminder (participants with mobile phone only) |  | X |  |
| Reminder to post diary |  |  | X |
| AE assessment |  |  | X |
| Narrative feedback |  |  | X |

**Blinding**

Due to the physical characteristics of honey it is not possible to blind subjects to the treatment they are receiving. The randomised treatment will be revealed by opening a sealed opaque envelope. Investigators will not have access to the randomisation schedule during the study. The statistical analysis will be undertaken blind to the treatment allocation.

**Outcome Measures**

Primary:

- Healing time from randomization to the return to normal skin.

Secondary:

- Total healing time, defined as the time from development of first sign or symptom to the return to normal skin.
- Total healing time stratified by stage of the lesion at onset of treatment.
- Highest pain severity.
- Time to pain resolution (defined as the time from first experiencing pain to total resolution of pain)
- Time to stage 4 from randomisation
- Time to stage 7 from stage 4
- Acceptability of treatments

**Sites & Recruitment**

Up to 100 community pharmacies in New Zealand will take part in the study. Sites will be initially allocated a 20 participants after which further randomisation codes will be provided. It is anticipated that the study will take up to 36 months to complete after regulatory approvals are obtained. Sites will be visited by an investigator from the Co-ordinating Centre before the start of the study for a start-up meeting and to provide GCP training and a study file. This will include logs of enrolment and also the potential participants who are screened but do not meet the entry criteria, or who meet entry criteria but decline to take part and all other essential documents. Ongoing monitoring by the Co-ordinating Centre will take place throughout the study, both remotely and on-site, according to the study monitoring plan.

**Concomitant Medications**

The following concomitant medications are not allowed during the study, and if taken or required, the participant must stop the study treatment:

- any topical treatment, medical or complementary, on the cold sore within the last 2 weeks (prior to enrolment)

- oral aciclovir or other antiviral medicine within the last 2 weeks (prior to enrolment)

- oral complementary medicines for cold sores, e.g. lysine supplements. If participant is taking these they must not have been taken for the current cold sore, but there is no washout period.

- any other medication which in the opinion of the investigator, could affect the cold sore

- participants will be asked where possible not to apply any makeup, foundation or other masking product to cover the cold sore. If they so use it, it should be removed before the cold sore stage is assessed for the daily diary entry

Participants who cease treatment due to the above will remain in the study for follow-up purposes and will complete Visit Two as per their follow-up schedule.

Data will be analysed for all participants randomised into the study via both per protocol and intention to treat principles.

**Safety monitoring**

**Adverse Events**

An adverse event is any untoward medical occurrence in a study subject temporally associated with participation in the trial and the administration of study medication, whether or not considered related to the medicine. An adverse event can therefore be any unfavourable and unintended sign, symptom or disease temporally associated with the use of the study treatment.

Adverse event data will be collected and analysed with efficacy data at the end of the study. Serious Adverse Events will be notified to the ethics and regulatory committees according to their current guidelines.

**Serious Adverse Events (SAEs)**

For the purposes of this study the following events will be considered to be SAEs:

- Death
- Life-threatening event
- Permanently disabling or incapacitating event
- Hospitalisation or prolongation of hospitalisation. Hospitalisation for the purposes of SAE reporting is defined as an admission to hospital and does not include a presentation to the Emergency Department followed by discharge without admission or an admission for elective reasons
- Any event considered serious by the study investigator

Reporting of SAEs to the Ethics and Regulatory Committees will take place in accordance with the conditions of ethical and regulatory approval for the study. Serious Adverse Events will be reported to MRINZ within 24 hours of study staff becoming aware of the event. SAEs will be reviewed by an independent Investigator (at MRINZ), on an ongoing basis, within 7 days of the event being reported. There will be no formal interim analysis or safety review during the study, due to the low risk of the investigational products. Should the independent Investigator perceive any ongoing or in-

creased risk to participants, the Sponsor will be made aware and the trial may be ceased for safety reasons. Should a female subject on the trial become pregnant during the course of the trial, the pregnancy itself will not be regarded as an SAE. The subject will be asked to contact the researchers after the birth of the baby and any congenital anomaly or birth defect will be considered to be an SAE.

**Collection of Adverse Event Data**

All Adverse Events (AEs and SAEs) will be elicited and recorded at Visit Two by the Follow-Up Centre and sent to the Co-ordinating Centre for data entry and analysis, unless a participant contacts a study Investigator during the intervention period, in which case the events will be recorded at that time.

Follow-up of SAEs will take place as far as possible, by the Co-ordinating and Follow-Up Centres. Should it be necessary, the participant will be advised to contact their GP to receive any further care or assessments as part of their usual healthcare.

**General Health Care**

Participants will receive usual general practitioner care during the study.

**Potential risks**

*Honevo™* - In general, honey is well tolerated for daily consumption and has generally recognized as safe (GRAS) status in the United States. It has been in widespread general usage, as a food and as a medical product for centuries. Honey is extremely safe to use for nutritional and medical purposes. In the 500-plus cases reported in publications on using honey on wounds, and the 140-plus cases reported of using honey in ophthalmology, there has been no mention of any serious adverse effects. With honey, there are no reported cytotoxic effects that would slow the healing process, whereas all

antiseptics in common use can be harmful to body eg. silver as released from nano-crystalline silver dressings.

Potential adverse effects of Honevo:

- Pain - there have been reports of honey causing a stinging pain when applied to wounds. This appears to be due to the acidity of honey, as pain is not experienced when neutralised honey is used.
- Allergies - the components of honey responsible for allergic reactions are thought to be pollens, glandular secretions and bee body material. There are rare reports of occupational asthma, urticaria on the hands and angioedema.
- Disease worsening – there is no reason to expect topical honey to cause disease worsening but this could occur as an unexpected adverse event.

*Topical aciclovir* - side effects may include mild pain or stinging at the site where it is applied. The U.S. Food and Drug Administration (FDA) approved aciclovir cream to treat recurrent cold sores in people older than age 12. The cream may cause temporary skin irritation.

In the event of any adverse events these would be treated according to usual care by the primary care practitioner. The subject or primary care practitioner can chose to withdraw the participant from the trial if felt appropriate. Reporting would be as outlined above.

**Power and Statistical methods**

**Sample Size**

Assuming that the median control duration of symptoms is five days and looking to achieve a one day median difference, with associated Hazard ratio of 1.25, 423 participants would be needed per arm of treatment, therefore the total required is 846. As

this is a short study lasting only for the duration of one cold sore episode, then dropouts from the study should be low, and so assuming that the dropout rate is around 10%, then 950 participants will be randomized

This study size is consistent with similar 2-arm or 3-arm cold sore studies have required around 300-350 patients per treatment arm.[4]

## Statistical Methods

Kaplan-Meier survival plots and estimates of median healing times and Cox Proportional Hazards with a random effect for participants to take into account the parallel design, compared time to healing and pain duration between treatments. Paired t-tests will be used to compare the continuous variables. Sensitivity analysis will be performed for both primary and secondary outcome variables, considering important potential confounders such as age, time to presentation from reported onset of symptoms and stage of cold sore at presentation. Interaction analysis will be performed for primary and secondary outcome variables, to assess potential differences in treatment effects.

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**Appendices**

Appendix 1 – Cold sore stages

| | | |
|---|---|---|
| **Stage 1**<br><br>Prodrome | Initial onset of tingling and itching. No sore is present and skin appears normal. Not everyone gets these symptoms. | |
| **Stage 2**<br><br>Redness | Skin has some redness and swelling. No blister. May itch or feel tender. | |
| **Stage 3**<br><br>Small blister | Small blisters form in the affected area, clear at first turn yellow with time. Usually painful. | |
| **Stage 4**<br><br>Ulcer | Completely formed cold sore with open areas where blisters were. Sore and painful. | |
| **Stage 5**<br><br>Crust | After some days a flaky crust develops resembling a scab. | |
| **Stage 6**<br><br>Drying up & Healing | Crust flakes off with healing skin underneath. Cold sore cycle is complete, maybe some residual redness of skin for a few days. | |
| **Stage 7**<br><br>Healed—skin back to normal | Skin is entirely back to normal. | |

Appendix 2 – Visit 1 Data Collection

The following information will be recorded on the Visit 1 worksheet:

1      Age

2      Gender

3      Initials

4      Ethnicity

5      Number of cold sores per year, on average

6      Months since last cold sore

7      Exact time of onset of symptoms (to the nearest hour - participants will be asked to give their best guess)

8      Current state of cold sore

## Appendix 3 – Patient Diary

The patient diary will collect the following information:

| | How painful has the cold sore been on average over last 24 hours? *(Circle answer)* | How many times have you applied treatment in the last day | What is the stage of the cold sore? (1-7, see photos) |
|---|---|---|---|
| Day 1 | 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 | | |
| Day 2 | 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 | | |
| Day 3 | 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 | | |
| Day 4 | 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 | | |
| etc to Day 14 | 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 | | |

Pain: Score 0-10, where 0 = no pain at all, 10 = severe pain

Stage: use the number that correlates with the pictures that you were given. If completely healed, record as "7"

Appendix 4 – Follow-up phone call (Visit Two)

- Thank participant for participation
- Ask that the diary is posted or emailed to the research centre
- Record any Adverse Events (which will be recorded and reported as per the 'Safety Monitoring' section)
- Record any use of concomitant medications
- Obtain narrative feedback including would you use this product again
- Advise that a copy of the results will be emailed to them (or if no email address a hard copy can be posted)
- Advise that their General Practitioner will be posted a notification of trial participation and progress report

## 9.2 APPENDIX 2: COSMETIC TOILETRY AND FRAGRANCE ASSOCIATION CERTIFICATION



CTFA
Cosmetic Toiletry and Fragrance Association Inc

# GMP CERTIFICATION

This is to certify that

**Zealand Health Manufacturing Ltd**
1 Whakakake Street
Tauriko
Tauranga 3110

A company having its manufacturing/exporting license registered by the New Zealand Food Safety Authority is a legal company authorised by Cosmetic Toiletry & Fragrances Association of New Zealand Inc.

All products manufactured by the above-named Company are in accordance with the Code of Good Manufacturing Practices and conform to all applicable health and substance laws in New Zealand.

This document is valid for 5 years from the date of issue below.

Cosmetic Toiletry & Fragrances Association of New Zealand Inc

Executive Director

**Dated: 8 December 2014**

Cosmetic Toiletry & Fragrances Association of New Zealand Inc
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## 9.3    APPENDIX 3: INVESTIGATOR BROCHURE FOR HONEVO

**INVESTIGATOR'S BROCHURE - HoneyLab Medical-grade Honey & 10% Glycerin**

Edition Number: 1

Release Date: 8/7/13

Author: Prof. Shaun Holt

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**Summary**

Honey has been used for centuries, orally and topically, for a variety of medical conditions. It is extremely safe to use, as would be expected from a common food. Safety risks when it is applied topically are miniscule, and these are reduced further when medical-grade (purified and sterilized) honey is used. 10% glycerine is added to reduce stickiness and increase temperature stability, without losing efficacy.

**Physical, Chemical, and Pharmaceutical Properties and Formulation**

*Honey* is a substance produced by bees to store as a food source. The Codex Alimentarius, produced by the Food and Agriculture Organisation of the United Nations, defines honey as

> *"the natural sweet substance produced by honey bees from nectar of blossoms or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honey bees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature".*

Honey is collected by bees as nectar from flowers and the sap of plants and since the earliest recorded times, humans have taken this honey for use, not only as a food product, but also as a medicine, especially for wound care. Bees concentrate the dilute sugar solutions they collect from the plants by evaporating off most of the water and honey typically consists of 17% water and 80% sugars.

The sugar molecules in solution bind up water molecules, thereby denying microbes the water that is essential for their survival. Bees also add the enzyme glucose oxidase, which converts some of the glucose to gluconic acid, making honey too acidic for microbes to grow (honey has a pH of about 3.5) and, as a by-product of this reaction, forms hydrogen peroxide, a sporicidal antiseptic that sterilises the honey. These factors not only ensure the preservation of honey in the comb but are also responsible for its medical efficacy in terms of suppressing microbial growth.

Additionally, there are 'herbal' factors present which may contribute to its efficacy. As honey is the concentrated juice from plants, it contains various nutrients and herbal chemicals that come from the plants, such as: amino acids, other organic acids, enzymes, vitamins, acetylcholine, flavonoids, carotenoids, polyphenols, minerals and a wide variety of organic chemicals in trace quantities. These are what give different honeys their characteristic colours, flavours and aromas. Some of the plant-derived chemicals have antioxidant properties and some are known to have antibacterial properties.

Honey normally contains between 2,000 and 10,000 pollen grains per gram, which is equivalent to 0.006% – 0.03% w:w of the honey, and this can be reduced to even lower levels by filtration of honey.

Dose - There is no proven safe or effective medicinal dose for honey in adults. Commercial preparations of honey are available, and honey is typically taken by mouth or applied on the skin. Doses for topical use are often unspecific, but 15-30 milliliters is a common dose.

*Glycerin* (or glycerine, glycerin) is a simple polyol (sugar alcohol) compound. It is a colorless, odorless, viscous liquid that is widely used in pharmaceutical formulations. Approximately 950,000 tons per annum are produced in the USA and Europe. Glycerol is used in medical and personal care preparations, mainly as a means of improving smoothness,

providing lubrication and as a humectant. It is found in allergen immunotherapies, cough syrups, elixirs and expectorants, toothpaste, mouthwashes, skin care products, shaving cream, hair care products, soaps and water-based personal lubricants.

**Manufacture of HoneyLab Medical-grade kanuka honey & 10% Glycerin**

See the attached " Manufacturing Specification"

**Safety**

In general, *honey* is well tolerated for daily consumption and has generally recognized as safe (GRAS) status in the United States. It has been in widespread general usage, as a food and as a medical product for centuries.

Honey is extremely safe to use for nutritional and medical purposes. In the 500-plus cases reported in publications on using honey on wounds, and the 140-plus cases reported of using honey in ophthalmology, there has been no mention of any adverse effects. With honey, there are no reported cytotoxic effects that would slow the healing process, whereas all antiseptics in common use can be harmful to body eg. silver as released from nanocrystalline silver dressings.

- Pain - there have been reports of honey causing a stinging pain when applied to wounds. This appears to be due to the acidity of honey, as pain is not experienced when neutralised honey is used.
- Allergies - the components of honey responsible for allergic reactions  are thought to be pollens, glandular secretions and bee body material. There are rare reports of occupational asthma, urticaria on the hands and angioedema.
- Honey intoxication - this has been documented in the literature as an adverse effect of consuming toxic honey also known as 'mad honey,' which is produced from the nectar of certain flowering plants such as those of the genus Rhododendron .
- Leprotic ulcers - there is a concern with some third world countries that the topical use of honey on deep leprotic ulcers may increase the risk of maggot infestation in the wound by houseflies and bluebottle flies.
- Diarrhoea - honey contains fructose in excess of glucose, which may lead to incomplete fructose absorption associated with abdominal symptoms and/or diarrhoea.
- Infant botulism - there are reports that this can be caused by consumption of honey containing *Clostridium botulinum* spores which can proliferate in the intestines of infants and cause botulism poisoning. In the UK, only six cases have been reported between 1976 and 2006. However, this potential risk does not apply to older children or adults. Because of this risk honey is not recommended for infants under 12 months of age. Medical-grade honey can is treated with gamma radiation to reduce the risk of botulinum spores being present without affecting honey's antibacterial activity.

*Glycerin* is not considered a health or environmental hazard. Side effects are rare but possible from oral use, but not topical.

**Efficacy**

Honey has a number of specific physicochemical characteristics which give it unique properties that are responsible for its effectiveness in treating some medical conditions:

- Osmotic effect – honey is a concentrated sugar solution predominantly consisting of the monosaccharides fructose and glucose. Few water molecules are available for micro-organisms and therefore it is a poor environment for their growth
- Hydrogen peroxide – this is slowly released when the honey comes into contact with body fluids and has antiseptic properties
- Acidity – honey is very acidic with a pH of 3.2 to 4.5, as acidic as some vinegars, which also makes micro-organism growth difficult
- Antioxidants - contains bioflavanoids and other antioxidants which may contribute to its activity
- Methylglyoxal - MGO, found in high levels in certain honeys, such as New Zealand manuka and kanuka honeys, has potent antibacterial effects

There is evidence in the medical literature of efficacy for the following conditions:

- Burns - a meta-analysis of the use of medical honey for burns found markedly greater efficacy of honey compared with alternative dressing treatments for superficial or partial thickness burns
- Cough - honey was found to be more effective at reducing the symptom of nighttime cough in children than the commonly used opiate dextromethorphan. Honey has a demulcent effect, soothing irritated mucous membranes
- Wounds - many studies have demonstrated the effectiveness of honey as a treatment for wounds. In addition to its antimicrobial properties which prevent and treat infections, it's hygroscopic properties assist healing by keeping the wounds moist. It is used, usually in the form of a tulle or gauze dressing, for surgical wounds, infected wounds, wound healing after chemotherapy, pressure sores and skin ulcers
- Diabetes - there is evidence that honey might be a good alternative to sugar for people with glucose intolerance or diabetes
- Cold sores - honey has been shown to be superior to the widely used antiviral agent acyclovir, for both oral and genital herpes, for a number of outcome measures including healing time
- Peptic ulcers - manuka honey has been shown to be effective against the bacteria Helicobacter pylori in vitro and studies are looking at whether honey can help to treat peptic ulcers in vivo
- Constipation - honey is a mild laxative
- Weight loss - it is sweeter than sugar but contains fewer calories

In addition, there are case reports and anecdotal claims based on historical use taht it may be effective in the following conditions: Athlete's Foot, Poison Ivy/Oak, Sore Throats, Dermatitis, Gum Disease, Arthritis, Skin Ulcers, Ringworm, Insect Bites, Jock Itch, Dry Skin, Acid Reflux Disease, Acne, Eczema, Nail Fungus, Muscle Stiffness, Tinea, Pressure Sores, Blisters, Scrapes, Abrasions

**Marketing Experience**

Medical-grade *honey* is sold globally and in many countries wound dressings that are impregnated with honey are classified as medical devices. Some of the major companies which manufacture and market medical-grade honey products are:

Comvita - http://www.comvita.co.nz          Honeymark - http://www.honeymark.net/

Revamil - http://www.revamil.nl/                    Medihoney - http://www.medihoney.com/

Manuka Health - http://www.manukahealth.co.nz/

Topical glycerine is used extensively globally in hundreds of medical and personal care preparations.

## Risk identification and management

Based on the widespread historical use of honey and glycerine and knowledge of their potential side effects, no important side effects would be expected in clinical studies. Any possible side effects, such as allergic reactions, would be mild and managed by the investigator. Even this possibility is remote in adults, who are likely to know if they have an unusual hypersensitivity to honey, especially if medical-grade honey is used in the studies.

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**honey lab™**

**Manufacturing Specification**

**Honevo™ Pharmaceutical Honey**

**Receiving**

For each batch of pharmaceutical grade kanuka honey forwarded to Zealand Health Manufacturing Ltd in Tauranga, all drums or pails will have accompanying identification:

## HONEY

Extracted by:

BUZZ APIARIES LTD
KATIKATI PH: 075492996
**RMP NO: BUZZAP1/01**

| | |
|---|---|
| EMPTY CHECK: | |
| BEEKEEPER: | |
| DATE: | |
| BATCH NO: | |
| DRUM NO: | |
| NET WEIGHT: | |

Product of New Zealand

Each shipment shall be accompanied by a Certificate of Analysis, issued by Eurofins Ltd. This will identify by batch:
  1. Total activity - % phenol equivalent using University of Waikato std assay

**Pre-Processing**

All honey undergoes high temperature flash pasteurization during the extraction process at Buzz Apiaries (flashed to >80 ° C and cooled to <35 ° C in line before being stored in sterile food rade drums or pails.

All drums and pails have intact tamper proof lids.

Any drums or pails that appear damaged or open are returned unused to Honeylab Ltd C/- Buzz Apiaries, 63 Rea Road, Katikati

Version 1.2

## Testing

Each unique batch is tested as follows for New Zealand retail markets:

| Item | Range/target |
|------|--------------|
| pH | 3.4-3.8 |
| % water | <18% |
| B.P. | Required BP for honey monograph |

## Testing Methods

pH
The pH values should be measured using a standard similar to the method published in Feás et al. (2010). Five grams of honey samples should be diluted with 20 mL of distilled water and mixed thoroughly (AOAC Official Method 962.19). The pH values for the samples should be measured using Digital pH Meter, this meter having been calibrated at pH 4.0, 7.0, and 10.0 prior to use.  Triplicate
sub-sampling and measurement should be carried out for each batch

water content
Water content (moisture) is determined by refractometry according to AOAC methods (AOAC, 1980a) using a Bellingham and Stanley standard model Abbe-type refractometer.

B.P.
As specified

## Manufacturing

Zealand Health Manufacturing mix finished product in 20kg batches under NZFSA approved and certified wet-room conditions as follows:
   1. 90% kanuka pharmaceutical grade honey
   2. 10% glycerin
Product is immediately used to fill finished goods packages.

## Environmental Controls

Honeylab pharmaceutical honey should be stored either prior to packaging or as finished packaged product in temperatures that may range between 6°C and 35°C

During preparation for processing Honeylab pharmaceutical honey many be warmed to a maximum of 40°C for no longer than 48 hours. Maintaining the honey at 35°C to 40°C for 24-36 hours will return the honey to fluidic state if it has been stored in cool conditions.

During packaging processes, Honeylab pharmaceutical honey may be warmed to a maximum of 45°C for periods not exceeding 4 hours.

## 9.4     9.4 CHEMICAL CLASSIFICAITON OF KĀNUKA HONEY

ANALYTICA
LABORATORIES

## Chemical Classification of Kānuka Honey

October 2016

Report Version 1.0

Jacob Jaine, Ph.D., & Terry Braggins, Ph.D.

Analytica Laboratories Ltd.

## Executive Summary

HoneyLab is a New Zealand company developing medical products from monofloral Kānuka honey. The honey used for their products is acquired predominantly from the Bay of Plenty, though there are plans to extend acquisition elsewhere. To verify the quality of honey from new suppliers, a screening test is required. Analytica Laboratories was commissioned to undertake this work, focusing on the development of an authenticity test using chemical markers. This report details the progress of the project.

Over the last three years, more than 130 kānuka samples were submitted to Analytica for screening. These samples have recently been analysed by UPLC-HRMS, along with a collection of other floral honeys supplied by Analytica. Selected chemical markers were quantified from the data, and statistical analyses performed. Results indicated that no individual compound would be sufficient to distinguish kānuka honeys from others, though a combination of marker compounds may suffice.

A decision tree model was built using data from three quarters of the samples. Five different marker compounds were incorporated into the model, which was then used to classify the samples as kānuka or not. The accuracy of the model was then tested using the remaining quarter of the samples, and was shown to correctly classify samples in >92% of instances. All of the markers have known structures, and most are commercially available. A targeted MS method for the analysis of these compounds is already available at Analytica, and could easily be adapted for their routine analysis. Alternatively, a cheaper UV-based method could be developed providing the same information.

Additionally, the data was perused in a second attempt to identify novel chemical markers. Twenty markers were selected which show desirable statistical properties, and some cursory attempts made to elucidate their structures. Several compounds were conclusively identified, including a novel analogue of phenylalanine which has never been reported in honey before. The accuracy of the model could potentially be improved by inclusion of these compounds, but only pending further development work.

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# 1. Introduction

Kānuka is a monofloral honey produced in New Zealand from the nectar of *Kunzea ericoides*. It has one of the highest total antibacterial activities of all native honeys (Allen and Molan, 1991), which has led to its use in topically-applied medications. One such example is HoneyLab's Honevo®, which has been used effectively for the treatment of acne (Semprini *et al.,* 2016) rosacea (Braithwaite *et al.,* 2015), and cold sores (Fingleton *et al.,* 2014). The success of this product requires the ongoing acquisition of high-quality kānuka honey, for which an authenticity test is required.

One method for testing the authenticity of honey involves using biomarkers (Donarski *et al.,* 2010). These are naturally-occurring chemical compounds which are unique to specific floral honeys, and can therefore be used to verify the authenticity of an unknown honey. Such an approach has been used for the classification of New Zealand honeys using various methodologies, including liquid chromatography (Kato *et al.,* 2014) , gas chromatography (Wilkins, Lu, and Molan, 1993) or nuclear magnetic resonance spectroscopy (Spiteri *et al.,* 2017). While the majority of this work has focused on the verification of mānuka honey, a small number of papers have also investigated kānuka honeys.

There are three main papers which have investigated biomarkers for kānuka honey. The earliest study on this matter was from Tan *et al.*, who studied methylated honey extracts by GC-MS (Tan *et al.,* 1988). The conclusion of this paper was that mānuka and kānuka honeys are both high in aromatic acids, notably syringic acid, 2'-methoxyacetophenone, 2-methoxybenzoic acid, and 3-phenyllatic acid, though the authors did not conclude it was possible to distinguish the two. Many years later, Stephens *et al.* published a paper specifically on the differentiation of mānuka and kānuka honey by LC/MS (Stephens *et al.,* 2010). Their conclusion was that the two honeys contained six phenolic acids in common, most prominently 3-phenyllactic acid and methyl syringate, and that kānuka honey contained elevated levels of 4-methoxyphenyllactic acid. In the more recent past, Beitlich *et al.* conducted a similar study using HS-SPME-GC/MS (Beitlich *et al.,* 2014). Their study found that the headspace above kānuka honeys contained elevated levels of 4-anisaldehyde, 4-oxoisophorone, and phenylethyl alcohol. Additionally, they analysed the honeys by UPLC-PDA-MS, finding 4-methoxyphenyllactic acid, 4-anisic acid, and lumichrome to be elevated in the kānuka honeys.

In 2014, Analytica was commissioned by HoneyLab to investigate authenticity testing for kānuka honey using the aforementioned biomarker approach. This research produced ten potential marker compounds, though ultimately the work was not found to be reproducible. Reconsideration of the experimental approach in 2015 lead to the development of a preliminary classification system which could identify samples as 'kānuka' or 'other' with 100% accuracy, using a combination of markers identified by other research groups. Subsequently, a broader range of samples have been acquired, which have since been used to refine the classification model. The present report details the outcome of this research.

The experimental approach taken thus far has revolved around UPLC-HRMS analysis of honey extracts. This allows the simultaneous detection of thousands of components within the samples, which can be assessed by statistical methods to determine their suitability as biomarkers. Importantly, compounds with known structures can be readily identified within the data by comparing their high-resolution masses and retention times with known standards. This allows the identification and quantification of previously-reported marker compounds, the concentrations of which can then be used to construct a classification model.

## 2. Methodology

### 2.1 Honey Collection

A total of 210 honey samples were collected for the project. Kānuka samples were supplied predominantly by BeeNZ and Buzz Apiaries, while honeys of other floral types were supplied by Analytica. A full list of samples and supporting information is given in Appendix 1, and a summary in Table 1.

It is well-known that kānuka honey shares many characteristics with mānuka honey; it is less well known that the same is true of their chemical composition. For this reason, a relatively large number of mānuka honeys were included in the experiment. Varying grades were used, including several low-grade "non-active mānuka" samples. It is probable that these contain high portions of kānuka honey, and are therefore important to include from a classification perspective. Fourteen honeys were of uncertain floral origin, including eight which were submitted as kānukas, but revised by the apiarists to "not kānuka" after receiving their 3-in-1 results.

**Table 1:** List of honey types analysed in the present research.

| Floral Type | Number |
| --- | --- |
| Borage | 1 |
| Clover | 5 |
| Honeydew | 1 |
| Kamahi | 5 |
| Kānuka | 126 |
| Mānuka | 30 |
| Multifloral | 5 |
| Pohutukawa | 1 |
| Rātā | 5 |
| Rewarewa | 7 |
| Tawari | 4 |
| Thyme | 6 |
| Unknown | 14 |

Analysis of the sample IDs showed that many of the kānuka samples received were drum duplicates from a single extraction batch. These were chemically similar, and therefore of little value to include in the experiment. The samples were therefore split into two groups. The first group contained all of the unique samples submitted, and were used for the initial portion of the experiment. The data from these samples was used for building the classification model, and is referred to as the 'training set'. The remaining samples were used to test the accuracy of the model, and are therefore referred to as the 'test set'.

### 2.2 Chemical Profiling (UPLC-HRMS)

After their receipt at the laboratory, samples were heated briefly to 40 °C to dissolve any crystallised sugar crystals, and stirred thoroughly to homogenise. An aliquot (1.00 g) was diluted with aqueous methanol (9.3 mL of 10%), centrifuged and 15,0000 RCF for 15 minutes, then taken into silanized LC-MS vials. Training samples and test samples were prepared by different technicians on different days using different equipment, thus reducing any potential bias in the analysis.

The extracts were separated by UPLC on an Ultimate 3000 system with Zorbax Eclipse C18+ column. A 16 minute gradient was used, with a flow rate of 0.4 mL/min, beginning at 5% B (ACN with 0.1% HOAc) and increasing to 100% B (water with 0.1% HOAc). The eluent was passed through a diode array detector running in 3D field acquisition mode, to a Thermo qExactive Orbitrap Mass Spectrometer. Full-scan mass spectra were recorded at 1 Hz at 70,000 resolution using positive/negative switching. Samples were randomised within the analytical sequence to remove potential drift effects on subsequent data analysis. Every six samples a composite sample was run as a control to monitor instrumental drift. The control was composed of equal aliquots of the extracts of all the individual honey sample extracts. The training and test samples were also run on different days, after shutting down and re-tuning the mass spectrometer.

## 2.3 Semi-Quantitative Analysis

Initially, thirteen chemical markers reported by other research groups were quantified from the data. These were a mixture of compounds reported to be either kānuka or mānuka markers, for which authentic standards were available. A nine-point standard curve containing all thirteen compounds was prepared, with concentrations distributed logarithmically from 0.1 mg/L up to 5 mg/L. Areas were calculated from extracted ion chromatograms of the base peak ions using a 5 ppm window. Quantification was performed by interpolation from the curve. The limit of quantification (LOQ) for each analyte was estimated from 5x the baseline noise in the standard samples. The details of the compounds and their performance properties are given in Table 2. All analysis was performed with Thermo Xcalibur, version 3.0.63.

**Table 2:** Previously reported marker compounds quantified in the current work, along with relevant mass spectral and method performance information.

| Compound | Quantifier Ion [m/z] | Qualifier Ion [m/z] | $R^2$ | Precision [% 2RSD] | LOQ [mg/kg] |
|---|---|---|---|---|---|
| 2'-methoxyacetophenone | 151.0753+ | 133.0648+ | 0.9995 | 3.8 | 0.002 |
| 2-methoxybenzoic acid | 135.0442+ | 151.0393- | 0.9952 | 7.8 | 0.01 |
| 4-methoxyphenyllactic acid | 195.0658- | 177.0552- | 1.0000* | 9.2 | 0.0005 |
| 3-phenyllactic acid | 165.0550- | 147.0444- | 1.0000* | 7.2 | 0.001 |
| Syringic acid | 197.0451- | 182.0215- | 0.9955† | 5.4 | 0.01 |
| 2-phenylethanol | 105.0699+ | 118.0651+ | 0.9962 | 10.5 | 0.2 |
| 4-anisaldehyde | 137.0598+ | 109.0650+ | 0.9999 | 14.7 | 0.05 |
| Methyl syringate | 213.0751+ | 211.0602- | 0.9958 | 12.6 | 0.01 |
| Lumichrome | 243.0875+ | 241.0731- | 0.9949† | 6.6 | 0.001 |
| 4-oxoisophorone | n.d. | n.d. | n.a. | n.a. | n.a. |
| Trimethoxybenzoic acid | 211.0602- | 213.0751+ | 0.9954† | 10.3 | 0.01 |
| Gallic acid | 169.0131- | 125.0231- | 0.9751 | 11.9 | 0.2 |
| Kojic acid | 143.0339+ | None | 0.9934 | 24.4 | 0.2 |

*Cubic spline calibration used, where $R^2$ is necessarily unity.

† Calibration performed on 7-pt curve with 1/x weighting after rejecting top 2 standards.

## 2.4 Untargeted and Qualitative Analysis

In addition to measuring the concentration of previously-reported markers, another attempt was made at choosing novel chemical markers. Emphasis was placed on finding high-abundance markers with good chromatography, as well as realistic statistical properties.

Raw data files were processed using the chemometric analysis package 'Progenesis QI' from Nonlinear Dynamics. Peak picking was performed on the data from a digital average sample, representing a composite of all the samples of each type analysed. Individual files were then back-integrated to build a table of mass features. The deisotoped and deconvoluted peak table was then manually searched for markers of interest. The following criterion were used:

- A mean fold-change greater than 2x;
- A P-value less than $1\times10^{-5}$;
- A mean abundance greater than the equivalent of 100 ppb;
- Free from chromatographic and mass spectral interferences.

The top ten most abundant markers satisfying these criterion were chosen, and attempts made to identify their structure. Firstly, the mass spectra were examined to determine whether each ion was a quasimolecular ion, or whether it was a fragment of a larger compound. $MS^2$ patterns were obtained by high energy dissociation of quasimolecular ions at 20-80 V; $MS^3$ patterns were obtained in the same way, but from fragments generated in-source by collision-induced dissociation. Using this information the molecular formulae were calculated, which were used to putatively assign a structural class. Spectral matching against online databases was used to narrow the range of candidate compounds, and then authentic standards run where available. Confirmation was given by an exact match in retention time, peak shape, and fragmentation pattern.

## 2.5 Statistical Analysis and Modelling

To determine whether the markers were able to discriminate between floral types, inferential statistical methods were used. Firstly the data was checked for normality using a Shapiro-Wilk test, and secondly, tested for distribution equality using an independent-samples Mann-Whitney U test. The ability for each marker to discriminate between floral types was assessed by constructing receiver operating characteristic (ROC) curves and calculating the area under the curve (AUC) by the trapezoidal method. Markers which best separated the desired classes were used to train a decision tree for classifying honeys as kānuka or not. Each node utilised a linear univariate classifier, with threshold concentrations on the branches. The choice of values was guided by calculating the empirical probability distribution functions for each marker and each class, and assessing the relative true positive and false positive rates at varying concentrations.

# 3. Results and Discussion

## 3.1 Semiquantitative Analysis

The specifically quantified marker compounds showed a mixture of statistical properties. Only three of the compounds had higher average abundances in the kānuka honeys than the other floral types, but none were sufficiently unique to be useful classifiers, at least when used individually. This is supported by the P-values, which showed two of three compounds exhibited no significant difference between floral types, and also by the ROC AUCs, which show the compounds are only 8-16% better a classifiers than random chance (0.5 is equivalent to random chance, 1.0 is a perfect classifier). The data from these analyses are summarised in Table 3.

**Table 3:** Previously reported marker compounds quantified in the current work, along with relevant statistical information. All values calculated comparing kānuka honey to all other floral types taken as a single group. P values from Mann-Whitney U test. Fold change between mean concentrations. Insufficient amounts of 2-phenylethanol and 4-oxoisophorone were found to calculate statistical parameters.

| Compound | Floral Type | Rt | P-Value | FC | AUC |
|---|---|---|---|---|---|
| 2'-methoxyacetophenone | Mānuka | 8.52 | $1.7 \times 10^{-10}$ | -9.4 | 0.18 |
| 2-methoxybenzoic acid | Mānuka | 7.01 | $5.0 \times 10^{-2}$ | -5.1 | 0.40 |
| 4-methoxyphenyllactic acid | Both | 6.92 | $2.8 \times 10^{-4}$ | -2.8 | 0.68 |
| 3-phenyllactic acid | Mānuka | 6.39 | $3.6 \times 10^{-2}$ | -1.0 | 0.55 |
| Syringic acid | Mānuka | 5.50 | $1.3 \times 10^{-3}$ | -1.1 | 0.66 |
| 2-phenylethanol | Kānuka | *n.a.* | *n.a* | *n.a.* | *n.a.* |
| 4-anisaldehyde | Kānuka | 8.15 | $6.0 \times 10^{-2}$ | +2.5 | 0.58 |
| Methyl syringate | Kānuka | 7.95 | $2.0 \times 10^{-1}$ | +1.3 | 0.66 |
| Lumichrome | Kānuka | 7.66 | $3.2 \times 10^{-3}$ | +1.8 | 0.65 |
| 4-oxoisophorone | Kānuka | *n.a.* | *n.a.* | *n.a.* | *n.a.* |
| Trimethoxybenzoic acid | Mānuka | 7.72 | $4.4 \times 10^{-7}$ | -2.8 | 0.25 |
| Gallic acid | Mānuka | 1.22 | $5.0 \times 10^{-9}$ | -2.4 | 0.21 |
| Kojic acid | Mānuka | 1.05 | $2.7 \times 10^{-2}$ | -1.6 | 0.39 |

The poor performance of the kānuka markers can be ascribed to their presence in many of the mānuka honeys at similar concentrations, or higher. For example, 3-phenyllactic acid *is* significantly elevated in the kānuka honeys compared to most other floral types *except* mānuka, in which it was even more concentrated. If a model could be constructed which firstly accounted for whether a honey was mānuka, classification of the remaining samples as kānuka or not would be relatively trivial using the existing markers.

## 3.2 Model Construction

A decision tree model was built for the classification, as it provides a simple and intuitive way to interpret classification problems with multiple steps, as was likely to be the case for the present data. A summary of the model is shown graphically in Figure 1.

As a first step, the ROC curves for each marker were re-calculated, comparing the mānuka honeys to all others. The AUCs indicated that the compound exhibiting the greatest discriminating power was leptosperin, a glycoside of methyl syringate reported by Kato in 2012 (Kato *et al.*, 2012). Calculation of the CDFs, as described earlier, suggested that 150 mg/kg would be an appropriate threshold for this compound. Based on

the current data set, honeys with leptosperin greater than this level are probably monofloral mānuka, while honeys below this level are *possibly* kānuka. Leptosperin was therefore included as the first node within the model.

To reduce the false positive rate of the model, a second orthogonal mānuka-exclusion step was incorporated. The ROC curves suggested the next most powerful marker was 2-methoxyacetophenone (2'-MAP), a compound first reported in New Zealand honey by Tan *et al.* in 1988 (Tan *et al.*, 1988). Calculation of the CDFs indicated that a threshold of 2 mg/kg would be appropriate for removing some of remaining mānukas and the samples likely to be mānuka-kānuka blends. In combination, these two markers effectively separated the mānuka honeys from all others, and so 2'-MAP was incorporated as the second node in the model.

Statistical analysis of the remaining honeys showed they could be classified most effectively using methyl syringate (MSYR), the methyl ester of syringic acid. This compound was first reported in mānuka honey in the late 80s (Tan *et al.*, 1988) but has also been reported in foreign honeys including Italian Asphodel (Tuberoso et al., 2009) and Australian Leatherwood (Rowland et al., 1995), Blue Gum, and Yellow Box (D'Arcy et al., 1997).
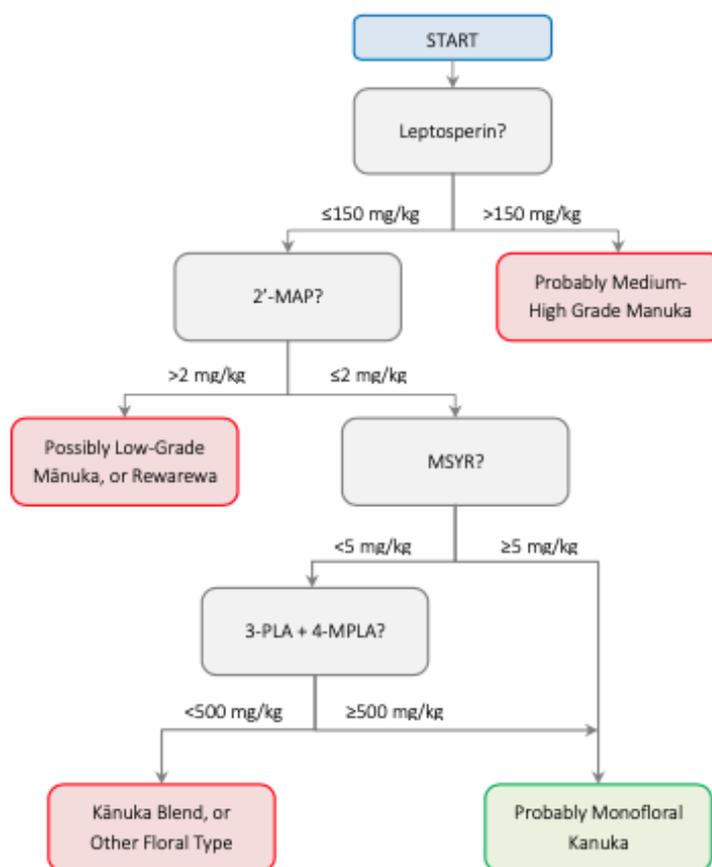


**Figure 1:** A visual representation of the classification model. For a honey of unknown floral type, begin at the top of the tree and trace downwards through the various branches. Grey boxes (nodes) each represent a different chemical marker which must be measured, and the appropriate arrow (branch) followed depending on the concentration. The final outcomes are either coloured red (not kanuka) or green (kanuka).

Most of the kānuka honeys had elevated MSYR compared to those of other floral types, with the CDFs indicating that an appropriate threshold would be 5 mg/kg. This compound was thus incorporated as the third node in the model.

A handful of the kānuka honeys did not have sufficient MSYR to meet the threshold, but were high in other markers. Notably, five of the kānuka honeys had high levels of the compounds 3-phenyllactic acid and 4-methoxyphenyllactic acid, which are two of the major extractable organic substances detectable in mānuka and kānuka honeys. There may therefore be two types of kānuka honey within the current data set, one classified by high methyl syringate, and the other by high phenyllactic acids (PLAs). These sum of these two two compounds were therefore incorporated as a combined fourth and final node within the classification scheme.

## 3.3 Model Performance and Limitations

The combination of these five analytes was shown to be sufficient to accurately classify most of the honey samples according to their designated floral type. When the model was tested on the training data, the proportion of correct classifications was 84%; when the misclassified kānuka samples were removed, the accuracy rose to an excellent 93%. The latter figure is probably a more accurate representation of the accuracy of the model, since samples which fail the classification are more likely to be of dubious quality, and thus less suitable for use in training a model in the first place. Similarly, 92% accuracy was achieved when the model was tested on the test set, showing that the model generalises well to other samples. Confusion matrices showing further details of the classification performance are given in Tables 4, 5, and 6.

In all instances where duplicate samples were received, the model was shown to classify them as the same floral type. This may be unsurprising given their origins, though it shows the analytical workflow has sufficient precision to produce the same result for different samples. Analysis of the relative percent differences (RPDs) between the five analytes used shows that in most instances the range of values measured for duplicate batch samples were in the region of 2-8%.

Classifications results from the model also indicate that "kānuka" honey from some suppliers is more likely to be true to label than those from some others. These can be identified in Appendix 1, by comparing the floral type as supplied by the apiarist to those calculated by the model.

These values are calculated based on the floral types provided by the clients, assuming that all honeys are monofloral; this is of course not the case. Kānuka honeys sent in by clients *hoping* they have kānuka rather than *knowing* will contribute to an artificially inflated false negative rate. Conversely, floral

**Table 4:** Confusion matrix showing the performance of the model on the training data.

| Accuracy: 84% | | Actual | |
|---|---|---|---|
| | | Kanuka | Other |
| Predicted | Kānuka | 69 | 15 |
| | Other | 7 | 50 |

**Table 5:** Confusion matrix showing the performance of the model on the training data after removing kānuka honeys of dubious quality.

| Accuracy: 93% | | Actual | |
|---|---|---|---|
| | | Kanuka | Other |
| Predicted | Kānuka | 73 | 9 |
| | Other | 0 | 47 |

**Table 6:** Confusion matrix showing the performance of the model on the test data.

| Accuracy: 92% | | Actual | |
|---|---|---|---|
| | | Kanuka | Other |
| Predicted | Kānuka | 30 | 4 |
| | Other | 0 | 17 |

honeys which may contain some proportion of mānuka, or indeed low-grade 'non active mānuka', may contribute to an artificially inflated false positive rate. For this reason, the false positive rate will always be higher, as it will be difficult to distinguish monofloral kānuka honeys from mānuka blends.

In both of the test and training sets, the false positive rate was several times larger than the false negative rate. This is an unavoidable consequence of the classification approach taken, which in turn relates to the fact that kānuka does not appear to contain any truly unique chemical markers. The practical consequence of this issue is that the model may misclassify multifloral honeys containing small portions of mānuka as being monofloral kānuka. The parameters of the model may be adjusted to reduce these instances, though this would simultaneously raise the requirement for what is considered a kānuka. As such, the balance of false positives to false negatives should be further considered, as well as the potential consequences.

It is possible that many of the issues described could be ameliorated by the use of alternative marker compounds. As the model already incorporates the best markers from the available scientific literature, the only alternative would be to search for new, unreported, marker compounds. These could supplement or even supplant those used in the current model, providing greater statistical power and increase classification accuracy. The choice of alternate novel markers is discussed in the following section.

## 3.4 Untargeted and Qualitative Analysis

Qualitative analysis of the samples revealed their chromatographic profiles were consistent with honey samples previously analysed at Analytica. Most of the major peaks represent previously reported compounds, though there are some peculiarities. For example, phenylalanine has been reported in many floral types of honey (Cotte *et al.*, 2004) but not in mānuka, though this is probably becayse no-one has specifically looked for it. Sebacic acid was found in many of the honey samples, despite being the major extractable component of rewarewa honey (Wilkins *et al.*, 1995), and one of the major flavonoid components, assigned as sakuranetin, has been reported in Serbian honeys (Kečkeš et al., 2013) but not those from New Zealand. A typical chromatogram with some of the features assigned is shown in Figure 2.
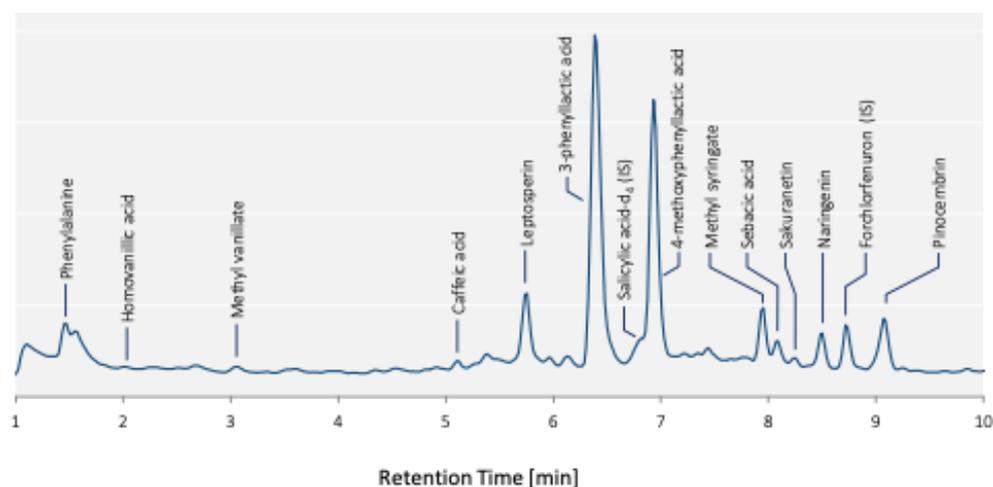


**Figure 2:** Typical total negative ion chromatogram of the honeys used in this study.

In addition to the specifically quantified markers, the data was also searched for novel marker compounds. Given previous issues with repeatability, emphasis was placed on finding markers with 'realistic' properties, that is, chromatographically and statistically similar to those already reported. Additionally, only compounds with relatively low molecular weights (generally <300 Da) were chosen, as these would be easier to identify by mass spectrometry alone, and would be more likely to be commercially available.

A total of 12,046 mass features were found in the data set, split evenly between positive and negative ion mode. Of these compounds, 1,037 were sufficiently enriched in the kānuka honeys that they may be *potentially* useful "positive" markers. Conversely, 551 were sufficiently depressed in the kānuka honeys that they may be useful as "negative" markers. A cursory assessment of their discriminating power suggests that some may be slightly better than the compounds used in the present model, though none are vastly better. Regardless, additional markers may be used to refine the model at a later date, pending further work on their identification and quantitation.

### 3.4.1 Positive Markers

The data contained an array of mass features potentially suitable as marker compounds. None of these compounds were sufficiently elevated in the kānuka honeys that they could individually conclusively distinguish them from all other floral types, just like those described earlier in this report. Once again, in most cases this was because they were also present in mānuka honeys. Though this is the case, it may be that some of these compounds are still more powerful than those used in the model described in the previous section, and so are still worthy of investigation. Ten markers were therefore chosen, and are listed in Table 7.

Attempts to elucidate the structures of the markers suggested they generally belonged to one of three classes:

o   Nitrogen-containing compounds (K1, K5-7);

o   Phenolic compounds, and derivatives thereof (K2-4, K9);

o   Degraded carotenoids (K8, K10).

**Table 7:** Novel positive markers of kānuka honey.

| Code | Base Peak m/z | Neutral Formula | Rt [min] | P-Value* |
|------|---------------|-----------------|----------|----------|
| K1 | 215.0813+ | $C_{11}H_{10}O_2N_2$ | 4.78 | $5.3 \times 10^{-15}$ |
| K2 | 165.0543+ | $C_9H_8O_3$ | 5.69 | $7.5 \times 10^{-12}$ |
| K3 | 327.1084- | $C_{15}H_{20}O_8$ | 5.36 | $2.4 \times 10^{-11}$ |
| K4 | 181.0499- | $C_9H_{10}O_4$ | 4.53 | $4.2 \times 10^{-11}$ |
| K5 | 166.0860+ | $C_9H_{11}NO_2$ | 1.46 | $8.7 \times 10^{-10}$ |
| K6 | 194.0816- | $C_{10}H_{13}O_3N$ | 2.26 | $1.3 \times 10^{-10}$ |
| K7 | 105.0700+ | $C_8H_{11}N$ | 1.93 | $5.6 \times 10^{-9}$ |
| K8 | 241.1431+ | $C_{13}H_{20}O_4$ | 7.12 | $5.7 \times 10^{-8}$ |
| K9 | 197.0813- | $C_{10}H_{14}O_4$ | 4.45 | $1.0 \times 10^{-8}$ |
| K10 | 107.0493+ | $C_{13}H_{20}O_3$ | 6.71 | $6.5 \times 10^{-7}$ |

*Student's t-test

Compounds belonging to all three of these classes have been detected in New Zealand honeys previously, though generally not matching the masses reported herein. Despite this, the structures of two markers were conclusively identified, as follows.

Marker K5 was initially detected as the $120.0808^+$ ion, one of the major features of the chromatograms. Analysis of the mass spectra showed this ion was a fragment of the $166.0860^+$ ion, for which the corresponding neutral formula would be $C_9H_{11}NO_2$. Database searches suggested the compound may be phenylalanine, one of the naturally-occurring amino acids. Comparison with an authentic standard confirmed this assignment, producing an identical retention time, peak shape, as well as $MS^2$ and $MS^3$ patterns. A coarse estimate of the concentration suggests that kānuka honeys contain 10-100 mg/kg of phenylalanine, while other floral types contain 5-50 mg/kg.
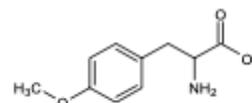


**Figure 3:** Structure of the novel marker compound 4-methoxy phenylalanine (4-MPALA).

Marker K6 was initially detected as the 194.0816- ion. No higher-mass parent ions could be found, indicating this was the quasimolecular ion. Calculation of the neutral formula gave $C_{10}H_{13}O_3N$, suggesting the compound is the methoxylated version of phenylalanine. This was supported by the $MS^2$ data, which showed a similar fragmentation pattern to phenylalanine, but 30 mass units higher. Considering the large quantities of the structurally-related compound 4-methoxyphenyllactic acid (4-MPLA) in kānuka honey, this was considered a plausible assignment. An authentic standard was therefore purchased, and was spiked into the control honey at 5 mg/kg. An excellent match in retention time was found, and the $MS^2$ patterns at 40-80 V were indistinguishable, confirming the assignment. A coarse estimate of the concentration suggests the honeys contain approximately 0.1 mg/kg of 4-methoxyphenylalanine (4-MPALA). The structure of this compound is shown in Figure 3. This compound has never been reported in honey before.

These markers all appear to be enriched in kānuka honeys over those of other floral types. As such, they could act to give a positive confirmation than that a sample is kānuka, in the way that nodes 3 and 4 act in the current model. Though their structures are not currently known, they could be semi-quantified using a surrogate external standard. For example, the carotenoids could be quantified against ionone or damascone, while the phenolics could be quantified against gallic acid or similar.

### 3.4.2 Negative Markers

The data also contained a small number of 'negative' markers, that is, ones which are lowest in kānuka honey. These could be used to specify maximum thresholds, in the same way that leptosperin is utilised in the currently presented model. Ten markers were chosen, and are listed in Table 8.

Marker K11 was initially identified by the 135.0442- ion. The characteristic mass of this ion indicated it was a fragment, and analysis of the mass spectra indicated the parent ion was the 179.0345- ion. The corresponding neutral formula was $C_9H_8O_4$, which suggested a hydroxylated phenylpropanoid. Analysis of several standards produced an exact match with caffeic acid. This compound is a known component of honey, and has been previously detected in Australian and New Zealand honeys (Yao et al., 2003). Though it is proposed this compound may contribute to the antibacterial qualities of honey (Wahdan, 1997), we estimate it is only present in the current honey samples at around 1 mg/kg.

**Table 8:** Novel negative markers of kānuka honey.

| Code | Base Peak m/z | Neutral Formula | Rt [min] | P-Value* |
|---|---|---|---|---|
| K11 | 135.0442- | $C_9H_8O_4$ | 6.21 | $2.1 \times 10^{-15}$ |
| K12 | 205.0499- | $C_{11}H_{10}O_4$ | 6.95 | $9.6 \times 10^{-14}$ |
| K13 | 151.0752+ | $C_9H_{10}O_2$ | 8.53 | $3.7 \times 10^{-11}$ |
| K14 | 167.0701+ | $C_9H_{10}O_3$ | 7.41 | $1.1 \times 10^{-9}$ |
| K15 | 161.0595+ | $C_{10}H_8O_2$ | 7.29 | $1.7 \times 10^{-9}$ |
| K16 | 205.0857+ | $C_{12}H_{12}O_3$ | 7.84 | $9.9 \times 10^{-8}$ |
| K17 | 127.0390+ | $C_6H_6O_3$ | 2.40 | $9.9 \times 10^{-8}$ |
| K18 | 219.0659- | $C_{12}H_{12}O_4$ | 7.64 | $1.5 \times 10^{-8}$ |
| K19 | 185.0812- | $C_9H_{14}O_4$ | 4.85 | $2.3 \times 10^{-8}$ |
| K20 | 239.0911+ | $C_{12}H_{14}O_5$ | 6.84 | $6.4 \times 10^{-7}$ |

*Student's t-test

Marker K13 was initially detected as the quasimolecular 151.0752$^+$ ion. The corresponding neutral formula was $C_9H_{10}O_2$, suggesting an aromatic ketone. Database searches suggested 2'-methoxyacetophenone as a likely candidate, and so an authentic standard was run. An exact match was found, confirming the identity of the compound. This compound is has been detected by many different groups, and is a known marker of mānuka honey. It is probable that it appears elevated in the current kānuka honeys, on average, because there are a far greater number of kānuka samples in the current experiment than any other floral type, but most probably contain a small portion of mānuka.

## 5. Conclusions and Recommendations

HoneyLab have commissioned Analytica to investigate developing an authenticity test for kānuka honey. A total of 210 honey samples have been supplied for the project, which have been chemically profiled by UPLC-HRMS. A selection of chemical markers were quantified from the data, and used to train a decision tree. The model was shown to be sufficiently selective to correctly classify honeys as kānuka or not in >92% of instances. The results of the classification are listed in this report. The compounds used for the classification are summarised in Table 8.

**Table 8:** Chemical markers used for the classification of kānuka honey.

| Compound | Required Concentration [mg/kg] |
|---|---|
| Leptosperin | ≤150 |
| 2'-methoxyacetophenone | ≤2 |
| Methyl syringate | ≥5 |
| 3-phenyllactic acid plus 4-methoxyphenyllactic acid | ≥500* |

\* Only required if methyl syringate is less than 5 mg/kg.

All of the compounds used for the model have known structures and are commercially available. A targeted mass spectrometry method is already available at Analytica for their analysis, which could be adapted for routine or high-throughput analysis of samples. Alternatively, it may be possible to develop a UV-based method for their analysis. Regardless of the method chosen, the results described in this report would need to be validated with the new method before 'going live'.

The method could be used routinely for screening all drums of honey from new clients, as well as batches of honey from existing clients. A small amount of representative honey (*ca.* 50 g) would be supplied to the laboratory, and passed through the method. A report would be produced giving the levels of the five analytes, as well as whether the unknown sample is consistent with being a monofloral kānuka as per the honeys supplied to develop the model. Ongoing comparison of the test results with the perceived quality of the honey will give an indication of whether or not the model is too stringent, with parameters adjusted accordingly.

If the model is found to perform insufficiently, or if interest dictates, then further development work could focus on the novel chemical markers which have been chosen. These could be retrospectively quantified from the data, statistically analysed, and their merits assessed. If results indicate that the novel markers could supplement or even supplant the existing markers used in the model, then some further attempts to identify their structures would be beneficial. If the model is found to perform adequately, then there may be no benefit to this exercise.

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Appendix 1. Sample Register.

| Lab ID | Client Sample ID | Client | Supplied Floral Type | Calculated Floral Type | Usage | DHA [mg/kg] | MGO [mg/kg] | NPA [equiv. [% phenol] | HMF [mg/kg] | Leptosperin [mg/kg] |
|---|---|---|---|---|---|---|---|---|---|---|
| 14-02382_001 | B5 D1 | Honeylab | Kānuka | Kānuka | Training | 24 | 32 | 2.8 | 21.7 | 77 |
| 14-02382_002 | B5 D2 | Honeylab | Kānuka | Kānuka | Test | 24 | 34 | 2.9 | 22.3 | 77 |
| 14-02382_003 | B5 D3 | Honeylab | Kānuka | Kānuka | Test | 24 | 34 | 2.9 | 21.7 | 77 |
| 14-02382_004 | B5 D4 | Honeylab | Kānuka | Kānuka | Test | 23 | 32 | 2.8 | 19.4 | 80 |
| 14-02382_005 | B5 D5 | Honeylab | Kānuka | Kānuka | Test | 24 | 33 | 2.9 | 22.7 | 77 |
| 14-02382_006 | B5 D6 | Honeylab | Kānuka | Kānuka | Test | 24 | 33 | 2.8 | 20.1 | 77 |
| 14-02382_007 | B13 D1 | Honeylab | Mānuka | Mānuka | Training | 2373 | 731 | 18.5 | 9.8 | 1270 |
| 14-02382_008 | B13 D2 | Honeylab | Mānuka | Mānuka | Test | 2341 | 746 | 18.8 | 9.9 | 1260 |
| 14-02382_009 | B13 D3 | Honeylab | Mānuka | Mānuka | Test | 2184 | 699 | 18 | 10.3 | 1290 |
| 14-02382_010 | B6 D2 | Honeylab | Mānuka | Mānuka | Training | 1696 | 514 | 15 | 10.9 | 1020 |
| 14-02382_011 | B6 D3 | Honeylab | Mānuka | Mānuka | Test | 1714 | 511 | 14.9 | 10.3 | 1020 |
| 14-02382_012 | B6 D4 | Honeylab | Mānuka | Mānuka | Test | 1694 | 507 | 14.9 | 10.3 | 1030 |
| 14-02382_013 | B12 D1 | Honeylab | Multifloral | Kānuka | Training | <10 | <4 | <0.8 | 8.8 | <20 |
| 14-02382_014 | B12 D1 | Honeylab | Multifloral | Kānuka | Test | <10 | <4 | <0.8 | 10.4 | <20 |
| 14-02382_015 | B12 D3 | Honeylab | Multifloral | Kānuka | Test | <10 | <4 | <0.8 | 10.3 | <20 |
| 14-02382_016 | B12 D3 | Honeylab | Multifloral | Kānuka | Test | <10 | <4 | <0.8 | 9.6 | <20 |
| 14-02382_017 | B12 D4 | Honeylab | Multifloral | Kānuka | Test | <10 | <4 | <0.8 | 10.7 | <20 |
| 14-02445_001 | Kanuka 1 | Honeylab | Kānuka | Kānuka | Training | <10 | <4 | <0.8 | 6 | <20 |
| 14-02445_002 | Kanuka 2 | Honeylab | Kānuka | Kānuka | Test | <10 | <4 | <0.8 | 6 | <20 |
| 14-02445_003 | Thyme 1143 | Honeylab | Thyme | Other | Training | <10 | <4 | <0.8 | 3 | <20 |
| 14-02445_004 | Thyme 1145 | Honeylab | Thyme | Other | Test | <10 | <4 | <0.8 | 3 | <20 |
| 14-02445_005 | Thyme 1146 | Honeylab | Thyme | Other | Test | <10 | <4 | <0.8 | 3 | <20 |
| 14-02445_006 | Thyme 1148 | Honeylab | Thyme | Other | Test | <10 | <4 | <0.8 | 3 | <20 |
| 15-01256_001 | Batch 12 (1) | Buzz Apiaries | Kānuka | Kānuka | Training | 38 | <4 | <0.8 | 3 | <20 |
| 15-01256_002 | Batch 12 (2) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | <20 |
| 15-01256_003 | Batch 12 (3) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 21 |
| 15-01256_004 | Batch 12 (4) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | <20 |
| 15-01256_005 | Batch 12 (5) | Buzz Apiaries | Kānuka | - | None | - | - | - | - | - |
| 15-01256_006 | Batch 12 (6) | Buzz Apiaries | Kānuka | - | None | - | - | - | - | - |

| Lab ID | Client Sample ID | Client | Supplied Floral Type | Calculated Floral Type | Usage | DHA [mg/kg] | MGO [mg/kg] | NPA [equiv. [% phenol] | HMF [mg/kg] | Leptosperin [mg/kg] |
|---|---|---|---|---|---|---|---|---|---|---|
| 15-01256_007 | Batch 10 (1) | Buzz Apiaries | Kānuka | Kānuka | Training | 62 | 12 | 1.6 | 2 | 30 |
| 15-01256_008 | Batch 10 (2) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 26 |
| 15-01256_009 | Batch 10 (3) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 27 |
| 15-01256_010 | Batch 10 (4) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 28 |
| 15-01256_011 | Batch 10 (5) | Buzz Apiaries | Kānuka | - | None | - | - | - | - | - |
| 15-01256_012 | Batch 10 (6) | Buzz Apiaries | Kānuka | - | None | - | - | - | - | - |
| 15-01256_013 | Batch 13(1) | Buzz Apiaries | Kānuka | Kānuka | Training | 53 | 12 | 1.6 | 3 | 26 |
| 15-01256_014 | Batch 13(2) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 23 |
| 15-01256_015 | Batch 13(3) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 22 |
| 15-01256_016 | Batch 13(4) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 23 |
| 15-01256_017 | Batch 13(5) | Buzz Apiaries | Kānuka | - | None | - | - | - | - | - |
| 15-01256_018 | Batch 13(6) | Buzz Apiaries | Kānuka | - | None | - | - | - | - | - |
| 15-01256_019 | Batch 15 (1) | Buzz Apiaries | Kānuka | Mānuka | Training | 280 | 31 | 2.8 | 2 | 151 |
| 15-01256_020 | Batch 15 (2) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 141 |
| 15-01256_021 | Batch 15 (3) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 138 |
| 15-01256_022 | Batch 15 (4) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 139 |
| 15-01256_023 | Batch 15 (5) | Buzz Apiaries | Kānuka | - | None | - | - | - | - | - |
| 15-01256_024 | Batch 15 (6) | Buzz Apiaries | Kānuka | - | None | - | - | - | - | - |
| 15-01256_025 | Batch 14 (1) | Buzz Apiaries | Kānuka | Kānuka | Training | 31 | 10 | 1.4 | 2 | 31 |
| 15-01256_026 | Batch 14 (2) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 27 |
| 15-01256_027 | Batch 14 (3) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 26 |
| 15-01256_028 | Batch 14 (4) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 26 |
| 15-01256_029 | Batch 14 (5) | Buzz Apiaries | Kānuka | - | None | - | - | - | - | - |
| 15-01256_030 | Batch 14 (6) | Buzz Apiaries | Kānuka | - | None | - | - | - | - | - |
| 15-01256_031 | Batch 16 (1) | Buzz Apiaries | Kānuka | Kānuka | Training | 60 | 12 | 1.6 | 3 | 34 |
| 15-01256_032 | Batch 16 (2) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 38 |
| 15-01256_033 | Batch 16 (3) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 36 |
| 15-01256_034 | Batch 16 (4) | Buzz Apiaries | Kānuka | Kānuka | Test | - | - | - | - | 43 |
| 15-01256_035 | Batch 16 (5) | Buzz Apiaries | Kānuka | - | None | - | - | - | - | - |

| Lab ID | Client Sample ID | Client | Supplied Floral Type | Calculated Floral Type | Usage | DHA [mg/kg] | MGO [mg/kg] | NPA [equiv. [% phenol] | HMF [mg/kg] | Leptosperin [mg/kg] |
|---|---|---|---|---|---|---|---|---|---|---|
| 15-01256_036 | Batch 16 (6) | Buzz Apiaries | Kānuka | - | None | - | - | - | - | - |
| 15-10252_001 | 2012 Batch 8 Drum 1 | Buzz Apiaries | Kānuka | Kānuka | Training | <10 | 15 | 1.8 | 20 | 36 |
| 15-10252_002 | 2012 Batch 5 Drum 3 | Buzz Apiaries | Kānuka | Kānuka | Test | <10 | 4 | 0.8 | 18 | <20 |
| 15-10252_003 | 2013 Batch 5 Drum 2 | Buzz Apiaries | Kānuka | Kānuka | Training | 24 | 28 | 2.6 | 15 | 53 |
| 15-10252_004 | 2013 Batch 9 Drum 7 | Buzz Apiaries | Kānuka | Kānuka | Training | 33 | 31 | 2.7 | 15 | 77 |
| 15-10252_005 | 2013 Batch 11 Drum 4 | Buzz Apiaries | Kānuka | Kānuka | Training | 70 | 50 | 3.7 | 13 | 86 |
| 15-10252_006 | 2013 Batch 15 Drum 1 | Buzz Apiaries | Kānuka | Kānuka | Training | 81 | 58 | 4 | 13 | 138 |
| 15-10252_007 | 2014 Batch 13 Drum 4 | Buzz Apiaries | Kānuka | Kānuka | Training | <10 | 5 | 0.9 | 8 | <20 |
| 15-10252_008 | 2014 Batch 14 Drum 2 | Buzz Apiaries | Kānuka | Kānuka | Training | <10 | <4 | <0.8 | 10 | <20 |
| 15-10252_009 | 2015 Batch 14 | Buzz Apiaries | Kānuka | Kānuka | Training | <10 | 15 | 1.8 | 3 | 31 |
| 15-10252_010 | 2015 Batch 15 | Buzz Apiaries | Kānuka | Kānuka | Training | 216 | 47 | 3.6 | 3 | 148 |
| 15-10252_011 | 2015 Batch 18 | Buzz Apiaries | Kānuka | Kānuka | Training | <10 | <4 | <0.8 | 4 | <20 |
| 15-10252_012 | 2015 Buzz/Lee Batch 6 | Buzz Apiaries | Kānuka | Kānuka | Training | 201 | 59 | 4.1 | 1 | 99 |
| 15-10252_013 | 2015 Buzz/Lee Batch 8 | Buzz Apiaries | Kānuka | Kānuka | Training | 135 | 38 | 3.1 | 2 | 75 |
| 15-10252_014 | 2015 Buzz/Lee Batch 9 | Buzz Apiaries | Kānuka | Kānuka | Training | 184 | 46 | 3.5 | 3 | 125 |
| 15-11527_001 | BUZZ batch 4   8.2.12  TA24.1 | Honeylab | Kānuka | Kānuka | Training | 75 | 55 | 3.9 | 29 | 99 |
| 15-11527_002 | BUZZ batch 10  23.2.12  TA27.1 | Honeylab | Kānuka | Kānuka | Training | <10 | 7 | 1.2 | 18 | 27 |
| 15-11527_003 | BUZZ batch 12  22.2.13 TA19.1 | Honeylab | Kānuka | Kānuka | Training | <10 | <4 | <0.8 | 17 | 20 |
| 15-11527_004 | BUZZ batch 13  22.2.12  TA29.9 | Honeylab | Kānuka | Kānuka | Training | <10 | 12 | 1.5 | 24 | 35 |
| 15-11527_005 | BUZZ batch 11  26.2.12  TA23.5 | Honeylab | Kānuka | Kānuka | Training | <10 | 13 | 1.7 | 18 | 35 |
| 15-11527_006 | BUZZ batch 8   20.2.13  TA12.5 | Honeylab | Kānuka | Kānuka | Training | <10 | 9 | 1.3 | 30 | 27 |
| 15-11527_007 | BUZZ batch 9   9.2.12   TA27.2 | Honeylab | Kānuka | Kānuka | Training | <10 | 17 | 1.9 | 30 | 44 |
| 15-11527_008 | BUZZ batch 5   15.2.12  TA24.9 | Honeylab | Kānuka | Kānuka | Training | <10 | 8 | 1.2 | 18 | 27 |
| 15-11527_009 | BUZZ batch 15  17.2.12  TA26.5 | Honeylab | Kānuka | Kānuka | Training | <10 | 16 | 1.8 | 24 | 43 |
| 15-11527_010 | BUZZ batch 12  21.2.13  TA19.1 | Honeylab | Kānuka | Kānuka | Training | <10 | 4 | 0.9 | 24 | 22 |
| 15-11527_011 | BUZZ batch 7   10.2.12  TA28.4 | Honeylab | Kānuka | Kānuka | Training | <10 | 22 | 2.3 | 24 | 56 |
| 15-11527_012 | BUZZ batch 17  17.2.12  TA30 | Honeylab | Kānuka | Kānuka | Training | <10 | 33 | 2.9 | 30 | 68 |
| 15-11527_013 | BUZZ batch 6   10.2.12  TA29.2 | Honeylab | Kānuka | Kānuka | Training | <10 | 22 | 2.3 | 22 | 54 |
| 15-11527_014 | BUZZ batch 12  20.2.12  TA23.7 | Honeylab | Kānuka | Mānuka | Training | 158 | 127 | 6.5 | 35 | 164 |

| Lab ID | Client Sample ID | Client | Supplied Floral Type | Calculated Floral Type | Usage | DHA [mg/kg] | MGO [mg/kg] | NPA [equiv. [% phenol] | HMF [mg/kg] | Leptosperin [mg/kg] |
|---|---|---|---|---|---|---|---|---|---|---|
| 15-11527_015 | BUZZ batch 16  23.2.12  TA27.2 | Honeylab | Kānuka | Kānuka | Training | <10 | 25 | 2.4 | 17 | 67 |
| 15-11527_016 | BUZZ batch 14  14.2.12  TA30.8 | Honeylab | Kānuka | Kānuka | Training | <10 | 17 | 1.9 | 22 | 51 |
| 15-11527_017 | BUZZ batch 11  20.2.12  TA25.5 | Honeylab | Kānuka | Kānuka | Training | 71 | 70 | 4.5 | 34 | 110 |
| 15-11527_018 | TAHUNA batch 5  3.4.14  TA23.5 | Honeylab | Kānuka | Kānuka | Training | <10 | 8 | 1.2 | 18 | 26 |
| 15-11527_019 | TAHUNA batch 6  7.4.14  TA21.9 | Honeylab | Kānuka | Kānuka | Training | <10 | 5 | 1.0 | 18 | 22 |
| 15-14528_001 | Hamilton A | Honeylab | Unknown | - | None | - | - | - | - | - |
| 15-14528_002 | Hamilton B | Honeylab | Unknown | - | None | - | - | - | - | - |
| 15-14528_003 | Akaroa A | Honeylab | Unknown | - | None | - | - | - | - | - |
| 15-14528_004 | Akaroa B | Honeylab | Unknown | - | None | - | - | - | - | - |
| 15-14528_005 | Southland A | Honeylab | Unknown | - | None | - | - | - | - | - |
| 15-14528_006 | Southland B | Honeylab | Unknown | - | None | - | - | - | - | - |
| 16-03255_001 | TAHUNA 166 D2 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | <4 | <0.8 | 6 | <20 |
| 16-03255_002 | TAHUNA 168 D5 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 4 | 0.8 | 6 | <20 |
| 16-03255_003 | TAHUNA 167 D3 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | <4 | <0.8 | 5 | <20 |
| 16-03255_004 | BUZZ 1619 D3 | Bee NZ Ltd | Kānuka | Kānuka | Training | 16 | 13 | 1.7 | 6 | 37 |
| 16-03255_005 | BUZZ 1620 D2 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 7 | 1.1 | 5 | 22 |
| 16-03255_006 | BUZZ 1615 D2 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 6 | 1.0 | 5 | <20 |
| 16-03369_001 | BUZZ 1615 D4 | Bee NZ Ltd | Kānuka | Kānuka | Test | <10 | 7 | 1.1 | 6 | 30 |
| 16-03369_002 | BUZZ 1614 D5 | Bee NZ Ltd | Kānuka | Kānuka | Training | 66 | 27 | 2.6 | 7 | 78 |
| 16-03369_003 | BUZZ 1613 D2 | Bee NZ Ltd | Kānuka | Kānuka | Training | 20 | 19 | 2.0 | 5 | 61 |
| 16-03455_001 | Kcaltd B4 D32 | Bee NZ Ltd | NotKānuka | Mānuka | Training | 803 | 113 | 6.0 | 4 | 231 |
| 16-03455_002 | Kcaltd B6 D11 | Bee NZ Ltd | NotKānuka | Other | Training | 159 | 27 | 2.5 | 3 | 59 |
| 16-03455_003 | Kcaltd BBSTU D40 | Bee NZ Ltd | NotKānuka | Mānuka | Training | 425 | 92 | 5.3 | 6 | 167 |
| 16-03455_004 | Kcaltd B5 D25 | Bee NZ Ltd | NotKānuka | Other | Training | 224 | 35 | 3.0 | 4 | 61 |
| 16-03455_005 | Kcaltd B2 D17 | Bee NZ Ltd | NotKānuka | Kānuka | Training | 163 | 32 | 2.8 | 5 | 57 |
| 16-03455_006 | Kcaltd B1 D21 | Bee NZ Ltd | NotKānuka | Other | Training | 643 | 88 | 5.2 | 6 | 141 |
| 16-03455_007 | Kcaltd B3 D36 | Bee NZ Ltd | NotKānuka | Other | Training | 428 | 71 | 4.6 | 4 | 135 |
| 16-03455_008 | Kcaltd B7 D29 | Bee NZ Ltd | NotKānuka | Kānuka | Training | 51 | 11 | 1.5 | 4 | 24 |
| 16-06487_002 | Airborne Rātā | Analytica | Rātā | Other | Training | 43 | 23 | 2.3 | 10 | 37 |

| Lab ID | Client Sample ID | Client | Supplied Floral Type | Calculated Floral Type | Usage | DHA [mg/kg] | MGO [mg/kg] | NPA [equiv. [% phenol] | HMF [mg/kg] | Leptosperin [mg/kg] |
|---|---|---|---|---|---|---|---|---|---|---|
| 16-06487_003 | Hauora Blue Borage | Analytica | Borage | Other | Training | <10 | <4 | <0.8 | 42 | <20 |
| 16-06487_004 | Hauora Rewarewa | Analytica | Rewarewa | Kānuka | Training | <10 | 8 | 1.2 | 14 | <20 |
| 16-06487_006 | Nz Honey Co Clover | Analytica | Clover | Other | Training | <10 | <4 | <0.8 | 7 | <20 |
| 16-06487_007 | NZ Honey Co Kamahi | Analytica | Kamahi | Other | Training | <10 | 5 | 1.0 | 9 | <20 |
| 16-06487_008 | NZ Honey Co Rātā | Analytica | Rātā | Other | Training | 110 | 26 | 2.5 | 6 | 55 |
| 16-06487_010 | NZ Honey Co Honeydew | Analytica | Honeydew | Other | Training | <10 | <4 | <0.8 | 9 | <20 |
| 16-06487_011 | Happy Valley Tawari | Analytica | Tawari | Other | Training | <10 | 11 | 1.4 | 9 | <20 |
| 16-06487_013 | Cammels Rewarewa | Analytica | Rewarewa | Kānuka | Training | <10 | 33 | 2.9 | 68 | 43 |
| 16-06487_014 | Cammels Pohutukawa | Analytica | Pohutukawa | Kānuka | Training | <10 | 16 | 1.9 | 91 | <20 |
| 16-06487_016 | Mānuka Health Tawari | Analytica | Tawari | Other | Training | <10 | 8 | 1.3 | 17 | <20 |
| 16-06487_017 | Mānuka Health Rewarewa | Analytica | Rewarewa | Kānuka | Training | 35 | 42 | 3.3 | 17 | 50 |
| 16-06487_018 | Mānuka Health Kamahi | Analytica | Kamahi | Other | Training | <10 | 16 | 1.9 | 14 | <20 |
| 16-06487_019 | Mānuka Health Clover | Analytica | Clover | Other | Training | 17 | 28 | 2.6 | 31 | <20 |
| 16-06487_021 | Watson and Sons Kamahi | Analytica | Kamahi | Other | Training | <10 | 13 | 1.6 | 12 | <20 |
| 16-06487_022 | Watson and Sons Rewarewa | Analytica | Rewarewa | Other | Training | 108 | 121 | 6.3 | 20 | 105 |
| 16-06487_024 | Airborne Tawari | Analytica | Tawari | Other | Training | <10 | 13 | 1.6 | 3 | <20 |
| 16-06487_038 | Clear Skys Clover | Analytica | Clover | Kānuka | Training | <10 | <4 | <0.8 | 16 | <20 |
| 16-06487_039 | Clear Skys Rātā | Analytica | Rātā | Other | Training | 11 | 12 | 1.6 | 5 | <20 |
| 16-06487_054 | Arataki Manuka 5+ | Analytica | Mānuka | Mānuka | Training | 29 | 142 | 6.9 | 73 | 151 |
| 16-06487_055 | Arataki Manuka 10+ 4181 | Analytica | Mānuka | Mānuka | Training | 125 | 290 | 10.6 | 61 | 198 |
| 16-06487_056 | Arataki Manuka 15+ 8928 | Analytica | Mānuka | Mānuka | Training | 689 | 506 | 14.8 | 22 | 405 |
| 16-06487_057 | Airborne Manuka 162641 | Analytica | Mānuka | Mānuka | Training | 138 | 47 | 3.6 | 7 | 170 |
| 16-06487_058 | Mossops Manuka 160622 | Analytica | Mānuka | Other | Training | 170 | 112 | 6.0 | 27 | 89 |
| 16-06487_059 | Mossops Manuka 5+ 160419 | Analytica | Mānuka | Mānuka | Training | 453 | 262 | 10 | 22 | 238 |
| 16-06487_062 | Honey Centre Mānuka | Analytica | Mānuka | Kānuka | Training | 24 | 45 | 3.4 | 21 | 63 |
| 16-06487_066 | 100% Pure New Zealand Thyme | Analytica | Thyme | Other | Training | <10 | <4 | <0.8 | 3 | <20 |
| 16-06487_067 | 100% Pure New Zealand Rewarewa | Analytica | Rewarewa | Other | Training | <10 | 18 | 2.0 | 19 | <20 |
| 16-06487_068 | 100% Pure New Zealand Kamahi | Analytica | Kamahi | Other | Training | <10 | 25 | 2.5 | 7 | <20 |
| 16-06487_069 | 100% Pure New Zealand Clover | Analytica | Clover | Other | Training | <10 | 15 | 1.8 | 13 | <20 |

| Lab ID | Client Sample ID | Client | Supplied Floral Type | Calculated Floral Type | Usage | DHA [mg/kg] | MGO [mg/kg] | NPA [equiv. [% phenol] | HMF [mg/kg] | Leptosperin [mg/kg] |
|---|---|---|---|---|---|---|---|---|---|---|
| 16-06487_070 | 100% Pure New Zealand Tawari | Analytica | Tawari | Other | Training | <10 | 12 | 1.6 | 13 | <20 |
| 16-06487_071 | 100% Pure New Zealand Rata | Analytica | Rātā | Other | Training | <10 | 18 | 2.0 | 25 | <20 |
| 16-06487_072 | 100% Pure New Zealand Manuka UMF 10+ | Analytica | Mānuka | Mānuka | Training | 873 | 390 | 12.7 | 27 | 385 |
| 16-06487-037 | ClearSkys Kamahi | Analytica | Kamahi | Other | Test | - | - | - | - | <20 |
| 16-06487-040 | Clear Skys Thyme | Analytica | Thyme | Other | Test | - | - | - | - | <20 |
| 16-06487-041 | ClearSkys Rewarewa | Analytica | Rewarewa | Other | Test | - | - | - | - | <20 |
| 16-06487-046 | J. Friend White Clover | Analytica | Clover | Other | Test | - | - | - | - | <20 |
| 16-06487-047 | J. Friend Northern Rata | Analytica | Rātā | Other | Test | - | - | - | - | <20 |
| 16-06487-074 | Nz Honey Co. Manuka 10+ | Analytica | Mānuka | Mānuka | Test | - | - | - | - | 465 |
| 16-06487-075 | NZ Honey Co. Manuka 5+ | Analytica | Mānuka | Mānuka | Test | - | - | - | - | 251 |
| 16-06487-076 | Melita Manuka 10+ | Analytica | Mānuka | Mānuka | Test | - | - | - | - | 301 |
| 16-06487-079 | Manawa Manuka 5+ | Analytica | Mānuka | Mānuka | Test | - | - | - | - | 158 |
| 16-06487-080 | Manawa Rewarewa | Analytica | Rewarewa | Other | Test | - | - | - | - | 88 |
| 16-09614_001 | 1503 Kaikoura | T. Murray | Kānuka | Mānuka | Training | 253 | 111 | 5.9 | 4 | 313 |
| 16-09614_002 | 1507 Kaikoura | T. Murray | Kānuka | Mānuka | Training | 327 | 136 | 6.7 | 5 | 383 |
| 16-09614_003 | 1527 Kaikoura | T. Murray | Kānuka | Mānuka | Training | 374 | 167 | 7.6 | 3 | 426 |
| 16-09614_004 | 1604 Kaikoura | T. Murray | Kānuka | Mānuka | Training | 124 | 34 | 2.9 | 3 | 155 |
| 16-09614_005 | 1615 Kaikoura | T. Murray | Kānuka | Other | Training | 99 | 37 | 3.1 | <1 | 134 |
| 16-09614_006 | 1616 Kaikoura | T. Murray | Kānuka | Other | Training | 134 | 43 | 3.4 | 1 | 146 |
| 16-09616_001 | 160058 No. 1 Waipu Northland | D. Herbert | Kānuka | Kānuka | Training | 51 | 20 | 2.1 | 5 | 47 |
| 16-09616_002 | 160058 No. 2 Waipu Northland | D. Herbert | Kānuka | Kānuka | Training | 12 | 9 | 1.3 | 6 | <20 |
| 16-09616_003 | 160058 No. 3 Waipu Northland | D. Herbert | Kānuka | Kānuka | Training | <10 | 6 | 1.0 | 4 | <20 |
| 16-09616_004 | 160073 No 1 Waipu Northland | D. Herbert | Kānuka | Kānuka | Training | <10 | 9 | 1.3 | 6 | <20 |
| 16-09616_005 | 160073 No 2 Waipu Northland | D. Herbert | Kānuka | Kānuka | Training | <10 | 5 | 0.9 | 6 | <20 |
| 16-09772_001 | BUZZ B1619 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 11 | 1.4 | 5 | 30 |
| 16-09772_002 | HARVEY B1615 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | <4 | <0.8 | 4 | <20 |
| 16-09772_003 | MENEFY B4 | Bee NZ Ltd | Kānuka | Other | Training | 190 | 33 | 2.9 | 3 | 95 |
| 16-09772_004 | PINACLE B165 | Bee NZ Ltd | Kānuka | Kānuka | Training | 34 | 10 | 1.4 | 7 | 30 |
| 16-09772_005 | BARTLEM B1415 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 7 | 1.2 | 7 | <20 |

| Lab ID | Client Sample ID | Client | Supplied Floral Type | Calculated Floral Type | Usage | DHA [mg/kg] | MGO [mg/kg] | NPA [equiv. [% phenol] | HMF [mg/kg] | Leptosperin [mg/kg] |
|--------|------------------|--------|---------------------|------------------------|-------|-------------|-------------|------------------------|-------------|---------------------|
| 16-10019_001 | Tahuna B162-D4 | Bee NZ Ltd | Kānuka | Kānuka | Training | 98 | 21 | 2.2 | 5 | 70 |
| 16-10019_002 | Tahuna B166-D6 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 5 | 0.9 | 5 | <20 |
| 16-10019_003 | Tahuna B167-D3 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 4 | 0.8 | 4 | <20 |
| 16-10019_004 | Tahuna B168-D6 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 8 | 1.2 | 4 | 27 |
| 16-10019_005 | Tahuna B169-D2 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 5 | 0.9 | 6 | <20 |
| 16-10019_006 | Tahuna B1610-D2 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 10 | 1.4 | 6 | 30 |
| 16-10019_007 | Tahuna B1611-D2 | Bee NZ Ltd | Kānuka | Other | Training | <10 | 5 | 1 | 8 | <20 |
| 16-10019_008 | Tahuna B1612-D5 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 5 | 0.9 | 7 | <20 |
| 16-10019_009 | Tahuna B1613-D5 | Bee NZ Ltd | Kānuka | Other | Training | <10 | 0 | <0.8 | 6 | <20 |
| 16-10019_010 | Tahuna B1614-D3 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 8 | 1.2 | 7 | <20 |
| 16-10019_011 | Tahuna B1615-D6 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 7 | 1.1 | 10 | 21 |
| 16-10379_010 | Composite 25 | Analytica | Mānuka | Mānuka | Training | 1770 | 637 | 17.1 | 11 | 761 |
| 16-10379_011 | Composite 26 | Analytica | Mānuka | Mānuka | Training | 1990 | 685 | 17.8 | 11 | 693 |
| 16-10379_012 | Composite 27 | Analytica | Mānuka | Mānuka | Training | 1670 | 634 | 17 | 18 | 754 |
| 16-10379_013 | Composite 28 | Analytica | Mānuka | Mānuka | Training | 1750 | 622 | 16.8 | 12 | 597 |
| 16-10379_014 | Composite 29 | Analytica | Mānuka | Mānuka | Training | 1290 | 691 | 17.9 | 20 | 600 |
| 16-10379_015 | Composite 30 | Analytica | Mānuka | Mānuka | Training | 2100 | 791 | 19.4 | 13 | 990 |
| 16-10379_016 | Composite 31 | Analytica | Mānuka | Mānuka | Training | 1810 | 749 | 18.8 | 19 | 770 |
| 16-10379_017 | Composite 32 | Analytica | Mānuka | Mānuka | Training | 811 | 820 | 19.9 | 34 | 605 |
| 16-10379_018 | Composite 33 | Analytica | Mānuka | Mānuka | Training | 1200 | 715 | 18.3 | 27 | 575 |
| 16-10379_019 | Composite 34 | Analytica | Mānuka | Mānuka | Training | 1480 | 525 | 15.2 | 23 | 801 |
| 16-10379_020 | Composite 35 | Analytica | Mānuka | Mānuka | Training | 1540 | 668 | 17.6 | 23 | 519 |
| 16-10379_000 | Composite 40 | Analytica | Mānuka | Mānuka | Training | 1920 | 351 | 11.9 | 4 | 651 |
| 16-10411_001 | B169 D3 Harvey | Bee NZ Ltd | Kānuka | Kānuka | Training | 214 | 59 | 4.1 | 5 | 80 |
| 16-10411_002 | B1610 D2 Harvey | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 11 | 1.5 | 6 | <20 |
| 16-10411_003 | B1616 D6 Harvey | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 0 | <0.8 | 2 | <20 |
| 16-10411_004 | B1616 D11 Harvey | Bee NZ Ltd | Kānuka | Kānuka | Test | <10 | 0 | <0.8 | 4 | <20 |
| 16-10411_005 | B1616 D15 Harvey | Bee NZ Ltd | Kānuka | Kānuka | Test | <10 | 6 | 1 | 2 | <20 |
| 16-10791_001 | P1073 McDonald South Island Oamaru | Bee NZ Ltd | Kānuka | Kānuka | Training | 109 | 22 | 2.3 | 1 | 73 |

| Lab ID | Client Sample ID | Client | Supplied Floral Type | Calculated Floral Type | Usage | DHA [mg/kg] | MGO [mg/kg] | NPA [equiv. [% phenol] | HMF [mg/kg] | Leptosperin [mg/kg] |
|--------|------------------|--------|---------------------|------------------------|-------|-------------|-------------|------------------------|-------------|---------------------|
| 16-11354_001 | D15 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 8 | 1.2 | 3 | <20 |
| 16-11354_002 | D16 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 5 | 0.9 | 3 | <20 |
| 16-11354_003 | D17 | Bee NZ Ltd | Kānuka | Kānuka | Training | <10 | 5 | 0.9 | 5 | <20 |
| 16-11721-001 | SGA3 16-K-1 (Raglan Harbour) | SummerGlow | Kānuka | Kānuka | Training | <10 | 8 | 1.2 | 3 | <20 |
| 16-11721-002 | SGA3 16-K-2 (Raglan Harbour) | SummerGlow | Kānuka | Kānuka | Training | <10 | 14 | 1.7 | 4 | <20 |
| 16-11721-003 | SGA3 16-K-3 (Raglan Harbour) | SummerGlow | Kānuka | Kānuka | Test | <10 | 8 | 1.2 | 3 | <20 |
| 16-11721-004 | SGA3 16-K-4 (Raglan Harbour) | SummerGlow | Kānuka | Kānuka | Test | <10 | 6 | 1.1 | 6 | <20 |

## 9.5    INVESTIGATOR BROCHURE FOR VIRABAN COLD SORE CREAM

**Viraban Cold Sore Cream**

Aciclovir 5% w/w

**What is in this leaflet**
Please read this leaflet carefully before you start to use your medicine. It answers some common questions about Viraban cream.
Keep the leaflet with the medicine.
You may need to read it again.
This leaflet only applies to Viraban cream.

**What the Viraban cold sore cream is used for and how it works**
Your medicine is called Viraban cold sore cream. The active ingredient is aciclovir. Aciclovir belongs to the class of medicines called antivirals. Antivirals are used to treat infections caused by viruses. Viraban cream is used to treat the symptoms of cold sores. Viraban cream may also be used for other conditions as determined by your doctor.

**Advice before using Viraban cold sore cream**
Make sure it is safe for you to use Viraban cream. Read the following questions carefully.

Have you previously had an allergic reaction to aciclovir or any other ingredient such as polyethylene glycol? (An allergic reaction may include rash, itching, swelling or breathing difficulties).
Are you pregnant or intend to become pregnant?
Are you currently breast-feeding?
Are you taking any other medicine, especially any other preparation that is to be applied to the same area of the skin?
Do you have any condition that might weaken your immune system? e.g. HIV infection, a bone marrow transplant or cancer treatment?

If you answer YES to any of the above questions or are not sure, do not start treatment. Talk to your doctor or pharmacist first.

**How to use the Viraban cold sore cream properly**
Use your medicine as instructed by your doctor or pharmacist. Apply a thin layer of Viraban cream to the affected area(s), five times a day for five days. Use Viraban cream as soon as the first symptoms of a cold sore appear, such as burning, tingling or itching. You should wash your hands before and after applying the cream, and avoid unnecessary rubbing of the lesions or touching them with a towel, to avoid aggravating or transferring the infection. Special care should be taken to avoid contact of Viraban Cream with your eye. If you get some cream in your eyes, wash it out with cool water. If any irritation persists, contact your doctor.
Do not use Viraban cream inside the mouth or vagina; it may irritate.
Do not mix Viraban cream with any other cream or lotion.
It is important to continue using Viraban cream for the full time of treatment (five days), even if your symptoms begin to clear up after a few days.

Do not forget to use the cream. If you do forget to use the cream at any time, use it as soon as you remember. However, if it is almost time for cream to be used, skip the missed dose and go back to your normal schedule.
Do not use Viraban cream more often or for a longer time than recommended.

This medicine is for you. Never give it to others. It may harm them, even if their symptoms are the same as yours.

### Side effects
Viraban cream can occasionally cause mild pain, drying, flaking, burning or stinging at the area where it is applied. These side effects are usually mild. Check with your doctor or pharmacist if they continue or, are intense or bothersome.
Less commonly, itching and rarely skin rash may occur. Consult your doctor if the effects persist or are bothersome. Other side effects that are not listed above may also occur in some patients. If you notice any other side effects, check with your doctor or pharmacist. If your symptoms do not improve within 10 days or if, they become worse, check with your doctor or pharmacist.

### Storage conditions
Store below 25°C.

DO NOT USE IN EYES
FOR EXTERNAL USE ONLY
Keep your medicine out of the reach of children.

Do not keep/use this medicine when no longer needed or after its expiry date.

### List of other ingredients
Besides aciclovir, Viraban cold sore cream also contains PEG-5-glyceryl stearate, dimeticone, cetyl alcohol, liquid paraffin, white soft paraffin and propylene glycol as inactive ingredients.

### If you want to know more
If you are concerned about any aspect of your treatment with Viraban cold sore cream, talk to your doctor or pharmacist.

Viraban Cream is made by Pinewood Healthcare, Ballymacarbry, Clonmel,
Co. Tipperary, Ireland.
Distributed in New Zealand by AFT Pharmaceuticals, Auckland, New Zealand.
Ph 09 4880232      Fax 09 4880234

Medicine classification: General Sales Medicine

Date of last revision: September 2006

## 9.6 PARTICIPANT INFORMATION SHEET AND CONSENT FORM

**PARTICIPANT INFORMATION SHEET**

### 5% Aciclovir or Honevo™ as a treatment for cold sores (KH10)

Thank you for considering taking part in this study of cold sore treatment, sponsored by HoneyLab®. This information sheet provides you with summary information about the study to allow you to decide whether to take part. Please ask the pharmacists any questions at any time. Participation in this study is free and completely your choice. If you decide to take part your pharmacist will need to run through some questions to ensure you are eligible to take part (box 1). You may withdraw from the study at any time without this affecting your healthcare. This study has been approved by the XXX Heath and Disability Ethics Committee (reference: XX/XXX/XX).

| ✓ | To take part in this study you must: |
|---|---|
| ✓ | • Be aged 16 or over |
| ✓ | • Require treatment for an active coldsore |
| ✓ | • Have coldsore symptoms that have started over the past 72 hours |
| ✗ | **You will will not be able to take in the study if:** |
| ✓ | • You are pregnant or breastfeeding |
| ✓ | • You are allergic to honey glycerin or aciclovir |
| ✓ | • You have used oral aciclovir or similar for the current cold sore |
| ✓ | • You are planning to use medicine that may affect your cold sore during your participation in the study |
| ✓ | • You have any condition that presents a safety risk or may impact the study results |

A cold sore is a painful condition caused by the *Herpes Simplex Virus* that often recurs in many people. Current treatment, in the form of aciclovir cream (e.g. Viraban), only seems to shorten cold sore symptoms by half a day. Honey has been shown in a previous study to improve healing times and pain in *Herpes Simplex infection* and this study aims to determine if medical grade (sterilised) Kanuka honey is more effective than aciclovir cream for the treatment of cold sores. 950 participants will be recruited in total to this study across New Zealand.

You will be allocated, by equal chance, to either the aciclovir cream treatment or the honey treatment. Both treatments need to be applied five times a day for 14 days or until the skin has completely healed – whichever is sooner. You will be asked to complete a diary to note down the:
- number of times you applied the treatment in the last 24 hours
- characteristics of the cold sore such as pain and appearance (according to given pictures).

We will give you a paper diary, but if you are able to connect to the internet you can complete the diary online (e-diary) instead on a computer or your smartphone. We will also send you a daily text message reminding you to apply the treatment and fill in the diary. On or around 14 days after you start the study you will receive a follow-up phone call from study Investigators at Optimal Clinical Trials.

You will be required not to use any medication or natural treatments for you cold sore during the study, or if you do to make a note of them and inform us in the follow up phone call.

Honey and aciclovir cream may cause mild pain, stinging and drying/flaking of skin. If you have a known allergy to aciclovir, honey or glycerine, you cannot take part in this study. In the unlikely event of a reaction to your treatment, or you require medical advice please see your regular medical practicioner. The study investigators may need to contact your usual healthcare practicioner if an adverse event occurs during the study for the purposes of follow up.

The treatments are provided free of charge and you will be reimbursed $25 by cheque for your time at the end of the study, on receipt of your paper diary/completion of the online ediary.

Your personal information will be securely held by pharmacy staff, researchers at the Medical Research Institute of New Zealand (Co-ordinating Centre) and Optimal Clinical Trials (Follow-up Centre), for study analysis and follow-up purposes. All documentation will be held for at least 15 years then destroyed. You will not be identifiable in any published study results and may receive a copy of these if you wish. A regulatory authority may access your medical records in relation to the study – solely for audit purposes to check the study has been run properly. Investigators will contact you if new information is discovered during the study which may affect your participation.

Although unlikely, if injury was to occur as a result of the study the ACC will not provide compensation. HoneyLab® will instead offer compensation as per pharmaceutical industry guidelines (these can be provided for your information).  If you wish to take part, please complete and sign the study Consent Form.

If you have any questions, concerns or complaints about the study at any stage, you can contact:

Name:                          Alex Semprini

Telephone number:    04 805 0232

Email:                         coldsore@mrinz.ac.nz

Or you can contact an independent health and disability advocate on:

Phone:                       0800 555 050
Fax:                           0800 2 SUPPORT (0800 2787 7678)
Email:                         advocacy@hdc.org.nz

You can also contact the <<insert committee>> Health and Disability Ethics Committee on:

Phone:                       0800 4 ETHICS (0800 438 442)

Email:                        hdecs@moh.govt.nz

Māori  support number – <<insert number here>>

# PARTICIPANT CONSENT FORM

## Study Title: 5% Aciclovir or Honevo™ as a treatment for cold sores (KH10)

**Participant Screening Number:** [ ][ ][ ]

### Please tick to indicate you consent to the following:

| | |
|---|---|
| I have read and understood the Participant Information Sheet | |
| I understand I may use a legal representative, whānau/ family support or a friend to help me ask questions and understand the study having read the detailed leaflet | |
| I am satisfied with the answers I have been given regarding the study and I have a copy of this consent form and participant information sheet | |
| I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care | |
| I consent to research staff (from the Pharmacy, Medical Research Institute of New Zealand and Optimal Clinical Trials) collecting and processing my information, including information about my health | |
| If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed | |
| I agree to an approved auditor appointed by the New Zealand Health and Disability Ethic Committees, or any relevant regulatory authority or their approved representative reviewing my relevant medical records for the sole purpose of checking the accuracy of the information recorded for the study | |
| I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study | |
| I understand the compensation provisions in case of injury during the study | |
| I understand my responsibilities as a study participant | |
| I know who to contact if I have any questions about the study in general | |
| I give consent for my phone number to be used by study staff to receive a free daily text message reminder to complete the study diary | |
| I give consent for my GP or usual health care provider to be informed of my participation and contacted should it be necessary to follow up for safety reasons | |

| | YES | NO |
|---|---|---|
| I would like to receive a copy of the study results via post | | |

## *Declaration by participant:*

I hereby consent to take part in this study

_____

Participant name (print)

_____          _____

Participant signature                                                    Date

## Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate

_____

Name of person conducting informed consent discussion (print)

_____          _____

Signature of person conducting informed consent discussion          Date

## 9.7    PAPER BACK-UP STUDY DIARY

**MEDICAL RESEARCH INSTITUTE OF NEW ZEALAND**

*5% Aciclovir or Honevo™ as a treatment for cold sores*
*(KH10)*

**Subject Diary**

Subject ID:

Subject Initials:

Please use this diary when unable to access the online
eDiary

Please return this diary after your Day 14 follow up call

| | |
|---|---|
| **Study contact person:** | **Alex Semprini** |
| Phone: | 04 805-0260 |
| E-mail: | coldsore@mrinz.ac.nz |

## Instructions:

① Please apply your provided cream to the affected area *five times daily*

② Please record how painful the cold sore has been over the previous 24 hours

③ Please record the stage of the cold sore as per the supplied chart

④ Please remember not to use any other cold sore treatments during this study but make a note if you do need to

⑤ Please remember to also complete the online diary if available to you

⑥ Please complete the diary after the final application of the day

| Stage 1 Prodrome | Initial onset of tingling and itching. No sore is present and skin appears normal. Not everyone gets these symptoms. |  |
|---|---|---|
| Stage 2 Redness | Skin has some redness and swelling. No blister. May itch or feel tender. |  |
| Stage 3 Small blister | Small blisters form in the affected area, clear at first turn yellow with time. Usually painful. |  |
| Stage 4 Ulcer | Completely formed cold sore with open areas where blisters were. Sore and painful. |  |
| Stage 5 Crust | After some days a flaky crust develops resembling a scab. |  |
| Stage 6 Drying up & Healing | Crust flakes off with healing skin underneath. Cold sore cycle is complete, maybe some residual redness of skin for a few days. |  |
| Stage 7 Healed—skin back to normal | Skin is entirely back to normal. |  |

**Day 1**   Date:___ /___ /___

| | |
|---|---|
| How painful has the cold sore been on average over last 24 hours?<br><br>0 no pain → 10 severe pain *(Circle answer)* | 0  1  2  3  4  5  6  7  8  9  10 |
| How many times have you applied treatment in the last 24 hours? | |
| What is the stage of the cold sore? (1-7) see photos | |

**Day 2**   Date:___ /___ /___

| | |
|---|---|
| How painful has the cold sore been on average over last 24 hours?<br><br>0 no pain → 10 severe pain *(Circle answer)* | 0  1  2  3  4  5  6  7  8  9  10 |
| How many times have you applied treatment in the last 24 hours? | |
| What is the stage of the cold sore? (1-7) see photos | |

**Day 3**   Date:___ /___ /___

| | |
|---|---|
| How painful has the cold sore been on average over last 24 hours?<br><br>0 no pain → 10 severe pain *(Circle answer)* | 0  1  2  3  4  5  6  7  8  9  10 |
| How many times have you applied treatment in the last 24 hours? | |
| What is the stage of the cold sore? (1-7) see photos | |

KH10                Subject Diary V1                05 May 2015

**Day 4**     Date:___ /___ /___

| How painful has the cold sore been on average over last 24 hours?<br><br>0 no pain → 10 severe pain *(Circle answer)* | 0  1  2  3  4  5  6  7  8  9  10 |
|---|---|
| How many times have you applied treatment in the last 24 hours? | |
| What is the stage of the cold sore? (1-7) see photos | |

**Day 5**     Date:___ /___ /___

| How painful has the cold sore been on average over last 24 hours?<br><br>0 no pain → 10 severe pain *(Circle answer)* | 0  1  2  3  4  5  6  7  8  9  10 |
|---|---|
| How many times have you applied treatment in the last 24 hours? | |
| What is the stage of the cold sore? (1-7) see photos | |

**Day 6**     Date:___ /___ /___

| How painful has the cold sore been on average over last 24 hours?<br><br>0 no pain → 10 severe pain *(Circle answer)* | 0  1  2  3  4  5  6  7  8  9  10 |
|---|---|
| How many times have you applied treatment in the last 24 hours? | |
| What is the stage of the cold sore? (1-7) see photos | |

**Day 7**     Date:___ /___ /___

| | |
|---|---|
| How painful has the cold sore been on average over last 24 hours?<br><br>0 no pain → 10 severe pain *(Circle answer)* | 0 1 2 3 4 5 6 7 8 9 10 |
| How many times have you applied treatment in the last 24 hours? | |
| What is the stage of the cold sore? (1-7) see photos | |

**Day 8**     Date:___ /___ /___

| | |
|---|---|
| How painful has the cold sore been on average over last 24 hours?<br><br>0 no pain → 10 severe pain *(Circle answer)* | 0 1 2 3 4 5 6 7 8 9 10 |
| How many times have you applied treatment in the last 24 hours? | |
| What is the stage of the cold sore? (1-7) see photos | |

**Day 9**     Date:___ /___ /___

| | |
|---|---|
| How painful has the cold sore been on average over last 24 hours?<br><br>0 no pain → 10 severe pain *(Circle answer)* | 0 1 2 3 4 5 6 7 8 9 10 |
| How many times have you applied treatment in the last 24 hours? | |
| What is the stage of the cold sore? (1-7) see photos | |

**Day 10      Date:___ /___ /___**

| | |
|---|---|
| How painful has the cold sore been on average over last 24 hours?<br><br>0 no pain → 10 severe pain *(Circle answer)* | 0  1  2  3  4  5  6  7  8  9  10 |
| How many times have you applied treatment in the last 24 hours? | |
| What is the stage of the cold sore? (1-7) see photos | |

**Day 11      Date:___ /___ /___**

| | |
|---|---|
| How painful has the cold sore been on average over last 24 hours?<br><br>0 no pain → 10 severe pain *(Circle answer)* | 0  1  2  3  4  5  6  7  8  9  10 |
| How many times have you applied treatment in the last 24 hours? | |
| What is the stage of the cold sore? (1-7) see photos | |

**Day 12      Date:___ /___ /___**

| | |
|---|---|
| How painful has the cold sore been on average over last 24 hours?<br><br>0 no pain → 10 severe pain *(Circle answer)* | 0  1  2  3  4  5  6  7  8  9  10 |
| How many times have you applied treatment in the last 24 hours? | |
| What is the stage of the cold sore? (1-7) see photos | |

| Day 13 Date:___ /___ /___ | |
|---|---|
| How painful has the cold sore been on average over last 24 hours?<br><br>0 no pain → 10 severe pain *(Circle answer)* | 0 1 2 3 4 5 6 7 8 9 10 |
| How many times have you applied treatment in the last 24 hours? | |
| What is the stage of the cold sore? (1-7) see photos | |

| Day 14 Date:___ /___ /___ | |
|---|---|
| How painful has the cold sore been on average over last 24 hours?<br><br>0 no pain → 10 severe pain *(Circle answer)* | 0 1 2 3 4 5 6 7 8 9 10 |
| How many times have you applied treatment in the last 24 hours? | |
| What is the stage of the cold sore? (1-7) see photos | |

## 9.8    PHARMACY VISIT ONE WORKSHEET

MEDICAL RESEARCH
**INSTITUTE**
OF NEW ZEALAND

**Participant Initials:**

**Randomisation ID:**

**CONSENT FORM:**

*I_____ (investigator) confirm that this clinical trial was fully discussed with the participant, they were provided with the participant information sheet version _____ and had the opportunity to review this and ask questions about the study. Their written consent to participate was provided prior to the commencement of any study specific procedures.          Investigator signature:                         Date:*

**Date of Visit:**                                          **Time (24h):** [  ] : [  ]

Date and time of onset of symptoms for current cold sore (to nearest hour):

**Date**                                                    **Time (24h):** [  ] : [  ]

Current pain score of coldsore: 0 (no pain) to 10 (severe)

Current stage of coldsore from 1 – 7 (see diary staging chart):

**ASSESSMENT NARRATIVE**

**Medical History (e.g. allergies, pregnancy) – Please list any medical conditions below:**

_____
_____
_____

**Current Medications (e.g. antivirals, lysine supplements)–please list medications currently being taken below:**

_____
_____

KH10 MRINZ Visit 1 Worksheet Version 3.0, 08/06/2016                        Page **1** of **3**

**MEDICAL RESEARCH INSTITUTE OF NEW ZEALAND**

**Participant Initials:**    **Randomisation ID:**

## INCLUSION / EXCLUSION CRITERIA ASSESSMENT

### Inclusion Criteria

To be eligible all inclusion criteria should be **YES**

| | |
|---|---|
| 1.  Signed informed consent form | YES / NO |
| 2.  Presentation to a pharmacy for treatment of a cold sore | YES / NO |
| 3.  Age $\geq$ 16 | YES / NO |
| 4.  First cold sore symptoms (including prodromal symptoms such as tingling or pain) within 72 hours | YES / NO |

### Exclusion Criteria

To be eligible all exclusion criteria should be **NO**

| | |
|---|---|
| 1.  Pregnant or breastfeeding | YES / NO |
| 2.  Known or suspected allergy to honey, bees, glycerin or acyclovir | YES / NO |
| 3.  Any other condition which, at the investigators' discretion, it is believed may present a safety risk or impact feasibility of the study or the study results | YES / NO |
| 4.  Patient has used oral acyclovir or other antiviral medicine, or any topical treatment, medical or complementary, on the current sore | YES / NO |
| 5.  Patient planning to take/use during the course of the trial unallowed concomitant medications: any topical product, medical or complementary medicines for colds sores, eg. **Lysine supplements, any other medication which in the opinion of the investigator, could affect the cold sore** | YES / NO |

**Did the subject meet all eligibility criteria?   YES ☐      NO ☐**

**Comments:** _____

_____

_____

_____

**Signature (Investigator):**_____

**Date:**

**MEDICAL RESEARCH**
**INSTITUTE**
**OF NEW ZEALAND**

**Participant Initials:** ☐☐☐    **Randomisation ID:** ☐☐☐☐☐☐

## DEMOGRAPHICS

**Date of Birth:** ☐☐ ☐☐ ☐☐☐☐

**Gender (pls tick):**   Male ☐   Female ☐

**Ethnicity:**   NZ European ☐   Maori ☐   Samoan ☐   Cook Island Maori ☐

Tongan ☐   Niuean ☐   Chinese ☐   Indian ☐

Other (such as Dutch, Japanese, Tokelauan). Please State:_____

**No. of cold sores per year:** ☐☐

**No. of months since last cold sore:** ☐☐

**Able to access eDiary:**   YES ☐   NO ☐

**Participant email address:** _____

**Participant mobile phone number:** _____

**Emergency contact number:** _____

**Would the participant like a copy of the study results:**   YES ☐   NO ☐

**Patient address (for $25 reimbursement via cheque):**

_____

_____

_____

**Signature (Investigator):**_____

**Date:** ☐☐ ☐☐ ☐☐☐☐

## 9.9    TELEPHONE FOLLOW-UP VISIT WORKSHEET

**MEDICAL RESEARCH INSTITUTE OF NEW ZEALAND**

**5% acyclovir or Honevo™ as a Treatment for Cold Sores (KH10)**
**Visit 2 Day 14 Worksheet**

**RANDOMISATION ID:** ☐☐☐☐          **PATIENT INITIALS:** ☐☐☐

**D14 SCHEDULED DATE:** ☐☐ ☐☐☐☐ ☐☐☐☐

**DATE OF PHONE CALL:** ☐☐ ☐☐☐☐ ☐☐☐☐    Success: Yes / No

**DATE OF PHONE CALL:** ☐☐ ☐☐☐☐ ☐☐☐☐    Success: Yes / No

**DATE OF PHONE CALL:** ☐☐ ☐☐☐☐ ☐☐☐☐    Success: Yes / No

**Adverse Events**

Has the participant experienced an AE/SAE since visit 1?    No ☐  Yes ☐▶ Complete an AE and/or SAE form for each event.

**AE Narrative**

_____
_____
_____
_____
_____

**Participant GP Details**

GP Name:          _____

Surgery Name:     _____

Address:          _____

                  _____

                  _____

Phone:            _____

Email:            _____

Fax:              _____

**Concomitant Medications**

Has the participant taken any concomitant medications during the study?

No ☐   Yes ☐▶ Complete a concomitant medication form for each medication.

**Concomitant Medication Narrative**

_____
_____
_____
_____
_____

**Narrative Feedback**

_____
_____
_____
_____
_____

**How acceptable was using this treatment for you on a scale of 1 (unacceptable) and 10 (acceptable)**

|  1    2    3    4    5    6    7    8    9    10 |

Reminded the participant to send back their diary?          Done ☐

**Visit completed by:**

Name:          _____

Signature: _____

Date:      [ ][ ] [ ][ ][ ] [ ][ ][ ][ ]

# 9.10 HEALTH AND DISABILITY ETHICS COMMITTEE SUBMISSION AND APPROVAL

**Health and disability research**

These screening questions will help determine whether HDEC review is required for your study. They are based on the rules contained in section three of the *Standard Operating Procedures for Health and Disability Ethics Committees*.

Don't hesitate to contact us if you'd like help answering these questions, or any others in the HDEC form.

**A. Health and disability research**

Does your study aim to improve health outcomes, or outcomes for disabled people?

◉ Yes
◯ No

**Human reproductive research**

**B. Will your study involve the creation or use of a human gamete, a human embryo or a hybrid embryo?**

◯ Yes
◉ No

**Type of study**

**C. Is your study:**

◉ an intervention study?

*In intervention studies, the investigator controls and studies the preventive, diagnostic or therapeutic intervention(s) provided to participants for the purpose of adding to knowledge of the health effects of the intervention(s). Many intervention studies are clinical trials.*

◯ an observational study?

*In observational studies the researcher has no control over study variables, and merely observes outcomes.*

**Main Criteria**

**D.** Will your study involve **human participants** recruited in their capacity as:

- consumers of health or disability support services, or
- relatives and/or caregivers of consumers of health or disability support services, or
- volunteers in clinical trials (including bioequivalence and bioavailability studies)?

◉ Yes
◯ No

**E.** Does your study involve the use, collection or storage of **human tissue** (as defined by section 7 of the Human Tissue Act 2008)?

*Examples of human tissue include:*

- *all or any part of a body*

- *whole human organs or parts of them*
- *human stem cells or other human cells*
- *human blood*
- *human bone marrow*
- *human hair, nails, and skin*
- *human mucus, sputum, or urine.*

○ Yes

◉ No

**G.** Will your study involve the use or disclosure of **health information** (as defined by section 4(1) of the Health Information Privacy Code 1996)?

*Health information is about identifiable individuals. It includes:*

- *information about the health of an individual, including his or her medical history*
- *information about any disabilities that individual has, or has had*
- *information about any health services or disability services that are being provided, or have been provided, to that individual*
- *information in connection with the donation of any body part or any bodily substance of that individual*
- *information derived from the testing or examination of any body part, or any bodily substance of that individual*
- *information about the individual which is collected before or in the course of, and incidental to, the provision of any health service or disability service to that individual.*

◉ Yes

○ No

**H.** *You don't need HDEC approval to use health information for research if:*

- *informed consent to this use has already been obtained*
  *or*
- *the health information won't be disclosed\* to researchers in a form that would allow them to identify the individual(s) concerned, or to match the information with other datasets through a non-encrypted identifier (eg, an NHI number).*

Does one of these exceptions to the need to obtain HDEC approval apply to your study?

○ Yes

◉ No

\* See rule 11 of the Health Information Privacy Code 1996.

**Exemptions**

**I.** *Exemption for low risk medical devices*

Does your study involve evaluating a low-risk (class I) medical device?

*Low-risk (class I) medical devices are defined from page 77 of the Australian Therapeutic Goods Administration's Australian Regulatory Guidelines for Medical Devices.*

○ yes

◉ no

**INCLUSIONS**

| HDEC REVIEW |
|---|

**O. Your study requires HDEC review**

*The question below will determine the review pathway appropriate to your study.*

Does your study involve any of the following?    (select all that apply)

- ☑ a <u>new medicine</u>
- ☐ an approved medicine being used for a new indication or through a new mode of administration
- ☐ a medical device that is or would be classified as a <u>class IIb, class III, or active implantable medical device</u> by the Therapeutic Goods Administration (TGA)
- ☐ a new surgical intervention
- ☐ one or more participants who will not have given informed consent to participate
- ☐ one or more participants who are vulnerable (that is, who have a restricted ability to make independent decisions about their participation)
- ☑ standard treatment being withheld from one or more participants
- ☐ the storage, preservation or use of human tissue without consent
- ☐ Future Unspecified Use of Tissue
- ☐ none

**Full.** *Your study will be reviewed by the* **full review** *pathway described at section 5 of the* <u>Standard Operating Procedures for Health and Disability Ethics Committees</u>.

| a.1 Title and summary |
|---|

**a.1.1.**

Short study title:              5% Aciclovir or Honevo as a treatment for cold sores

**a.1.2.**

Formal study title:          An Open Label, Parallel Group, Randomised Controlled Trial of Topical 5% Aciclovir vs Honevo for the treatment of cold sores in adult participants.

**a.1.3.** A protocol must be uploaded in the "Documents" tab before submission to an HDEC.

*If this protocol has a unique identifier, please enter this below.*

Protocol number (if applicable):      KH10

**a.1.4.** Please provide the dates on which you plan to commence and conclude your study in New Zealand

Planned commencement date:      06/07/2015
Planned conclusion date:          06/07/2016

**a.1.5. Please provide a brief, plain English summary of your study.**

[< 2000 characters]

Herpes Simplex Labialis (HSL) is endemic worldwide causing characteristic blisters on the lips: 'cold sores'.

**a.2.1.1. Which of the following best describe your intervention study?**

*Blinding:*   ⦿ open-label      ◯ single-blind      ◯ double-blind

*Arms:*      ⦿ two-arm      ◯ multi-arm

*Design:*     ⦿ parallel      ◯ crossover      ◯ dose-ranging      ◯ cluster      ◯ factorial

*Control:*     ◯ placebo-      ⦿ active-controlled   ◯ uncontrolled
              controlled

*Randomisation:*  ⦿ randomised    ◯ non-
                              randomised

*Aim:*       ⦿ superiority     ◯ equivalence      ◯ non-inferiority

            ◯ none of the
            above

---

**a.2.2. Please select the ANZSRC field of research that best describes your study from the drop-down menus.**

Level 1: 11 Medical and Health Sciences

Level 2:  Complementary and Alternative Medicine

Level 3:  Complementary and Alternative Medicine not elsewhere classified

---

**a.3 Investigators**

---

Co-ordinating Investigator (CI)

*The CI has overall responsibility for the conduct of the study, including adherence to established ethical standards.*

*In student research, the student him- or herself is the CI.*

---

**a.3.1.** Are you the CI for this study?

◯ Yes

⦿ No

---

**a.3.1.1.** *The CI must authorise this application (through the "Authorisations" tab) before it can be submitted to an HDEC for review. You should request authorisation once you have completed all questions in the Online Form, or sign this form as the Co-ordinating Investigator in the Authorisations tab.*

Please provide the following information on the study's CI.

|  | Title: | Forename/Initials: | Surname: |
|---|---|---|---|
|  | Dr | Irene | Braithwaite |
| Mailing Address: | Medical Research Institute of New Zealand | | |
|  | Private Bag 7902 | | |

| | |
|---|---|
| Suburb/Town: | Wellington |
| Postcode: | 6012 |
| Country: | New Zealand |
| Organisation: | Medical Research Institute of New Zealand |
| Department*: | |

| | |
|---|---|
| Position: | Medical Research Fellow |
| E-mail: | irene.braithwaite@mrinz.ac.nz |
| Phone (BH): | 048050245 |
| Phone (AH)*: | |
| Mobile*: | |
| Fax: | 043895707 |

Other Investigator(s)

Other than the Co-ordinating Investigator, Investigators at all localities in a multi-centre intervention study must be listed as Investigators. Supervisors of student research must also be listed as Investigators.

You may list any other Investigators at your discretion.

---

**a.3.2.** Will any co-investigators be involved in conducting your study?

◉ Yes

○ No

---

**a.3.2.1.** You should request authorisation from each Investigator in your study (using the "Authorisations" tab) once you have completed all questions in the Online Form.

(For each co-investigator:)

| Other CI 1 | |
|---|---|
| | Title:  Forename/Initials:  Surname: |
| | Dr     Alex              Semprini |
| Mailing Address: | Medical Research Institute of New Zealand |
| | Private Bag 7902 |
| Suburb/Town: | Wellington |
| Postcode: | 6242 |
| Country: | New Zealand |
| Organisation: | Medical Research Institute of New Zealand |
| Department*: | |
| Position: | Medical Research Fellow |
| E-mail: | alex.semprini@mrinz.ac.nz |
| Phone (BH): | 021427527 |
| Phone (AH)*: | |
| Mobile*: | |
| Fax: | 043895707 |

---

### a.4 Primary contact person

**a.4.1. Are you the primary contact person for this study?**

◉ Yes

○ No

| | |
|---|---|
| | Title:  Forename/Initials:  Surname: |
| | Dr     Alex              Semprini |

Mailing Address:    MRINZ, Private Bag 7902


Suburb/Town:        Wellington
Postcode:           6242
Country:            New Zealand
Organisation:
Department*:
Position:
E-mail:             Alex.Semprini@mrinz.ac.nz
Phone (BH):         +64-4-8050241
Phone (AH)*:
Mobile*:
Fax:                +64-4-3895707

## a.5 Sponsor

*The sponsor has overall responsibility for the initiation, management, and financing arrangements of a study.*

**a.5.1.** Which of the following best describe the sponsor(s) of your study?

- ☐ pharmaceutical company
- ☐ medical device company
- ☐ academic institution
- ☐ collaborative research group
- ☐ district health board (DHB)
- ☐ other government agency
- ☐ non-governmental organisation (NGO)
- ☑ other

- ☐ no sponsor

**a.5.2. The sponsor(s) must authorise this application (through the "Authorisations" tab) before it can be submitted to an HDEC for review. You should request authorisation once you have completed all questions in the Online Form.**

Please provide the following details for your study's sponsor(s).

**Sponsor 1**

|  | Title: | Forename/Initials: | Surname: |
|---|---|---|---|
|  | Prof | Shaun | Holt |

Mailing Address:    305 Karaka Bay Road


Suburb/Town:        Wellington
Postcode:           6022
Country:            New Zealand
Organisation:       HoneyLab
Department*:
Position:           Director
E-mail:             holtshaun@gmail.com

Phone (BH):          0292001111
Phone (AH)*:
Mobile*:
Fax:                 0292001111

**Third party performing sponsor's duties or functions in New Zealand**

**a.5.3.** Will a third party (such as a contract research organisation) perform one or more of the sponsor's duties or functions in relation to this study in New Zealand?

- ⦿ Yes
- ○ No

**a.5.4.** This third party must authorise this application (through the "Authorisations" tab) before it can be submitted to an HDEC for review. You should request authorisation once you have completed all questions in the Online Form.

Please provide the following details for this third party.

| | Title: | Forename/Initials: | Surname: |
|---|---|---|---|
| | Prof | Richard | Beasley |

Mailing Address:   Medical Research Institute of New Zealand
                   Private Bag 7902

Suburb/Town:       Wellington
Postcode:          6242
Country:           New Zealand
Organisation:      Medical Research Institute of New Zealand
Department*:
Position:          Director
E-mail:            richard.beasley@mrinz.ac.nz
Phone (BH):        048050238
Phone (AH)*:
Mobile*:
Fax:               043895707

**a.6 Localities and participants**

New Zealand

It is a standard condition of HDEC approval that locality authorisation be obtained (through the "Authorisations" tab) **before a study commences at a locality**. This authorisation confirms that the locality has addressed research governance issues that may arise as a result of the study.

However, locality authorisation **does not** have to be obtained prior to submission of your application to an HDEC.

Other organisations involved in studies may prefer or require that their involvement in studies be recorded as an authorisation. You should check with these organisations before proceeding with your study.

Contact details for DHB research offices are available *here*

**a.6.1. At which type(s) of locality do you intend to conduct your study?**

☐ district health board

☐ tertiary education institution

☐ primary health care centre

☐ private organisation

☑ other - please specify: Community Pharmacies

---

**a.6.2. Approximately how many participants do you intend to recruit in New Zealand?**

950

---

Other countries

---

**a.6.3. Will your study also involve participants recruited in countries other than New Zealand?**

○ Yes

◉ No

---

### a.7 Prior review

**a.7.1. Is this application related to one or more previous applications for HDEC review?**

◉ Yes

○ No

---

**a.7.1.1. Please explain the relationship, giving the ethics reference number(s) of the previous application(s).**

[< 1200 characters]

15/NTB/43

'An Open Label, Parallel Group, Randomized Controlled Trial of Topical 5% Aciclovir vs a 5% Aciclovir/Honevo combination in the treatment of cold sores in adult participants'

Due to an unforeseen issue at the manufacturing site concerning stability testing of the investigational product we were unable to progress with combination product (acyclovir 5%/Honevo). This trial uses the same design as previously considered by the committee but the investigational product is honevo alone as compared to the active comparator acyclovir 5%.

---

**a.7.2. Has an application for this study (or a substantially similar study) previously been declined approval by an HDEC in New Zealand?**

○ Yes

◉ No

---

**a.7.3. Has an application for this study (or a substantially similar study) previously been declined approval by an overseas ethics committee?**

○ Yes

◉ No

---

## a.8 Clinical trials of new medicines

*You can apply for HDEC approval and regulatory approval(s) in any order. The PI and study sponsor are responsible for ensuring that all necessary regulatory approvals have been obtained before the study commences.*

**a.8.1.** Is your intervention study a clinical trial of a new medicine (as defined by the <u>Medicines Act 1981</u>)?

- ◉ Yes
- ○ No

**a.8.2.** *An Investigator's Brochure must be uploaded in the "Documents" tab before submission to an HDEC.*

*Your study must be approved under section 30 of the <u>Medicines Act 1981.</u>*
*"Section 30" approval is given by <u>Medsafe</u> on the recommendation of the <u>Health Research Council of New Zealand</u>, which maintains two standing committees for this purpose. These are the <u>Standing Committee on Therapeutic Trials (SCOTT)</u> and the <u>Gene Technology Advisory Committee (GTAC)</u>.*
*You can apply to SCOTT through the Online Forms website.*

## a.9 Open/closed meeting

*HDECs are public administrative bodies, and their meetings are open to the public. Your study may be reviewed in a closed meeting only if grounds may exist to withhold information about it under the <u>Official Information Act 1982.</u>*

**a.9.1. Do you want your application to be considered in a closed meeting?**

- ○ Yes
- ◉ No

## a.10 HDEC review preference

**a.10.1.** Please indicate your review preference.

- ◉ I request that this application be reviewed **as soon as possible**.
  I understand that this may mean that this application is not reviewed by the HDEC nearest to the CI

- ○ I request that this application be reviewed by the HDEC that meets **nearest to the CI.**

## b.1 Research should be based around a clear study question that can produce benefits.

**b.1.1.** Briefly and in plain English, what is the principal study question (hypothesis) that your study will test?
*You can refer to page numbers of your study's protocol for further detail if you need to.*

[< 2000 characters]

The primary outcome is a decrease in the healing time (from randomization to the return to normal skin). Secondary outcomes will also be analysed. These include pain severity, pain resolution, progression of the cold sore and narrative data on acceptability. More details can be found in the protocol under "Outcome Measures".

**b.1.2.** Please briefly describe the scientific basis for your study (including, where appropriate, brief discussion of previous research).
*You can refer to page numbers of your study's protocol for further detail if you need to.*

[< 2000 characters]

Honey has been used for centuries, orally and topically, for a variety of medical conditions. For example, Hippocrates recommended it for treatment of wounds, sores and other ailments. It is safe to use, as would be expected from a common food. Safety risks when it is applied topically are minimal, and these are reduced further when medical-grade (purified and sterilized) honey is used. Honey has a number of specific physicochemical properties that may be responsible for its effectiveness in treating some medical conditions:
Osmotic effect – honey is a concentrated sugar solution predominantly consisting of the monosaccharides fructose and glucose. Few water molecules are available for microorganisms and therefore it is a poor environment for their growth. Hydrogen peroxide – this is slowly released when the honey comes into contact with body fluids and has antiseptic properties. Acidity – honey is acidic with a pH of 3.2 to 4.5, which makes microorganism growth difficult. Kanuka honey contains bioflavonoids and other antioxidants which may contribute to its activity Methylglyoxal MGO, found in high levels in certain honeys, such as New Zealand manuka and Kanuka honeys, has potent antibacterial effects.
There is evidence in the medical literature of efficacy for a number of medical conditions, including burns, cough, wounds, diabetes, cold sores, peptic ulcers, constipation and weight loss (for details see Investigator's Brochure). Invitro and clinical studies show honey has antimicrobial properties, likely due to its low pH, hydrogen peroxide content and high osmolality. Honey may also have immunemodulatory properties.
Moreover, a small randomised controlled trial (RCT) from Dubai in 16 adult patients with recurrent attacks of herpetic lesions found topical application of honey was more effective than 5% aciclovir cream for HSL and genital herpes.

**b.1.3.** Please briefly explain how your study will contribute to new knowledge and improve health outcomes.

[< 2000 characters]

There is a need for novel, more effective therapies for the treatment and management of Cold sores, which are acceptable to patients. This study will ascertain the effectiveness of Kanuka honey in treating cold sores, potentially improving the health outcomes of those with the condition and contribute to the knowledge base around the use of honey for the treatment of skin conditions. It also has the potential to provide an effective and natural treatment choice for patients.

Direct benefits for participants: therapeutic and non-therapeutic studies

**b.1.4.** *Therapeutic studies are studies that examine interventions or procedures that hold the prospect of direct diagnostic, therapeutic or preventative benefit for individual participants.*

Is your intervention study a therapeutic study?
◉ Yes   ○ No

**b.1.4.1.** Please briefly describe the direct diagnostic, therapeutic or preventative benefits that your intervention study may have for participants.

[< 600 characters]

This study has the potential to offer subjects a better therapeutic benefit in reduction of cold sores symptoms and healing time than the current standard treatment of 5% aciclovir.

**b.2 Research should be well-designed, so that it can answer the study question.**

**b.2.1.** Please briefly describe and justify the design of your study.

[< 1200 characters]

Open label, randomised, 2way active comparator, parallel group trial.

Participants will be randomised 1:1 to topical treatment with either the medicalgrade kanuka honey (Honevo), or 5% aciclovir.

The median control duration of HSL symptoms is assumed as 5 days. To achieve a one day median difference, with associated Hazard ratio of 1.25, 423 participants would be needed per arm of treatment, therefore a total of 846 subjects. The study is deemed as short (one cold sore episode) so the drop out rate is assume to be around 10%, then 950 participants will be randomized. This study size is consistent with similar 2/3 arm cold sore studies have required around 300/350 patients per treatment arm.

Participants will be instructed to apply both the Honevo formulation and the 5% aciclovir five times per day for 14 days or until skin has returned to normal, which ever is sooner.

Due to physical characteristics of honey it is not possible to blind subjects to the treatment they are receiving.

---

**b.2.2.** Please indicate whether peer review of the scientific and statistical quality of your study has been obtained from one or more of the following.

- ☑ the Standing Committee on Therapeutic Trials (SCOTT)
- ☐ the study's funder (e.g. the Health Research Council)
- ☑ the study's sponsor
- ☑ experts within the research team
- ☑ senior colleague(s) in the field
- ☐ other

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**b.2.2.1.** *Evidence of favourable peer review for this study must be uploaded in the "Documents" tab before submission to an HDEC.*

Please briefly describe the peer review process that has been carried out for your study.

[< 1200 characters]

The study will be reviewed by SCOTT under current guidelines for new medicines.
Statistical methods and analysis, including power calculations, have been performed by the study statistician, Prof Mark Weatherall, of Otago University. The research team at the Medical Research Institute of New Zealand have developed and reviewed the protocol, along with Prof. Shaun Holt on behalf of the study Sponsor, Honeylab.

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**b.3 Research should be conducted by an appropriate Principal Investigator, to ensure that the study protocol is respected and followed.**

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**b.3.1.** *A CV for the study's Co-ordinating Investigator must be uploaded in the "Documents" tab before submission to an HDEC.*

Please briefly summarise the Co-ordinating Investigator's qualifications and experience relating to conducting studies of this nature.

[< 1200 characters]

Dr Braithwaite is an experienced researcher and deputy Director at the Medical Research Institute of New Zealand. She has experience working on both Investigator initiated research (as part of her ongoing PhD) and also pharmaceutical industry sponsored studies, being designated as PI. Dr Braithwaite was a subinvestigator in the Kanuka Honey Pilot studies investigating the use of Kanuka honey in various skin conditions (ethics ref: MEC/11/12/098; MEC/12/03/022; MEC/12/03/023; MEC/12/03/024; MEC/12/03/025) and was involved in the protocol design, analysis and publication of those studies.

Dr Braithwaite is also the a subInvestigator for three recently completed studies investigating the use of Kanunka honey in Rosacea, Acne and Nappy Rash (ethics ref: 13/CEN/118; 13/CEN/119; 13/CEN/120) and has been involved in the data analysis and manuscript writing of these studies.

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**b.4 Where possible, research should generate material that is useful for future research.**

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Reporting and dissemination of results

**b.4.1.** How do you intend to report or disseminate the results of your study?

- ☑ article(s) in peer-reviewed scientific journals
- ☑ internal reports
- ☑ conference presentations
- ☑ publication on website
- ☑ other publications
- ☑ submission to regulatory authorities (e.g. Medsafe, TGA, FDA, EMA)
- ☑ other
- ☐ no plans to report or disseminate results

**b.4.2.** Will any restrictions be placed (for example, by your study's sponsor or funder) on the publication of the results of your study?

○ Yes  ◉ No

Future research using data generated in your study

**b.4.4.**

Might data generated in your study be made available for use in future research?
◉ Yes  ○ No

**b.4.4.1.** *You should explain this clearly to potential participants.*

Which of the following best describes the form in which data generated by your study might be made available to other researchers?

- ○ identified
- ◉ potentially identifiable
- ○ partially de-identified
- ○ de-identified
- ○ anonymous
- ○ other – describe:

**b.4.6.** *Intervention studies must be registered prior to commencement.*
**Has your intervention study already been registered in a clinical trials** registry**approved by the World Health Organisation?**

- ○ yes
- ◉ no

**b.4.7.** You can obtain HDEC approval prior to registration, as long as you have obtianed a Universal Trial Number (UTN) for your study.

UTN: U1111-1170-1537

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**r.1 Risk of physical harm to participants**

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**r.1.1.** Briefly and in plain English, please describe:

- the procedures to be undertaken by participants in your study, and
- any risks associated with these procedures that potential participants may reasonably wish to be informed of.

*Do not describe procedures that will be undertaken as part of normal clinical care regardless of participation in your study, or the risks of such procedures.*

[< 2500 characters]

Participants will undergo the informed consent process prior to any study specific procedures being undertaken. Participants will then complete a questionnaire to collect information including demographic data, history of cold sores and their treatment, duration of current episode and prescribed medications (with time of onset and stage progression)along with allergy history.
Participants will be randomised to one of the two treatment arms with either the Honevo(HoneyLab) or the 5% aciclovir and will be instructed to apply their treatment five times per day. Participants will be asked not to use any other topical/systemic treatment for cold sores.
During this period, participants will complete a daily diary recording symptoms including pain, cold sore stage and number of treatment applications, until the lesion completely resolves or a maximum of 14 days.
Pain will be assessed by the patient using a 0 to 10 scale where 0=no pain and 10= severe pain.
The stage of the cold sores will be assessed by the patient using pictures with different stages of cold sores which the patient can correlate with their own cold sores.
At day 14, participants will be called by the study team to ask that diary is returned (via mail)to the research center, ask about any adverse events that the participant may have experienced and ask about narrative feedback.
The investigator will note any safety information as per the protocol, including any AEs and SAEs.
There are no perceived risks in undergoing these assessments.
The risk of applying the study treatments is deemed low, please see the investigators brochure and PISCF for more information.

---

**r.1.2.** Will you seek consent from participants to inform health practitioners with responsibility for their health care that they are taking part in your study?

⦿ Yes     ○ No

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**r.1.3.** Will your study involve withholding standard treatment from participants?

⦿ Yes     ○ No

---

**r.1.3.1.** Please briefly explain why it is appropriate to withhold standard treatment from participants.

[< 1200 characters]

Acyclovir provides a mean increased healing time in cold sores of half a day compared to no treatment in a self limiting condition. A previous pilot study has shown honey for the treatment of cold sores to be both acceptable, effective and without serious side effects. Testing this potential efficacy in a larger, powered trial is necessary to provide the evidence base in order to recommend honey as an appropriate treatment or not. Participants may, at any time, withdraw and take the standard treatment if they wish.

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Arrangements for monitoring serious adverse events

**r.1.4.** How will serious adverse events occurring in your study be monitored?

☑ independent data safety monitoring committee

☑ internal data safety monitoring committee

☐ other data safety monitoring arrangements

☐ no formal data safety monitoring arrangements

**r.1.5.** Please briefly explain *either*:

- the monitoring arrangements in place for your study, and explain why they are appropriate (including reference to your study's protocol where appropriate), or
- why you do not consider formal monitoring arrangements to be necessary for your study.

[< 1200 characters]

The study is deemed as low risk but given the size and novel recruitment methods an independent DSMB will be convened to review the data any any AE/SAEs at the point of 100 patients recruited. All AEs or SAEs will be recorded as part as the phone call session at week 2 (if not notified before by the participant), as per protocol requirements (See protocol section "Safety monitoring"). A study doctor will review the SAEs and assess for causality and grading. Study staff will follow up all SAEs and inform subject's GPs if appropriate. MRINZ will perform all safety analysis on the sponsor's behalf.
Adverse event data will be collected and analysed with efficacy data at the end of the study. Serious Adverse Events will be reported according to regulatory guidelines.

**r.1.6.** Please briefly outline the criteria (if any) for terminating your intervention study, including reference to your study's protocol where appropriate.

[< 600 characters]

The Sponsor may terminate the study at any point, except for reasons of commercial interest.

Compensation for injury to participants

**r.1.7.** Will any participants seek or be given treatment by or at the direction of a registered health professional (as defined in the Accident Compensation Act 2001) as part of your intervention study?

◉ Yes    ○ No

**r.1.7.1.** Will any of these participants have given written consent to participate?

◉ Yes    ○ No

**r.1.7.1.1.** Does your intervention study involve trialing a medicine or item?

◉ Yes    ○ No

**r.1.7.1.2.** Having regard to the following questions, will your study be carried out principally for the benefit of the manufacturer or distributor of the medicine or item being trialed?

- *Who is initiating the study?*
- *Who is designing and planning the research questions that the study will ask?*
- *Will the PI or other investigators receive remuneration from the manufacturer or distributor?*
- *Is the manufacturer or distributor putting any unreasonable restrictions or delays on the timely publication of*

*the results of the study?*
- *Is the manufacturer or distributor providing any funding and/or materials for the study?*

⦿ **yes**, my study will be carried out principally for the benefit of the manufacturer or distributor

of the medicine or item in question

◯ **no**, my study will **not** be carried out principally for the benefit of the manufacturer or distributor

of the medicine or item in question

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**r.1.8.** Please briefly explain your answer(s) to questions r.1.7 above.

[< 1200 characters]

As a Sponsored study principally for the benefit of the manufacturer of Kanuka Honey, the study will not be covered by ACC and therefore the Sponsor will provide adequate insurance for indemnity purposes.

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**r.1.9.** *Participants injured as a result of treatment given as part of your intervention study may not be eligible for publicly funded no-fault compensation from the Accident Compensation Corporation. Researchers and sponsors must ensure that they have arrangements in place to ensure that at least ACC-equivalent compensation would be available in case of such injury.*

In the event of injury to a participant in your intervention study, will compensation potentially be available for <u>all</u> of the following entitlements, which would be available through ACC?

- rehabilitation (comprising treatment, social rehabilitation, and vocational rehabilitation)
- first week compensation
- weekly compensation
- lump sum compensation for permanent impairment
- funeral grants, survivors' grants, weekly compensation for the spouse or partner, children and other dependents of a deceased claimant, and child care payments

⦿ Yes     ◯ No

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**r.1.10.** Please confirm that:

- the study's sponsor agrees to abide by Medicines NZ's <u>Guidelines on Clinical Trials Compensation for Injury Resulting from Participation in an Industry-Sponsored Clinical Trial</u>, and
- insurance cover will be in place for the duration of the study in New Zealand, and
- participation in the trial does not affect the right of participants to pursue legal remedies in respect of any injury alleged to have been suffered as a result of participation.

⦿ Yes     ◯ No

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**r.1.11.** *Evidence that the study sponsor holds insurance in respect of this intervention study must be uploaded in the "Documents" tab before submission to an HDEC.*

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**r.1.12.** *Evidence that the Principal Investigator is professional indemnified, for example through membership of the Medical Protection Society (MPS), must be uploaded in the "Documents" tab before submission to an HDEC.*

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<u>Ionising radiation not needed for normal clinical management</u>

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**r.1.13.** Will your study involve the administration of ionising radiation that is not needed for participants' normal clinical

management?

○ Yes    ⊙ No

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**r.2 Risk of breach of privacy and confidentiality**

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Before the study

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**r.2.1.** Will your study involve reviewing or screening health information, for example in order to identify potential participants?

*The term "health information" is defined in the Health Information Privacy Code*

⊙ Yes    ○ No

---

**r.2.1.1.** Please briefly explain how you will ensure the confidentiality of this health information before the study.

[< 600 characters]

Potentially eligible patients will be identified at the time of presentation to their pharmacy for treatment of a cold sore. Health information will be held on secure databases or on study worksheets/ documents at each pharmacy practice, remaining confidential to the study team at that site.
A start up visit will be undertaken by MRINZ to ensure all pharmacies involved have the adequate facilities for the confidentiality of participant's health information (locked storage etc) and adequate expertise and other resources.

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During the study

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**r.2.2.** During your study, who will have access to health information used in your study?

[< 600 characters]

Due to the nature of recruitment for this study, health information will be shared between the recruiting site and the coordinating center and follow up team. Identifiable information will be held securely at site, but also a copy sent to MRINZ and Optimal Clinical Trials for the purposes of the day 14 follow up, via fax. This ensures follow up, allows MRINZ to monitor recruitment and the consent process and perform data management during the study to ensure ethical and GCP guidelines are met.

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**r.2.3.** Please briefly explain how you will ensure the confidentiality of this health information during the study.

[< 600 characters]

The original source documentation will remain at site during the study and will be stored securely in a password protected database or in a locked cabinet. Information sent to MRINZ and Optimal Clinical Trials will be via fax to a secure location and will be held in password protected databases and/or locked cabinets. All data which can be de-identified by assigning a subject code. Only study team members will have access to this identifiable data

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**r.2.3.1.** Will your study involve the use of surveys or questionnaires?

⊙ Yes    ○ No

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**r.2.3.2.** *Copies of these surveys or questionnaires must be uploaded in the "Documents" tab before submission to an HDEC.*

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After the study

**r.2.4.** Which of the following best describes the form in which data generated in your study will be stored after the study has finished?

○ identified

◉ potentially identifiable

○ partially de-identified

○ de-identified

○ anonymous

○ other – describe:

---

**r.2.4.1.** Please briefly explain your answer above.

[< 600 characters]

Data stored after the study has finished will be coded by a unique subject number. Each site will maintain a subject code list that identifies the details of the subject against their unique subject number and this list remains securely stored at site. The code list means that any study data will be potentially identifiable if required, to allow monitoring and verification of source data, but the data and code list will be stored separately and the study data will not contain identifiable information.

---

**r.2.5.** The _Health (Retention of Health Information) Regulations 1996_ require that **some** health information be retained for a period of ten years.

For how long will health information generated in your study be stored?
[< 600 characters]

Source and study data will be stored for 15 years, as per Good Clinical Practice guidelines.

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Publication of results

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**r.2.6.** Will the results of your study be published in a form that identifies (or could reasonably be expected to identify) individual participants?

○ Yes    ◉ No

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**r.3 Risks associated with the use of human tissue**

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**r.4 Risk of unexpected clinically significant findings**

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**r.4.1.** Might any aspect of your study produce findings that may be both unexpected and clinically significant for participants, donors of existing stored human tissue, or their families?

◉ Yes    ○ No

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**r.4.1.1.** What might these findings be, and how will participants, donors of existing stored human tissue, or their families be informed of them?

[< 600 characters]

The study is minimal risk and involves low risk procedures throughout, however there may be instances where subjects have an unexpected reaction to the Honevo or aciclovir, which may be clinically significant. To mitigate this potential risk potential subjects will be asked about allergies and any subject who is known to be sensitive or allergic to the investigational products will be excluded from this study.

## r.5 Risk of potential conflict of interest

Funding and remuneration

**r.5.1.** Please briefly describe the main source(s) of funding for your study.

[< 600 characters]

The Sponsor, Honeylab, is fully funding the study.

**r.5.2.** Does the Co-ordinating Investigator, any Co-Investigator, or any direct member of their families have any commercial interest in the intervention(s) to be studied, or any financial relationship to the study sponsor or funder(s), that may inappropriately influence his or her conduct in the study?

○ Yes   ◉ No

**r.5.3.** Will the Co-ordinating Investigator or any Co-Investigator be remunerated for their involvement in the study in a way that may inappropriately influence his or her conduct in the study (for instance, bonuses for favourable results or high recruitment rates)?

○ Yes   ◉ No

Health or disability support service providers

**r.5.4.** Will the Co-ordinating Investigator or any Co-Investigator also be the usual health or disability support service provider for one or more participants in your study?

◉ Yes   ○ No

**r.5.4.1.** Please briefly describe how the risk of a conflict of interest between the research and clinical roles of such Investigators will be minimised and managed.

[< 600 characters]

The investigator/pharmacist will discuss with the subject and ensure that they are made aware of the voluntary nature of the study, and that whether or not they enroll in the study will not affect their ongoing healthcare. Pharmacist will abide by the GCP guidelines for informed consent.

**r.5.5.** Will the usual health or disability service provider for one or more participants in your study receive any remuneration (or any other valuable consideration) for referring potential participants to the research team in your study?

◉ Yes   ○ No

**r.5.5.1.** Please briefly describe how the risk of a conflict of interest will be minimised and managed.

[< 600 characters]

Each pharmacy will receive $40 as the result of consultation in order to reimburse them for time spent discussing the study with potential participants and the loss of revenue from the sale of a product that the study will be providing at no cost to the participant.

Other potential conflicts of interest

**r.5.6.** Please briefly describe any other potential conflicts of interest that may arise for researchers in your study, and describe how they will be minimised and managed.

[< 600 characters]

Recruiting sites will be reimbursed for their time in conducting the protocol procedures and recruiting subjects for the study, by the Sponsor. The is a fixed fee per subject ($40) and no bonuses will be awarded for high recruitment rates. The MRINZ will conduct study monitoring to ensure ethical and GCP guidelines are adhered to during the study, to minimise potential conflict of interest.

## r.6 Risk of stigmatisation

**r.6.1.** Please briefly indicate whether the results of your study may risk stigmatising individuals or population groups, and if so, how this risk will be minimised and managed.

[< 600 characters]

It is not envisaged that subjects will be at risk of stigmatisation by participating in the study, however the informed consent process will outline the balance of risks and benefits of taking part and potential subjects will be free to decide to participate or not.

## r.7 Risks to researchers and third parties

**r.7.1.** Please briefly indicate whether your study may pose any significant risks to researchers and/or third parties, and briefly explain how such risks will be minimised and managed.

[< 600 characters]

There are no perceived risks for researchers.

## r.8 Summary: the risks of research should be proportional to its expected benefits.

**r.8.1.** Please briefly explain why you consider the risks of your study to be proportional to its expected benefits.

[< 1200 characters]

Topical application of Kanuka honey is deemed low risk (see investigators brochure for more detail). Whilst there is always a risk of subjects experiencing adverse events that are unexpected, subjects will be asked about safety events at follow up and can contact investigators at any point during the study should they have concerns.
Data from a small study in Dubai has founded that for labial herpes, the mean duration of attacks and pain, occurrence of crusting, and mean healing time with honey treatment were 35%, 39%, 28% and 43% better, respectively, than with acyclovir treatment.
Another benefit of taking part in the study is the contribution of valuable information to a study which may help improve treatment outcomes for cold sores for which, current therapeutic benefit is modest.

## Participants should consent to their participation in research.

**p.1.1.** Briefly and in plain English, please describe what taking part in your study will involve for participants.

[< 1200 characters]

Participants will be consented and then randomized to received either Honevo or 5% aciclovir.
Participants in both treatment arms will be instructed to apply the topical treatment five times per day until the lesion completely resolves.
During this treatment period, participants will complete a diary (ediary or paper) recording compliance, pain score and cold sore stage (according to provided guidelines/ images). A text message will be sent daily as a reminder to complete the diary and provide a link to the ediary.
Participants will be contacted by phone on Day 14 to ensure compliance with the study treatment and completion of the diary and to remind them to post the paper diary to the research centre.
During each phone call participants will be asked if they have experienced any adverse effects, which will be recorded and reported to the Coordinating Centre for review, and as necessary to SCOTT/HDEC. Narrative feedback will also be recorded.

**p.1.2.** Will **all** participants in your study give their informed consent to participate?

◉ yes, all participants will give informed consent

○ no, one or more participants will not give informed consent

**p.1.9.** Will informed consent be recorded in writing?

◉ Yes     ○ No

Consent should be informed by adequate understanding of relevant information.

**p.2.1.** Briefly explain the process by which potential participants in your study will be provided with information on the study, have the opportunity to ask questions, and asked to give their informed consent.

[< 1200 characters]

Potential subjects will be approached by the investigator, who will explain the study with the information sheet, discuss any concerns and answer any questions. If the subject wishes to enrol, the informed consent form will be signed if all points are agreed. Participants are able to withdraw at any time. Subjects may contact the investigator or MRINZ at any point.

**p.2.2.** A **generic** version of the participant information sheet and consent form (PIS/CF) that you will provide to potential participants must be uploaded in the "Documents" tab before submission to an HDEC. You don't need to submit information sheets specific to each study locality.
*A suggested pro forma for your PIS/CF can be found here.*

**p.2.3.** How have you checked that the participant information sheet is appropriate for your study population?

[< 600 characters]

The MRINZ has developed the PIS/CF on behalf of the Sponsor. The MRINZ has extensive experience designing these types of documents and has specific experience from the Kanuka Honey Pilot studies on which to base these current documents. Care has been taken to ensure that the information is provided in lay terms and is appropriate for the patient population, including taking into account any potential cultural issues. The summary information sheet has been created to to balance the ability to provide relevant study information, ensure informed consent and enable recruitment via pharmacy sites.

**p.2.4.** How many words does your participant information sheet contain?

1298

**p.2.5.** What is the Flesch Reading Ease Score for your participant infornation sheet?
*You can use Microsoft Word to calculate this score.*
*While there are no hard and fast rules for the readability of information sheets, a score of 65 or above usually indicates that a document is written in plain English.*

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Withholding or concealing information from participants

**p.2.6.** Does your study involve deliberately withholding or concealing information from participants?
*Blinding procedures in randomised controlled trials are not normally considered to involve withholding or concealing information from participants.*

○ Yes     ◉ No

Information that becomes available during the study and that may be relevant to continued participation

**p.2.7.** How will you ensure that participants receive information that becomes available during the study and that may be relevant to their continued participation?

[< 1200 characters]

Any clinically significant information that becomes apparent will be communicated to the subjects by letter/phone call if necessary/urgent. In cases where this requires further treatment, information will be provided to the subjects GP, with their consent (or will be treated as appropriate by the Investigator, in cases where they are the subject's usual health care provider).
Any significant change to the study will be incorporated into an updated information sheet and consent form, which will be provided to the subject and explained as part of ongoing consent to the study.

Information about the results of the study

**p.2.8.** Will you inform participants of the results of your study?

⦿ Yes    ◯ No

**p.2.9.** Please *either* explain how you will inform participants or explain why you do not intend to do so.

[< 600 characters]

A lay summary of the trial results will be made available to all subjects, should they wish to receive this.

**Consent should be voluntary.**

**p.3.1.** *Generic copies of any advertising that you intend to use to encourage potential participants to take part in your study must be uploaded in the "Documents" tab before submission to an HDEC.*
Please explain how potential participants will be identified and approached in a way that ensures they can give informed consent free from undue influence.

[< 1200 characters]

Recruitment will take place at the pharmacy when a patient seeks cold sore treatment. Once approached, participants will be given an information sheet to read to consider whether they wish to be involved in the study and can ask the pharmacist any questions. A small card highlighting the study may also be placed around cold sore treatments on display, along with other general advertising to share information about the study in the vicinity of the pharmacy listed on the advertisement.
Consent will take place in the pharmacy (in appropriate private rooms/surroundings), with the Investigator/pharmacist ensuring the participant is fully aware of the study requirements and information.
Potential participants will also be reassured that whether they decide to take part or not, it will have no impact on their standard of care treatment. Participants are free to contact the study doctor and to withdraw at any time.

Potentially vulnerable people

**p.3.2.** Will your study involve potentially vulnerable people – that is, people who may have a restricted ability to make independent decisions about their participation?

◯ Yes    ⦿ No

Inducements

**p.3.3.** Will participants receive any payments, reimbursement of expenses or any other benefits or incentives for taking part in your study?

◉ Yes      ○ No

**p.3.3.1.** Please describe these, and explain why they are appropriate.

[< 600 characters]

The treatment is provided free. Patients will be reimbursed $25 by cheque for their time, at the end of the study.

**P.4 Population groups, particularly Māori, should be consulted in the design and conduct of research that is of relevance to them.**

Consultation with Māori

**p.4.1.** Please describe whether and how your study may benefit Māori.

[< 1200 characters]

Herpes Simplex Labialis (HSL) is a common condition in New Zealand and is likely to affect Māori to at least a similar extent to that of the wider population. Although the study is not specifically aimed at directly benefiting Māori, the results may possibly lead to a better understanding of the treatment of cold sores and Māori may benefit from this understanding, as better, more directed treatments may be developed.

**p.4.2.** Please identify the main cultural issues that may arise for Māori who may participate in your study, and explain how these issues will be managed.

*If Māori will be excluded from participating, please state this. You will be asked to explain your inclusion/exclusion criteria in the next section of the Form.*

[< 1200 characters]

As per consultation regarding the earlier related pilot studies, and for this study, there were no significant cultural issues raised regarding the procedures and enrolment of Maori subjects.

**p.4.3.** According to the Health Research Council's Guidelines for Researchers on Health Research Involving Māori, is formal consultation with Māori required for your study?

◉ Yes      ○ No

**p.4.3.1.** Please either describe your study's consultation process, or explain why you do not consider that formal consultation with Māori is required.

[< 1200 characters]

The protocol has been reviewed by the internal Honeylab group and as part of its development there has been input from Māori as to any cultural concerns that may be raised. Please see the attached documents outlining consultation with Māori, provided by the sponsor, alongside copies of the original correspondence and process as requested from the previous ethics review of our similar study. As consultation has taken place at the level of protocol development, local consultation at the level of each locality is not deemed necessary.

**p.4.4.** Does your study involve kaupapa Māori research methodologies?

○ Yes      ◉ No

Consultation with other relevant population groups

**p.4.5.** Will any other population groups be specifically targeted for recruitment into your study?

○ Yes     ⦿ No

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Collection of ethnicity status

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**p.4.6.** Will participants' ethnicity status be collected as part of your study?

⦿ Yes     ○ No

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Community intervention studies

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**p.4.7.** Is your study a community intervention study?

○ Yes     ⦿ No

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**f.1 Where possible, research should reduce health inequalities.**

**f.1.1.** Might your intervention study contribute to reducing inequalities in health outcomes between different populations, and particularly between Māori, Pacific peoples and other New Zealanders?

○ Yes     ⦿ No

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**f.1.2.** Please explain your answer above.

[< 1200 characters]

The purpose of this study is to prove the effectiveness of Kanuka Honey (Honevo)   as a treatment for cold sores, which will potentially be of benefit for all population groups experiencing the condition. The study does not specifically aim to reduce health inequalities between different populations, the study is open to all ethnic groups, as per the inclusion and exclusion criteria.

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**f.2 Participants and non-participants should be treated fairly compared to each other**

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Inclusion and exclusion criteria

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**f.2.1.** Please briefly describe the inclusion and exclusion criteria for your study.
*You can refer to page numbers of your study's protocol where further detail is required.*

[< 2000 characters]

Inclusion criteria:
Aged 16 or over at the time of enrollment.
Presentation to a pharmacy for treatment of a cold sore.
First cold sore symptoms (including prodromal symptoms such as tingling or pain) within 72 hours
Exclusion criteria:
Pregnant or breastfeeding
Known or suspected allergy to honey or aciclovir.
Any other condition which, at the investigators' discretion, is believed may present a safety risk or impact the feasibility of the study or the study results.
Patients taking/using or planning to take/use during the course of the trial unallowed concomitant medications: any topical product, medical or complementary, on the cold sore, oral aciclovir or other antiviral medicine, oral complementary medicines for cold sores, eg. lysine supplements, any other medication which in the opinion of the

investigator, could affect the cold sore.

**f.2.2.** Please explain how these inclusion and exclusion criteria ensure that the risks and benefits of your study are distributed fairly.

[< 1200 characters]

This study aims to capture all participants with early symptoms of cold sores and would could potential benefit from treatment with the Honevo treatment. All subjects whom may require or who have already taken a treatment for their cold sore will be excluded, so that efficacy can be tested. The risks are deemed low and all subjects with know allergies to the products under study will not be able to take part.

Placebo-controlled Studies

**f.2.3.** Does your study involve the use of placebo?

◯ Yes    ⊙ No

Impact on health and disability support service provision

**f.2.4.** Might your study adversely impact on the provision of health and disability services?

⊙ Yes    ◯ No

**f.2.4.1.** How will this possibility be minimised and managed?

[< 600 characters]

Pharmacy participation in this study will require an extended consultation time and therefore impinge on usual resources. To mitigate this we have made the information sheet and consenting process as streamlined as possible without impacting the necessary content, whilst enabling fully informed consent to be given. Each site will undergo feasibility and locality assessment to ensure they have appropriate resources and and facilities to conduct the study.

Best intervention standard

**f.2.5.** *An intervention study meets the best intervention standard if the intervention(s) in the study are tested against the best proven intervention(s) available outside the study.*
Please explain how your study meets the "best intervention standard".

[< 600 characters]

Participants will be randomised 1:1 to one of the 2 arms:
● Honevo(HoneyLab) formulation
● 5% aciclovir
There is no placebo, 5% aciclovir is the standard treatment for cold sores and a pilot study in Dubai and one from our group showed that honey was highly acceptable for the treatment of cold sores. All patients will be given a treatment considered as effective for cold sores, the purpose of the study if to find out which one is the more effective.

**f.3 Different groups of participants should be treated fairly compared to each other**

Post-study access for participants to best-proven intervention

**f.3.1. Will all participants have continued access to the best-proven intervention after the end of your intervention**

**study?**

⦿ Yes    ◯ No

---

Equipoise Standard

---

**f.3.2.** *An intervention study meets the equipoise standard if the evidence is 'equally poised' as to the overall balance of risks and benefits of each of the interventions offered in the study, so that it cannot be determined in advance which of the groups in a proposed study will be better off.*

Please briefly explain how your intervention study meets the equipoise standard.

[< 600 characters]

A recent feasibility study from our group identified that topical pharmaceutical grade kanuka honey for the treatment of HSL was highly acceptable to patients for the treatment of HSL.
However there is not yet enough definitive evidence to show that the Kanuka honey product is of greater benefit than applying standard care topical acyclovir.
This purpose of this study is to determine either the Honevo formulation is better than current standard treatment and so it is not yet known which group will be better off.

09 June 2015

Dr Irene Braithwaite
Medical Research Institute of New Zealand
Private Bag 7902
Wellington 6242

Dear Dr Braithwaite

| Re: | **Ethics ref:** | **15/NTB/93** |
|-----|-----------------|---------------|
| | Study title: | An Open Label, Parallel Group, Randomised Controlled Trial of Topical 5% Aciclovir vs Honevo for the treatment of cold sores in adult participants. |

I am pleased to advise that this application has been _approved_ by the Northern B Health and Disability Ethics Committee. This decision was made through the HDEC-Full Review pathway.

Summary of Study

- Dr Semprini explained that this is essentially the same study that was reviewed by the Committee earlier in the year, with the main change being to the investigational product.

Summary of ethical issues (resolved)

The main ethical issues considered by the Committee and addressed by the Researcher are as follows.

- The Committee asked for clarification on the data safety monitoring committee. Dr explained that there are two consultants within Wellington Hospital who have reviewed the protocol. After 100 patients have been recruited, they will give them the safety information and SAEs and how the study is going generally and they will generate reports from there.
- Dr Semprini advised that SCOTT review was underway and was looking at new investigational product. He did not expect any issues as the same formulation had been used for other studies.
- The Committee asked for clarification on the recruitment process. Dr Semprini explained that there will be notes on the pharmacy shelf where coldsore products are available advising customers that there is a trial taking place and to discuss it with the pharmacist if they are interested. The pharmacists will also tell potential participants about the study when they go to buy coldsore products. If a person says yes, there will be a suitable area within the pharmacy where the trial can be discussed. The pharmacist can then go through the PIS and answer any questions the participant may have. Pharmacists will be given pre-randomised packs which will be given sequentially to participants.

- The Committee asked what would stop participants from taking the study treatment and other coldsore treatments. Dr Semprini advised that they would be relying on participants not to and to report it in the study diary and in the final phone call at the end of the study.
- Dr Semprini advised that an organisation in Auckland will conduct the final phone call at the end of the study. This was because they found in a previous acne study that there was too much information recorded in the study diaries and the idea was to keep this information to a minimum and collect the information at the end of the study.
- The Committee commended the researcher for condensing the PIS, while still including key information, based on previous Committee feedback.
- Dr Semprini advised that he had spoken to the Maori Pharmacists Association and local universities as they had struggled to find a national contact number for Maori support that encompasses every iwi. He said he had discussed it with Te Ora, a health network charitable trust in Taranaki who had agreed to provide support and contact on an as needed basis. The plan was to include their contact details in the PIS and the Committee agreed this was acceptable.

Conditions of HDEC approval

HDEC approval for this study is subject to the following conditions being met prior to the commencement of the study in New Zealand. It is your responsibility, and that of the study's sponsor, to ensure that these conditions are met. No further review by the Northern B Health and Disability Ethics Committee is required.

Standard conditions:

1. Before the study commences at *any* locality in New Zealand, all relevant regulatory approvals must be obtained.

2. Before the study commences at *any* locality in New Zealand, it must be registered in a WHO-approved clinical trials registry (such as the Australia New Zealand Clinical Trials Registry, www.anzctr.org.au).

3. Before the study commences at *a given* locality in New Zealand, it must be authorised by that locality in Online Forms. Locality authorisation confirms that the locality is suitable for the safe and effective conduct of the study, and that local research governance issues have been addressed.

Summary of ethical issues (outstanding)

The main ethical issues considered by the Committee and which require addressing by the Researcher are as follows.

The Committee requested the following changes to the Participant Information Sheet and Consent Form:

- Please consider grouping information in the PIS under key headings. Please refer to the PIS and consent form template on the HDEC website for recommended headings.
- Please make the checklist on page 1 bigger so it is easier to read.
- Please include Te Ora contact details.

Non-standard conditions:

- Please amend the participant information and consent form, taking into account the suggestions by the Committee *(Ethical Guidelines for Intervention Studies, para 6.22)*.

Non-standard conditions must be completed before commencing your study. Non-standard conditions do not need to be submitted to or reviewed by HDEC before commencing your study. Do not submit non-standard conditions as a post approval form (PAF).

For information on non-standard conditions please see section 128 and 129 of the Standard Operating Procedures at http://ethics.health.govt.nz/home.

After HDEC review

Please refer to the *Standard Operating Procedures for Health and Disability Ethics Committees* (available on www.ethics.health.govt.nz) for HDEC requirements relating to amendments and other post-approval processes.

**Your next progress report is due by 03 June 2016.**

Participant access to ACC

This clinical trial is to be conducted principally for the benefit of the manufacturer or distributor of the medicine or item being trialled. Section 32 of the Accident Compensation Act 2001 provides that participants injured as a result of treatment received as part of this trial will **not** be eligible for publicly-funded compensation through the Accident Compensation Corporation (ACC).

Please don't hesitate to contact the HDEC secretariat for further information. We wish you all the best for your study.

Yours sincerely,

Mrs Raewyn Sporle
Chairperson
Northern B Health and Disability Ethics Committee

Encl:   appendix A:   documents submitted
        appendix B:   statement of compliance and list of members

**Appendix A**
**Documents submitted**

| Document | Version | Date |
|---|---|---|
| Evidence of sponsor insurance | 1 | 05 February 2015 |
| Investigator's Brochure: Viraban Product Leaflet | 1 | 01 September 2006 |
| PIS/CF: PISCF | 1 | 15 May 2015 |
| Protocol | 1 | 16 May 2015 |
| Survey/questionnaire: eDiary day 1 | 1 | 15 May 2015 |
| Survey/questionnaire: eDiary days 2-14 | 1 | 15 May 2015 |
| Survey/questionnaire: Text reminder | 1 | 15 May 2015 |
| Survey/questionnaire: Final phone call reminder | 1 | 15 May 2015 |
| Evidence of scientific review: Peer review | 1 | 05 April 2015 |
| Evidence of CI indemnity | 1 | 31 January 2015 |
| CVs for other Investigators: CVs for other Investigators: Dr Alex Semprini | 1 | 18 February 2015 |
| CV for CI: CI CV: Dr Irene Braithwaite | 1 | 31 January 2015 |
| Investigator's Brochure: Honevo IB | 1 | 08 July 2013 |
| Maori consultation process | 1 | 13 April 2015 |
| Maori consultation process 2 | 1 | 11 February 2015 |
| Pharmacy Advertisement | 1 | 11 February 2015 |
| Covering Letter: Covering letter | 1 | 15 May 2015 |
| Public advertisement | 1 | 20 May 2015 |

**Appendix B**
**Statement of compliance and list of members**

Statement of compliance

The Northern B Health and Disability Ethics Committee:

— is constituted in accordance with its Terms of Reference
— operates in accordance with the *Standard Operating Procedures for Health and Disability Ethics Committees*, and with the principles of international good clinical practice (GCP)
— is approved by the Health Research Council of New Zealand's Ethics Committee for the purposes of section 25(1)(c) of the Health Research Council Act 1990
— is registered (number 00008715) with the US Department of Health and Human Services' Office for Human Research Protection (OHRP).

List of members

| Name | Category | Appointed | Term Expires | Present on 02/06/2015? | Declaration of interest? |
|---|---|---|---|---|---|
| Mrs Raewyn Sporle | Lay (the law) | 01/07/2012 | 01/07/2015 | Yes | No |
| Mrs Maliaga Erick | Lay (consumer/community perspectives) | 01/07/2012 | 01/07/2015 | Yes | No |
| Mrs Phyllis Huitema | Lay (consumer/community perspectives) | 19/05/2014 | 19/05/2017 | Yes | No |
| Miss Tangihaere Macfarlane | Lay (consumer/community perspectives) | 19/05/2014 | 19/05/2017 | Yes | No |
| Mrs Kate O'Connor | Non-lay (other) | 01/07/2012 | 01/07/2015 | Yes | No |
| Mrs Stephanie Pollard | Non-lay (intervention studies) | 01/07/2012 | 01/07/2015 | Yes | No |
| Dr Paul Tanser | Non-lay (health/disability service provision) | 01/07/2012 | 01/07/2015 | Yes | No |

http://www.ethics.health.govt.nz

# 9.11 STANDING COMMITTEE ON THERAPEUTIC TRIALS SUBMISSION AND APPROVAL

## 1.1 Title and summary

**1.1.1.** Lay study title:

5% Aciclovir or Honevo as a treatment for cold sores

**1.1.2.** Formal study title:

An Open Label, Parallel Group, Randomized Controlled Trial of Topical 5% Aciclovir vs Honevo in the treatment of cold sores in adult participants

**1.1.3.** *A protocol and an Investigator's Brochure must be uploaded in the "Documents" tab before submission to SCOTT.*

Protocol number: KH10

**1.1.4.**

Planned study commencement date: 06/07/2015
Planned study conclusion date:      06/07/2016

**1.1.5.** Please provide a brief, plain English summary of your study.

[< 2000 characters]

Herpes Simplex Labialis (HSL) is endemic worldwide causing characteristic blisters on the lips: 'cold sores'. Recurrent episodes cause considerable pain and discomfort and risk auto-inoculation to different sites of the body and transmission to others.
Topical treatments (e.g. aciclovir) can reduce illness duration, though the therapeutic benefit is modest reducing time to healing by about half a day.
Honey is a potential topical treatment for cold sores as it has antimicrobial, anti-inflammatory, and immuno-modulatory effects.
A recent randomized controlled trial (RCT) from Dubai in 16 adult patients with recurrent attacks of herpetic lesions found topical application of honey was more effective than 5% aciclovir for HSL and genital herpes. HoneyLab (Sponsor) has also conducted a pilot study which showed that Kanuka honey was an acceptable treatment for cold sores.
This trial plans to investigate the effectiveness of medical-grade Kanuka honey (10% Glycerin and 90% honey) in the treatment of cold sores, in 950 participants recruited through pharmacies.
Participants will be approached at the pharmacy and after consenting and confirmation of eligibility, they may enroll into the study, if they wish. A questionnaire will be completed at Visit 1 to collect demographic data, history of cold sores, treatments used and duration of their current episode.
Participants will be randomized (1:1) to one of the treatments listed above. They will complete a daily diary for up to 2 weeks or until the cold sore heals, whichever is sooner. This captures pain, stage of cold sore and number of treatment applications. The diary will be completed in paper form or online for those with internet access via and eDiary.
On Day 14 the study team will call to ask the participant to return/complete the diary, to report any adverse events occurred during the study period and any other narrative feedback.

**1.1.6.** Please provide the Universal Trial Number (UTN) for this study.

U1111-1170-1537

## 1.2 Object, phase and design

**1.2.1.** Which of the following best describe your intervention study?

*Blinding:*     ⦿ open-label     ○ single-blind     ○ double-blind

*Arms:*     ⦿ two-arm     ○ multi-arm

| | | | | |
|---|---|---|---|---|
| *Design:* | ◉ parallel | ○ crossover | ○ dose-ranging | ○ cluster | ○ factorial |
| *Control:* | ○ placebo-controlled | ◉ active-controlled | ○ uncontrolled | | |
| *Randomisation:* | ◉ randomised | ○ non-randomised | | | |
| *Aim:* | ◉ superiority | ○ equivalence | ○ non-inferiority | | |
| | ○ none of the above | | | | |

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**1.2.2.** Which of the following best describe your study?

○ phase I

○ phase I/IIa

○ phase II

○ phase IIb

◉ phase III

○ phase IV

○ none of the above / not sure

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**1.3 Co-ordinating Investigator (CI)**

*The Co-ordinating Investigator (CI) has overall responsibility for the conduct of intervention studies, including adherence to established ethical standards.*

**1.3.1.** *The CI must authorise this application (through the "Authorisations" tab) before it can be submitted to SCOTT. You should request authorisation once you have completed all questions in the Online Form.*

Who is the Co-ordinating Investigator (CI) for this intervention study?

| | |
|---|---|
| | Title: Forename/Initials: Surname: |
| | Dr    Irene              Braithwaite |
| Mailing Address: | Medical Research Institute of New Zealand |
| | Private Bag 7902 |
| Suburb/Town: | Wellington |
| Postcode: | 6242 |
| Country: | New Zealand |
| Organisation: | Medical Research Institute of New Zealand |
| Department*: | |
| Position: | Medical Research Fellow |
| E-mail: | irene.braithwaite@mrinz.ac.nz |
| Phone (BH): | 048050245 |
| Phone (AH)*: | |
| Mobile*: | |
| Fax: | 043895707 |

**1.3.2.** Is the CI for this study registered with the New Zealand Medical Council?

○ yes – registration number:  42780

○ no

**1.4.1.** *The primary contact must authorise this application (through the "Authorisations" tab) before it can be submitted to SCOTT for review. You should request authorisation once you have completed all questions in the Online Form*

Who is the primary contact person for this application?

|  | Title: | Forename/Initials: | Surname: |
|---|---|---|---|
|  | Dr | Alex | Semprini |

| | |
|---|---|
| Mailing Address: | Medical Research Institute of New Zealand |
| | Private Bag 7902 |
| Suburb/Town: | Newtown/Wellington |
| Postcode: | 6021 |
| Country: | New-Zealand |
| Organisation: | Medical Research Institute of New Zealand |
| Department*: | |
| Position: | Medical Research Fellow |
| E-mail: | alex.semprini@mrinz.ac.nz |
| Phone (BH): | 021427527 |
| Phone (AH)*: | |
| Mobile*: | |
| Fax: | 043895707 |

**1.5.1. Applicant**

*The Applicant is responsible for the trial in New Zealand, and must be located in New Zealand.*

|  | Title: | Forename/Initials: | Surname: |
|---|---|---|---|
|  | Prof | Shaun | Holt |

| | |
|---|---|
| Mailing Address: | 305 Karaka Bay Road |
| | Karaka Bays |
| Suburb/Town: | Wellington |
| Postcode: | 6022 |
| Country: | New-Zealand |
| Organisation: | Honeylab |
| Department*: | |
| Position: | Director |
| E-mail: | holtshaun@gmail.com |
| Phone (BH): | 02920011111 |
| Phone (AH)*: | |
| Mobile*: | |
| Fax: | 043895707 |

**1.6.1. Trial sites**

Please provide the following details for each trial site.
*Lead (Principal) Investigators for all sites in this study must authorise this application (through the "Authorisations" tab) before it is submitted to SCOTT. You should request authorisation once you have completed all questions in the Online Form.*

---

**Name and address of site:** Medical Research Institute of New Zealand
CSB Level 7,
Wellington Hospital,
Wellington,
New Zealand
6012

**Name, address and contact person for site:1**

|   | Title: | Forename/Initials: | Surname: |
|---|--------|--------------------|----------|
|   | Dr | Alex | Semprini |

Mailing Address:        Medical Research Institute of New Zealand

                                    Private Bag 7902

Suburb/Town:            Wellington
Postcode:                   6242
Country:                     New Zealand
Organisation:             Medical Research Institute of New Zealand
Department*:
Position:                     Medical Research fellow
E-mail:                        alex.semprini@mrinz.ac.nz
Phone (BH):                021427527
Phone (AH)*:
Mobile*:
Fax:                            043895707

Principal Investigator (PI) at site: Irene Braithwaite

<u>Site certification status:</u>  certification not required

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**1.6.2.** Approximately how many participants do you intend to recruit in New Zealand?

950

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**1.6.2.1.** Please provide brief details of:

The proposed study period for individual participants: 14 days

The proposed treatment period for participants: up to 14 days (treatment ceases once lesion is healed)

The age range in years of participants: 16 years and older

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**1.6.2.2.** Are participants:

◯ male

◯ female

⦿ both male and female

---

**1.6.2.3.** Are participants:

◯ non-patient (healthy) volunteers

◉ patient volunteers

◯ both

**1.6.2.4.** Are contrast/control groups to be used?

◉ yes

◯ no

Other countries

**1.6.3.** Will your study also involve participants recruited in countries other than New Zealand?

◯ yes – how many countries?

◉ no

## 1.7 Prior review

**1.7.1.** Is this application related to one or more previous SCOTT applications?

◉ yes

◯ no

**1.7.1.1.** Please explain the relationship, giving the SCOTT reference number(s) of the previous application(s).

[< 1200 characters]

These studies are a follow on programme from the initial Pilot studies reference: TT508896(1246), Protocols KH01/KH02/KH03/KH04/KH05, and also from larger studies conducted more recently by HoneyLab/ MRINZ: 13/SCOTT/92 (Protocol KH06), 13/SCOTT/93 (Protocol KH07) and 13/SCOTT/94 (Protocol KH08).The recently reviewed KH09 trial was abandoned due to unforeseen issues with the availability of quality control for the novel investigational product combination (aciclovir and honey), therefore this trial is the same design but comparing aciclovir with honevo alone (TT50-8896(1759)).

**1.7.2.** Has an application for this study (or a substantially similar study) previously been declined approval by SCOTT?

◯ yes

◉ no

**1.7.3.** Has an application for this study (or a substantially similar study) previously been declined approval by an overseas regulator?

◯ yes

◉ no

## 1.8 Eligibility for abbreviated approval process

**1.8.1.** *Details of SCOTT's abbreviated approval process are set out here.*
Is the proposed trial a bioequivalence study utilising an investigational product that contains an active pharmaceutical ingredient included in a medicine that is approved for distribution in New Zealand?

◯ yes

◉ no

## 2.1 Trial purpose, design and publication

**2.1.1.** What is the principal study question (hypothesis) of your study?

[< 2000 characters]

That topical Kanuka honey will reduce the Herpes Simplex Labialis symptoms and will be acceptable to patients.
The primary outcome is healing time from randomization to the return to normal skin.
Secondary outcomes will be analysed as follows:
Total healing time, defined as the time from development of first sign or symptom to the return to normal skin.
Total healing time stratified by stage of the lesion at onset of treatment.
Highest pain severity.
Time to pain resolution (defined as the time from first experiencing pain to total resolution of pain).

**2.1.2.** Briefly describe the scientific basis for your study including, discussion of previous research on humans and/or animals.

[< 2000 characters]

Honey has been used for centuries, orally and topically, for a variety of medical conditions. For example, Hippocrates recommended it for treatment of wounds, sores and other ailments. It is safe to use, as would be expected from a common food. Safety risks when it is applied topically are minimal, and these are reduced further when medical-grade(purified and sterilized) honey is used. Honey has a number of specific physicochemical properties that may be responsible for its effectiveness in treating some medical conditions:
Osmotic effect – honey is a concentrated sugar solution predominantly consisting of the monosaccharides fructose and glucose. Few water molecules are available for microorganisms and therefore it is a poor environment for their growth. Hydrogen peroxide – this is slowly released when the honey comes into contact with body fluids and has antiseptic properties. Acidity – honey is acidic with a pH of 3.2 to 4.5, which makes microorganism growth difficult. Manuka honey contains bioflavonoids and other antioxidants which may contribute to its activity. Methylglyoxal MGO, found in high levels in certain honeys, such as New Zealand Manuka and Kanuka honeys, has potent antibacterial effects.
There is evidence in the medical literature of efficacy for a number of medical conditions, including burns, cough, wounds, diabetes, cold sores, peptic ulcers, constipation and weight loss (for details see Investigator's Brochure). Invitro and clinical studies show honey has antimicrobial properties, likely due to its low pH, hydrogen peroxide content and high osmolality. Honey may also have immune-modulatory properties.
Moreover, a small randomized controlled trial (RCT) from Dubai in 16 adult patients with recurrent attacks of herpetic lesions found topical application of honey was more effective than 5% aciclovir cream for HSL and genital herpes.

**2.1.3.** Please explain how your study will contribute to new knowledge and improve health outcomes.

[< 2000 characters]

There is a need for novel effective therapies for the treatment and management of Cold sores, which are acceptable to patients. This study will ascertain the effectiveness of Kanuka honey in treating cold sores, potentially improving the health outcomes of those with the condition and contribute to the knowledge base around the use of honey for the treatment of skin conditions.

**2.1.4.** Please briefly describe the design of your study, and explain why it is appropriate.

[< 2000 characters]

Open label, randomised, 2-way active comparator, parallel group trial.
Participants will be randomized 1:1 to topical treatment with either Medical grade Kanuka honey (90%/glycerin 10% (Honevo, HoneyLab)) or 5% acyclovir (Viraban) alone.
The median control duration of HSL symptoms is assumed as 5 days. To achieve a one day median difference, with associated Hazard ratio of 1.25, 423 participants would be needed per arm of treatment, therefore a total of 846. The study is deemed as short (one cold sore episode) so the drop out rate is assume to be around 10%, then 950 participants will be randomized. This study size is consistent with similar 2/3 arm cold sore studies have required around 300/350 patients per treatment arm.

Participants will be instructed to apply Honevo five times per day, and 5% aciclovir five times per day for 14 days or until the lesion completely resolves, whichever is earlier.
Due to physical characteristics of honey it is not possible to blind subjects to the treatment they are receiving.

**2.1.5.** Has a biostatistician been consulted on the design of your study?

- ◉ yes
- ○ no

**2.1.6.** Please provide details, and indicate who will be responsible for analysing data.

[< 2000 characters]

Professor Mark Weatherall, Otago University, Wellington, was consulted on the study design and power calculations and in regard to appropriate analysis if the outcome variables.
Prof Weatherall will conduct the statistical analysis.

**2.1.7.** How do you intend to report or disseminate the results of your study?

- ☑ articles in peer-reviewed scientific journals
- ☑ internal reports
- ☑ conference presentations
- ☑ publication on website
- ☑ other publications
- ☑ submission to regulatory authorities (e.g. Medsafe, TGA, FDA, EMA)
- ☐ other
- ☐ no plans to report or disseminate results

## 2.2 Technical information

**2.2.1.** Please provide the following details for each new medicine that is to be used in your study.

| | |
|---|---|
| Trade name (if any): | N/A |
| Dose form: | Topical |
| Active ingredient: | Medical Grade Kanuka honey + glycerine (Honevo) |
| Chemical name: | N/A |
| Biological name: | N/A |
| Non-proprietary name: | N/A |
| Identifying code: | N/A |
| Other descriptor: | N/A |
| Location of dispensing schedule in attached protocol/IB: | Protocol Page 6 |

Does the product contain a substance listed in a schedule to the Misuse of Drugs Act 1975?

- ○ yes
- ◉ no

**2.2.2.** Please indicate the location of the following information in the protocol and/or Investigator's Brochure by entering "PR" or "IB" followed by the page number in the relevant document.
If the information is not contained in these documents, please provide brief comment and indicate whether supplementary documents are provided.

| | | |
|---|---|---|
| **Chemical and pharmaceutical data** | Chemical structure | Not available |
| | Stereochemistry | Not available |
| | Physicochemical data (incl. solubility, kPa) | Not available |
| | Formulation of investigational product | IB3 and Manufacturing Specification |
| | Stability | Not available |
| | Bioavailability | Not available |
| **Animal data** | Pharmacology | Not available |
| | Toxicology | Not available |
| **Human data** | Pharmacokinetics | Not available |
| | Pharmacodynamics | Not available |
| | Efficacy | Not available |
| | Side Effects | IB3-5 |
| | Interactions | Not available |
| **Purpose of trial** | Statement of hypothesis to be tested | PR4/8 |
| | Justification for and significance of study | PR4 |
| **Recruitment and selection methods** | Inclusion criteria | PR4 |
| | Exclusion criteria | PR4/5 |
| | Criteria for exclusion during trial | PR8/9 |
| | Handling of emergencies during trial | PR9/10 |
| | Estimated time to recruit participants | PR8 |
| **The medicine** | Indication(s) for which medicine to be studied | PR5 |
| | Dosage schedule | PR6 |
| | Route of administration | PR6 |
| | Washout of existing medicines | PR8/9 |
| | Other medicines/treatments | PR8/9 |

|  |  |  |
|---|---|---|
|  | to continue during trial |  |
|  | Other medicines not permitted during trial | PR8/9 |
| **Assessments and when made** | Assessment of trial efficacy | PR7 |
|  | Assessment of toxicity/side effects | PR7/10 |
|  | Assessment of compliance | PR7 |
|  | Trial termination, if trial is hazardous (or obviously successful) | PR9/10 |
|  | Other | PR7 |
| **Data analysis** | Justification for number of participants | PR11 |
|  | How dropouts and discontinuations will be handled | PR9 |
|  | Summary table of phases, measures, and measurement points (optional, but desirable in any complex trail) | PR7 |
| **Informed consent** | How dropouts and discontinuations will be handled | PR5 |
|  | Consent form and procedure | PISCF attached |
|  | Patient information sheet | PISCF attached |

*Please ensure that the following documents are uploaded in the "Documents" tab before proceeding to submission.*

- *a protocol*
- *an Investigator's Brochure*
- *CVs for the Co-ordinating Investigator and all Principal Investigators*
- *site (re)certification for all trial sites, where applicable*
- *sample labels for all new medicines*
- *GMP certification for manufacturer(s)*
- *GMP certification for packer(s)*
- *Investigator consent form/signed protocol*

**MEDSAFE**
NEW ZEALAND MEDICINES
AND MEDICAL DEVICES
SAFETY AUTHORITY
A BUSINESS UNIT OF
THE MINISTRY OF HEALTH

Freyberg Building
20 Aitken Street
PO Box 5013
Wellington 6145, New Zealand
**T** +64 4 496 2000

3 June, 2015

TT50-8896
(1759)
15/SCOTT/14

Alex Semprini
Medical Research Institute of New Zealand
Private Bag 7902
Wellington 6242

Dear Mr Semprini

**Clinical Trial on Honey (Medical-Grade Kanuka Honey)**
**Protocol Number: KH10**

Further to your letter of 19 May 2015, SCOTT would like to thank you for your
response and, I am pleased to advise you that this clinical trial has been approved
by the Director-General of Health.

You are therefore authorised to distribute Honey (Medical-Grade Kanuka Honey) for
the purposes of this clinical trial to the following approved investigator(s):

| Approved Investigators | |
|---|---|
| Name | Site |
| Dr Irene Braithwaite | Medical Research Institute of New Zealand Level 7, CSB Building Wellington Hospital Riddiford Street Wellington 6021 |
| Ms Pam Bremford | Unichem Pharmacy 37 Bay Road Kilbirnie WELLINGTON |

Please note that it is your responsibility to obtain HDEC approval before your trial can commence in New Zealand.

**Legal reporting and record keeping requirement**

It is a requirement of the Medicines Act 1981 that you

1. report the progress of the trial to the Director-General of Health at six monthly intervals;

2. report the results of the trial to the Director-General of Health on completion of the trial;

3. report serious adverse reactions which occur during the trial to the Director-General in accordance with the requirements of the Guideline on the Regulation of Therapeutic Products in New Zealand, Part 11: Clinical trials – regulatory approval and good clinical practice requirements;

4. keep complete and accurate records of all quantities of the trial medicine supplied during the trial;

5. ensure that every label on every package or container of the trial medicine bears the words "To be used by qualified investigators only" or words of similar meaning.

**Additional reporting requirements**
If a patient of a medical practitioner who is not an investigator is a trial subject, that medical practitioner should be kept informed of the progress of the trial.

**Importation of the trial medicine**
If requested, you should present this letter to New Zealand Customs as evidence that the Ministry of Health has no objection to the importation of this clinical trial medicine.

Importation of any investigational product which contains controlled drugs that are scheduled under the Misuse of Drugs Act also require additional Licence(s). It is your responsibility to ensure that you have the appropriate licences before you commence importation of any investigational product which contains controlled drugs. For further information regarding this process and an application form please contact Medicines Control at medicinescontrol@moh.govt.nz

In all further correspondence concerning this medicine, please quote the file reference TT50-8896 (1759).

Yours sincerely
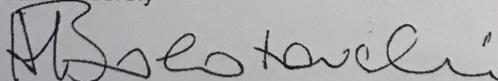
Dr Alexander Bolotovski
for Director-General of Health

**ANZCTR**
Australian New Zealand Clinical Trials Registry

**Please note:** All data submitted to this register will be made publicly available.

Questions in **bold** text are mandatory. **(*)**

| Request Number: | 368118 |
| --- | --- |
| Current Page: | Review |

## Trial from ANZCTR

| Trial ID | |
| --- | --- |
| Trial Status: | Submitted |
| Date Submitted: | 10/06/2015 |
| Date Registered: | |

### Page 1   Edit 🖉

| Public title | 5% Aciclovir or Honevo™ as a treatment for cold sores |
| --- | --- |
| Study title in 'Participant- Intervention- Comparator- Outcome (PICO)' format | In adult patients with cold sores will topical medical grade honey reduce healing time compared to topical 5% aciclovir alone. |
| Secondary ID [1] | None |
| UTN | U1111-1170-1537 |
| Trial acronym | KH10 |

### Page 2   Edit 🖉

| Health condition(s) or problem(s) studied: | |
| --- | --- |
| Herpes Labialis | |

| Condition category: | Condition code: |
| --- | --- |
| Skin | Dermatological conditions |

### Page 3   Edit 🖉

| Descriptions of intervention(s) / exposure | Topical medical grade Kanuka honey applied to the affected area five times daily for fourteen days or until skin returns to normal, whichever is sooner. A participant diary will record applications during the treatment period. |
| --- | --- |
| Intervention Code: | Treatment: Other |
| Comparator / control treatment | Standard treatment is 5% topical aciclovir cream. Participants in the control arm will apply 5% topical aciclovir to the affected area five times daily for fourteen days or until the skin returns to normal. |
| Control group | Active |

### Page 4   Edit 🖉

| Primary Outcome: | The primary outcome is a decrease in the healing time (from randomization to the return to normal skin) as measured daily by participant reported cold sore stage from a pictorial chart. |
| --- | --- |
| *Timepoint:* | Daily up to fourteen days. |
| Secondary Outcome: | Total healing time, defined as the time from development of first sign or symptom to the return to normal skin, as measured daily by participant reported cold sore stage from a pictorial chart. |
| *Timepoint:* | Daily up to fourteen days. |
| Secondary Outcome: | Total healing time stratified by stage of the lesion at onset of treatment, as measured daily by participant reported cold sore stage from a pictorial chart. |
| *Timepoint:* | Daily up to fourteen days. |
| Secondary Outcome: | Highest pain severity as recorded daily by participants using the scale 'o - 10' with 0 being no pain and 10 being most pain. |
| *Timepoint:* | Daily up to fourteen days. |
| Secondary Outcome: | Time to pain resolution (defined as the time from first experiencing pain to total resolution of pain), as recorded daily by participants using the scale '0 - 10' with 0 being no pain and 10 being most pain. |
| *Timepoint:* | Daily up to fourteen days. |

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| | |
|---|---|
| Key inclusion criteria | Aged 16 years or over at the time of enrolment.<br>Presentation to a pharmacy for treatment of a cold sore.<br>First cold sore symptoms (including prodromal symptoms e.g. tingling/pain) within 72 hours. |
| Minimum age | 16 Years |
| Maximum age | No limit |
| Gender | Both males and females |
| Healthy volunteers? | Yes |
| Key exclusion criteria | Any participant who is pregnant or breastfeeding.<br>Known or suspected allergy to honey, aciclovir and/or glycerin.<br>Any other condition which, at the investigators' discretion, it is believed may present a safety risk or impact the feasibility of the study or the study results.<br>Patient has used oral aciclovir or other antiviral medicine, or any topical treatment, medical or complementary, on the current sore.<br>Participants planning to take or use any concomitant medications, which in the opinion of the investigator, could affect the cold sore during the course of the trial. This includes any topical product, medical or complementary, on the cold sore, oral aciclovir or other antiviral medicine, oral complementary medicines for cold sores, such as lysine supplements. |

| | |
|---|---|
| Study type | Interventional |
| Purpose of the study | Treatment |
| Allocation to intervention | Randomised controlled trial |
| Describe the procedure for enrolling a subject and allocating the treatment (allocation concealment procedures) | Eligible participants will be randomised into the study in a 1:1 ratio, one arm receiving topical medical grade Kanuka honey and the second arm receiving topical 5% aciclovir. Both groups will apply their specific treatment five times a day. Each pharmacy site will be provided an allocation of randomised patient packs containing the treatment allocation and patient diary. Pharmacists will dispense the appropriate treatment as randomised to the participant upon opening of the concealed envelope. |
| Describe the methods used to generate the sequence in which subjects will be randomised (sequence generation) | Biostatistitian to generate randomisation schedule. |
| Masking / blinding | Open (masking not used) |
| Who is / are masked / blinded (choose all that apply) | |
| Assignment | Parallel |
| Other design features | Participants and study site staff will not be blinded to the allocated treatment due to the nature of the investigational product. Data analysis will be conducted by a statistician blinded to the allocation. |
| Type of endpoint(s) | Safety/efficacy |
| Statistical Methods/Analysis | Assuming that the median control duration of symptoms is five days and looking to achieve a one day median difference, with associated Hazard ratio of 1.25, 423 participants would be needed per arm of treatment, therefore the total required is 846. As this is a short study lasting only for the duration of one cold sore episode, then drop-outs from the study should be low, and so assuming that the drop-out rate is around 10%, then 950 participants will be randomized. This study size is consistent with similar 2-arm or 3-arm cold sore studies have required around 300-350 patients per treatment arm. Kaplan-Meier survival plots and estimates of median healing times and Cox Proportional Hazards with a random effect for participants to take into account the parallel design, compared time to healing and pain duration between treatments. Paired t-tests will be used to compare the continuous variables. |

| | |
|---|---|
| Phase | Phase 3 |
| Anticipated date of first participant enrolment | 6/07/2015 |
| Date of first participant enrolment | |
| Anticipated date last participant recruited/enrolled | 6/06/2017 |
| Actual date last participant recruited/enrolled | |
| Target sample size | 950 |
| Recruitment status | Not yet recruiting |

**Recruitment outside Australia**

| Country: | New Zealand |
|---|---|
| State/Province: | |

### Page 8   Edit ✏

| Funding Source: | Commercial sector/Industry |
|---|---|
| Name: | Honeylab Ltd |
| Address: | Honeylab Ltd<br>305 Karaka Bay Road<br>Wellington 6022 |
| Country: | New Zealand |
| **Primary Sponsor** | Commercial sector/Industry |
| Name: | Honeylab Ltd |
| Address: | Honeylab Ltd<br>305 Karaka Bay Road<br>Wellington 6022 |
| Country: | New Zealand |
| **Secondary Sponsor:** | None |
| Name: | |
| Address: | |
| Country: | |
| Other Collaborator: | Charities/Societies/Foundations |
| Name: | Medical Research Institute of New Zealand |
| Address: | Medical Research Institute of New Zealand<br>Private Bag 7902<br>Wellington 6242 |
| Country: | New Zealand |

### Page 9   Edit ✏

| Has the study received approval from at least one Ethics Committee? | Yes |
|---|---|
| Ethics Committee name: | Northern B Health and Disability Ethics Committee |
| Address: | Ministry of Health<br>Ethics Department<br>Freyberg Building<br>Reception – Ground Floor<br>20 Aitken Street<br>Wellington |
| Country: | New Zealand |
| Approval Date: | 09/06/2015 |
| Submitted Date: | |
| HREC: | 15/NTB/93 |
| Brief summary | A recent pilot study has shown that medical grade Kanuka honey is an acceptable treatment for cold sores. This large-scale randomised controlled trial has the potential to demonstrate a combination product of Kanuka honey and aciclovir (the current topical standard treatment) as an effective treatment for cold sores. In this study 950 participants seeking over the counter treatment for a cold sore will be randomised to receive either topical medical grade Kanuka honey or topical 5% aciclovir alone. Participants will apply their treatment five times a day for fourteen days or until the skin returns to normal, whichever is sooner. Participants will complete a paper or online diary recording the number of applications, the pain suffered that day and the stage of the cold sore based on a pictorial chart. At day fourteen each participant will receive a follow up telephone call to record adverse events and obtain narrative feedback. |
| Trial website | |
| Trial related presentations / publications | |
| Public Notes | |
| Attachments [1] | http://www.anzctr.org.au/AnzctrAttachments/368118-HDEC Letter 15NTB93 Approved FULL Application with Non Standard Conditions.pdf |

### Page 10   Edit ✏

**Principal Investigator**

| Title: | Dr |
|---|---|
| Name: | Irene Braithwaite |

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# 9.13    BIO US conference PRN brochure

The Pharmacy Research Network is run by MRINZ, New Zealand's leading independent medical research organisation.

In collaboration with the NZ Pharmacy Guild, Pharmaceutical Society and Community Pharmacy Industry we have the capacity to undertake randomised controlled trials of over the counter, complementary and alternative medicines with Global applicability.

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Dr Alex Semprini,

**Network Director
(MBBS, BSc Hons)**

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pharmacy research network

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## PRN Studies

**honey lab®**

950 patient study assessing efficacy of medical grade honey vs 5% aciclovir for the treatment of herpes labialis

**HIKURANGI**

Programme of research assessing efficacy of kanuka oil extracts in the topical treatment of acne and eczema

## PRN Partners

**PHARMACY GUILD OF NEW ZEALAND**

**GREEN CROSS HEALTH**

# PHARMACY RESEARCH NETWORK

The PRN was developed by the Medical Research Institute of New Zealand to facilitate large scale randomised controlled trials in therapeutic areas and patient groups where traditional methodology faced difficulty in data collection, recruitment and financial resourcing.

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The PRN delivers unique access to a diverse and representative patient population via the pharmacist - patient interface. Utilising our extensive patient database and investigator network provides fast and effective recruitment, with over 100 patients enrolled in one month.

### ROBUST AND RAPID DATA

Integrated informatics and a centralised database allows for real time data collection and monitoring.

### PROTOCOL TO PUBLICATION

The MRINZ will manage the entire study process from protocol development, obtaining all ethical and regulatory approvals, manage recruitment, study data, statistical analysis to manuscript preparation and publication.

# Kanuka honey versus aciclovir for the topical treatment of herpes simplex labialis: a randomised controlled trial

Alex Semprini,[1] Joseph Singer, Irene Braithwaite,[1] Nick Shortt,[1]
Darmiga Thayabaran,[1] Melanie McConnell,[2] Mark Weatherall,[3] Richard Beasley[1]

¹Medical Research Institute of New Zealand, Wellington, New Zealand
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## ABSTRACT

**Objective** To compare New Zealand medical grade kanuka honey with topical aciclovir for the treatment of herpes simplex labialis.

**Design** Prospective parallel randomised controlled open-label superiority trial.

**Setting** 76 community pharmacies across New Zealand between 10 September 2015 and 13 December 2017.

**Participants** 952 adults randomised within the first 72 hours of a herpes simplex labialis episode.

**Interventions** Random assignment 1:1 to either 5% aciclovir cream or medical grade kanuka honey (90%)/glycerine (10%) cream, both applied five times daily.

**Outcome measures** The primary outcome was time from randomisation to return to normal skin (stage 7). Secondary outcomes included time from randomisation to stage 4 (open wound), time from stage 4 to 7, maximal pain, time to pain resolution and treatment acceptability.

**Results** Primary outcome variable: Kaplan-Meier-based estimates (95% CI) for the median time in days for return to normal skin were 8 (8 to 9) days for aciclovir and 9 (8 to 9) for honey; HR (95% CI) 1.06 (0.92 to 1.22), p=0.56. There were no statistically significant differences between treatments for all secondary outcome variables. No related serious adverse events were reported.

**Conclusion** There was no evidence of a difference in efficacy between topical medical grade kanuka honey and 5% aciclovir in the pharmacy-based treatment of herpes simplex labialis.

**Trial registration number** ACTRN12615000648527;Post-results

## INTRODUCTION

Herpes simplex virus (HSV) is a pervasive human infection. The global prevalence is reported to be up to 90%.[1] The two forms of the virus, HSV-1 and HSV-2, traditionally manifest with orolabial and genital symptoms, respectively; however, anatomical overlap exists. Herpes simplex labialis (HSL) is a painful and incurable condition. The cosmetic appearance, affecting the mouth and lips, results in considerable stigma among the 30% who suffer from recurrent attacks.[2]

Some HSL episodes are severe and require oral antiviral treatment. However, the majority are self-limiting and treated with medications such as aciclovir cream. Aciclovir inhibits viral replication within host cells through a series of conversions to aciclovir triphosphate, a competitive inhibitor of viral DNA polymerase.[3] The topical gold standard for the treatment of HSL for over 20 years, aciclovir is available over-the-counter and there is moderate evidence that it reduces healing time and self-reported pain for HSL attacks.[4] Novel treatment approaches are needed because of emerging resistance to aciclovir in particular patient groups,[5] limited risk profiling available (the Food and Drug Administration gives aciclovir a pregnancy category B rating)[6] and consumer-driven desire to use complementary and alternative medicines (CAM).[7]

Honey has a long history of therapeutic use,[8] with an expanding contemporary evidence base, particularly for topical application in wound care, burns and dermatological diseases such as rosacea and HSV.[9–12] A small pilot study of HSL reported a reduction in healing time from a mean 5.9 days for aciclovir to a mean 2.6 days with topical application of honey.[12] The mechanisms of a therapeutic benefit for honey could include physiochemical properties such as low pH and high osmolality and mechanistically independent bioactive factors that vary between honeys; these bioactives may be anti-inflammatory, antiviral and promote wound healing.[13–15] Kanuka honey is derived from the kanuka tree (*Kunzea ericoides*) of the same family as the better studied manuka tree

(*Leptospermum scoparium*) and has been shown to have immunostimulatory properties in vitro.[16] The virucidal properties of manuka honey have been demonstrated in preclinical assays, with efficacy against influenza, varicella zoster and rubella viruses.[17–19] As a member of the same botanical family, Myrtaceae, kanuka-derived honey may offer similar antiviral effects. While the exact factors responsible have yet to be identified, compounds present in honey such as flavonoids have demonstrable antiviral activity; however, further research into the mechanism and clinical relevance is required.[20]

Here, we report a 952-participant randomised controlled trial (RCT) of medical grade, New Zealand kanuka honey cream for the treatment of HSL compared with 5% aciclovir cream, within a community Pharmacy Research Network. We wished to test the hypothesis based on the available study data[12] that topical kanuka honey applied to HSL lesions would reduce the healing time to normal skin by at least 1 day compared with topical 5% aciclovir.

## METHODS
### Trial design
This was a parallel randomised, open-label, active comparator trial in which participants were assigned to either topical medical grade honey or topical 5% aciclovir cream for the treatment of an active HSL lesion. Participants applied allocated treatment for up to a maximum of 14 days. The primary outcome variable was time from randomisation to complete healing of skin. The trial design, statistical methods and Pharmacy Research Network development have been reported previously.[21]

### Setting and participants
All participants were aged 16 years and over, recruited by a New Zealand-wide network of community pharmacy-based investigators within the first 72 hours from the onset of a new HSL episode. Exclusion criteria were current pregnancy or breast feeding; allergy to honey, bees glycerine or aciclovir; use of antiviral medication for the current cold sore or any oral antiviral medicine during the previous 2 weeks; planned use of additional cold sore medications during the study period; any condition that presented a risk to participant safety or the integrity of the study data. International Conference on Harmonisation Good Clinical Practice guidelines were adhered to for all study procedures and all investigators trained in Good Clinical Practice during the site initiation visit.

### Randomisation and blinding
A biostatistician, blinded to treatment allocation, electronically generated 1:1 randomisation schedules for each uniquely coded study site using a randomisation block of four (Minitab V.13). Allocations were concealed within brown opaque envelopes, opened only at the point of randomisation by an investigator. The study was open-label due to the characteristics of honey rendering masking impossible; therefore, both the pharmacist and the participant were aware of the randomised treatment. The central coordinating study team were blind to allocation at all times unless a safety issue required unmasking of the study physician.

### Interventions
Treatments were 5% aciclovir cream (Viraban, AFT Pharmaceuticals, Auckland, New Zealand) and 90% kanuka honey/10% glycerine cream (Honevo, Honeylab Ltd, Tauranga, New Zealand). Both study groups were instructed to apply their randomised treatment five times daily until the skin returned to normal as per a visual healing chart (figure 1) or 14 days had elapsed, which ever occurred sooner.

### Outcome variables
The primary outcome was healing time from randomisation to normal skin (stage 7). Secondary outcomes were time from randomisation to stage 4 (open wound); time from stage 4 to stage 7; time to pain resolution; highest pain severity on a scale from 0 (no pain) to 10 (severe pain) and acceptability of treatments on a scale from 1 (unacceptable) to 10 (acceptable). Simple data descriptions for median and IQR and minimum to maximum are shown for the continuous variables with counts and proportions for categorical variables, all by randomisation status. An amendment was approved to add time from randomisation to stage 4 (open wound) and time from stage 4 to stage 7 as outcome variables to ensure any stage-dependent benefits to healing were reported.

### Data collection
Following randomisation and dispensing of study treatment, the enrolment Case Report Form was transmitted via eFax with pdf conversion, directly to the coordinating investigators. This information was entered into the study database and a Short Message Service sequencer triggered, which sent 14 unique daily diary hyperlinks to the participant's smart phone at 18:00 hours. Participants self-recorded data for pain, number of applications and the stage of their HSL progression referenced to the seven-stage pictorial chart embedded within the hyperlink (figure 1). Each morning, missing entries for the day prior were screened and participants followed up directly via telephone to ensure real-time data entry. For those participants unable to use the designated study technology, a paper diary was used and sent back to the coordinating centre on completion of the study. A final follow-up contact was conducted via telephone at or on the closest working day to day 15, with two further attempts up to day 22 if unsuccessful, to record adverse events, concomitant medications and acceptability scoring.

### Safety monitoring
Adverse event data were collected at the follow-up visit and additional contact points during the study period. All were reviewed by the study physician, categorised via a standard severity reporting system and reported to the

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| Stage 1 Prodrome | Initial onset of tingling and itching. No sore is present and skin appears normal. Not everyone gets these symptoms. | |
|---|---|---|
| Stage 2 Redness | Skin has some redness and swelling. No blister. May itch or feel tender. | |
| Stage 3 Small blister | Small blisters form in the affected area, clear at first, turn yellow with time. Usually painful. | |
| Stage 4 Ulcer | Completely formed cold sore with open areas where blisters were. Sore and painful. | |
| Stage 5 Drying up & Healing | After some days a flaky crust develops resembling a scab | |
| Stage 6 Drying up & Healing | Crust flakes off with healing skin underneath. Cold sore cycle is complete, maybe some residual redness of skin for a few days. | |
| Stage 7 Healed – skin back to normal | Skin is entirely back to normal | |

**Figure 1** Visual staging chart for HSL progression. Participants recorded the stage of the lesion each evening during the study period within an electronic Case Report Form delivered via Short Message Service hyperlink. The pictorial chart was embedded within this link. All participants were provided a paper back up in case of unavailable technology or systems failure.

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HDEC according to national guidelines. An independent Data and Safety Monitoring Committee convened to review the data of the first 100 participants and recommended the addition of anaphylactic allergy to bee stings be added to the exclusion criteria. This was actioned and approved by the HDEC.

### Statistical analysis

Analysis was intention to treat (ITT). Participants who provided time-to-event data without reaching the end point were included as censored. Participants with no time-to-event data were excluded from the analysis. For the time to outcome variables in days: time to stage 7, time to stage 4, time from stage 4 to stage 7 and time to a pain score of zero, Kaplan-Meier plots, associated product limit-based estimates of the median time to event and the HR for the particular event by Cox proportional hazards were used for analyses. An interaction term was used to test if the stage at randomisation affected the response to treatment. A t-test was used to compare the maximum pain scores. The acceptability outcome measure was left skewed and Mann-Whitney test and the Hodges-Lehmann estimator of location were used. The sample size calculation was based on previously reported HRs of 1.23 and 1.24 for a median 5-day duration of symptoms, which implies a 1-day median reduction to 4 days.[22] Using this assumption of a 5-day median time to healing and clinically significant 1-day median difference in favour of honey, with an associated HR of 1.25, 80% power and 5% type I error rate, a total of 423 participants were required per arm. Nine hundred fifty in total were to be randomised, to take into account an assumed attrition rate of 10%.

### Post hoc analyses

Post hoc analyses included time from randomisation to stage 6 (loss of crust and residual erythema), in order to benchmark performance with the seminal aciclovir studies, which used this stage of healing as the primary outcome instead of return to normal skin.[22] In addition, aborted episodes, proportions of HSL episodes not reaching the blistering stage, were estimated, defined by those participants with a baseline stage of 3 or less that progressed to stage 7 without recording 4, 5 or 6.

SAS V.9.4 was used for analysis.

### Patient involvement

Participants were not involved in the design conduct or interpretation of the study. The main study results will be disseminated to those participants who requested this on enrolment.

### RESULTS

Participants were recruited from 10 September 2015 through to completion on 13 December 2017 within the Pharmacy Research Network, involving 76 community pharmacies across New Zealand. Nine hundred fifty-two participants were randomised to either 5% aciclovir

| Table 1 | Participant characteristics | |
|---|---|---|
| | **Median (IQR)** | |
| **Continuous variables** | Aciclovir, n=475 | Honey, n=477 |
| Age (years) | 42 (30–54) | 43 (29–55) |
| Time since last HSL episode (months) | 3 (1–7) | 3 (1–6) |
| Episodes of HSL in previous year (N) | 3 (1–5) | 3 (2–5) |
| Pain score at baseline | 2 (1–4) | 2 (1–4) |
| Time from onset of symptoms to randomisation (days) | 1 (0–1) | 1 (0–1) |
| **Categorical variables** | **N/475 (%)** | **N/477 (%)** |
| Female sex | 364 (76.6) | 350 (73.5) |
| Ethnicity | | |
| NZ European | 360 (75.8) | 354 (74.3) |
| Māori | 62 (13.1) | 66 (13.8) |
| Other | 53 (11.1) | 57 (11.9) |

HSL, herpes simplex labialis.

control (n=475) or honey (n=477). The participants are described in table 1. The participants had a median of three HSL episodes in the previous year and the median time since the last episode was 3 months.

The flow of participants is shown in figure 2. Four participants in the aciclovir group and one in the honey group were dispensed the incorrect treatment. There were 91 participants lost to follow-up (49 aciclovir, 42 honey) and nine withdrew from the study due to adverse events (three aciclovir, six honey). In the final ITT analysis, 852 participants provided data. The 100 participants excluded were defined as those that did not provide any time-to-event data and therefore unable to be included in the survival analysis of the primary outcome variable. All participants that provided data were analysed according to allocation in line with ITT principles.

### Primary outcome variable

There was no evidence of a difference in the time from randomisation to complete healing between aciclovir and honey. The Kaplan-Meier-based estimates (95% CI) for median healing time were 8 (8 to 9) days for aciclovir (33 censored) and 9 (8 to 9) days for honey (44 censored); HR (95% CI) 1.06 (0.92 to 1.22), p=0.56 (figure 3A). There was no interaction between the stage at randomisation treated as a continuous variable and randomised treatment (p=0.49).

### Secondary outcome variables
#### Healing time

The estimated median time from randomisation to stage 4 (open wound) was 2 days for aciclovir (three censored) and 2 days for honey (nine censored); HR (95% CI) 1.05 (0.92 to 1.20), p=0.46 (table 2, figure 3B). The estimated
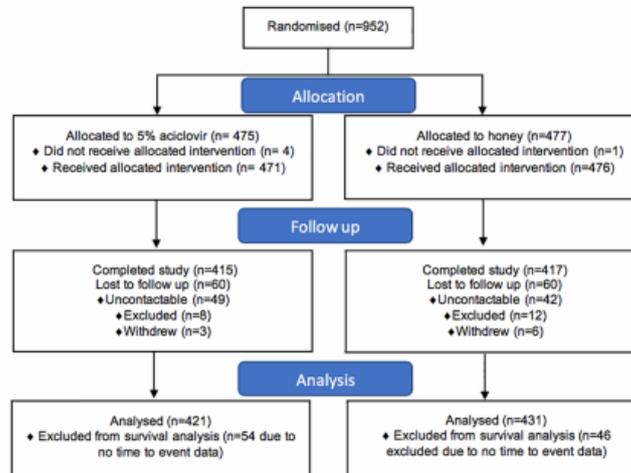
**Figure 2**   Study CONSORT diagram.

median time from stage 4 to complete healing was 7 days for aciclovir (35 censored) and for honey (40 censored); HR (95% CI) 1.03 (0.90 to 1.19), p=0.66 (figure 3C).

### Pain

The estimated median time (95% CI) to pain resolution was 9 (8 to 9) days for aciclovir (36 censored) and 9 days (8 to 9) for honey (42 censored); HR (95% CI) 1.04 (0.91 to 1.20), p=0.56 (figure 3D). The median (IQR) maximal pain for aciclovir was 3 (2 to 5) and for honey 3 (2 to 5), with a difference (95% CI) for aciclovir minus honey −0.02 (-0.32 to 0.28), p=0.90 (figure 4A).

### Acceptability

For overall acceptability of treatment, the median (IQR) score for aciclovir was 9 (8 to 10) and honey 9 (8 to 10) (figure 4B).

### Post hoc analyses
#### Healing time to stage 6

There was no difference in estimated median (95% CI) time from randomisation to stage 6 (some residual erythema): 5 days (5 to 6) for aciclovir (seven censored)
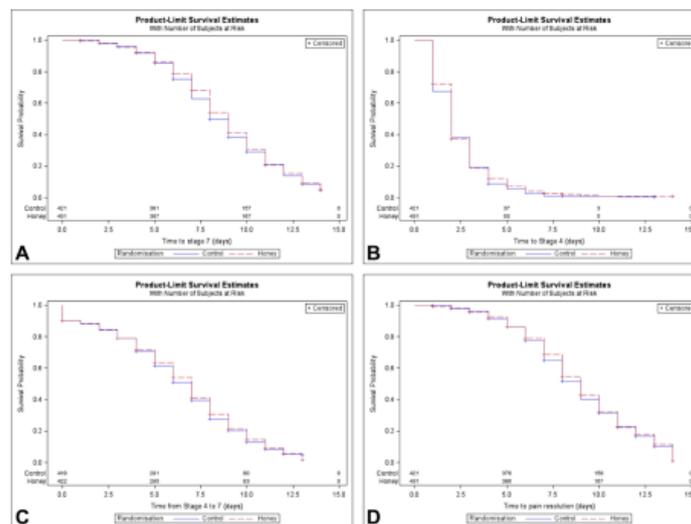


**Figure 3**   Kaplan-Meier survival plots for: (A) time to stage 7; (B) time to stage 4; (C) time from stage 4 to stage 7; (D) time to pain resolution.

**Table 2**  Censoring, estimated medians and HRs for all time-to-event outcome variables

| Time to stage 7 from randomisation | Control | Honey |
|---|---|---|
| N uncensored (censored) | 388 (33) | 387 (44) |
| Median time to complete healing in days (95% CI) | 8 (8 to 9) | 9 (8 to 9) |
| | **HR (95% CI)** | **P value** |
| Aciclovir versus honey | 1.06 (0.92 to 1.22) | 0.56 |
| **Time to stage 4 from randomisation** | **Control** | **Honey** |
| N uncensored (censored) | 418 (3) | 422 (9) |
| Median time to stage 4 in days (95% CI) | 2 (not estimable) | 2 (not estimable) |
| | **HR (95% CI)** | **P value** |
| Control versus honey | 1.05 (0.92 to 1.20) | 0.46 |
| **Time from stage 4 to stage 7** | **Control** | **Honey** |
| N uncensored (censored) | 383 (35) | 382 (40) |
| Median time from stage 4 to stage 7 in days (95% CI) | 7 (6 to 7) | 7 (6 to 7) |
| | **HR (95% CI)** | **P value** |
| Control versus honey | 1.03 (0.90 to 1.19) | 0.66 |
| **Time to pain resolution from randomisation** | **Control** | **Honey** |
| N uncensored (censored) | 385 (36) | 389 (42) |
| Median time to pain resolution in days (95% CI) | 9 (8 to 9) | 9 (8 to 9) |
| | **HR (95% CI)** | **P value** |
| Control versus honey | 1.04 (0.91 to 1.20) | 0.56 |

and 5 days (5 to 6) for honey (17 censored); HR (95% CI) 1.06 (0.93 to 1.22), p=0.39 (figure 5).

### Aborted episodes

There was no evidence of a difference in the proportions of participants recording stage 3 or less at baseline who reached the primary outcome variable with no recorded stage 4, 5 or 6 comprising 43/431 (10.0%) in the aciclovir group and 45/421 (10.7%) of participants in the honey group; relative risk (95% CI) 0.93 (0.63 to 1.39), p=0.73.

### Adverse events

There were two serious adverse events deemed unrelated to study treatments, one hospital admission for atrial fibrillation and one hospital admission for urinary retention, and there were 17 adverse events classified as 'definitely/probably/possibly' related to the investigational products, 6 for aciclovir and 11 for honey. These were further subcategorised into pain on application (nil aciclovir, three honey), swelling postapplication (two aciclovir, two honey) and worsening/further cold sores (four aciclovir, six honey).

### DISCUSSION

This study found no difference between the effectiveness of medical grade kanuka honey and topical 5% aciclovir in the community-based management of HSL. Both treatments had similar efficacy across all outcome variables including time to healing, pain resolution and proportion of aborted episodes between treatment groups. Both treatments were considered highly acceptable by participants.

For the primary outcome variable of time to complete healing, there was no difference between the randomised
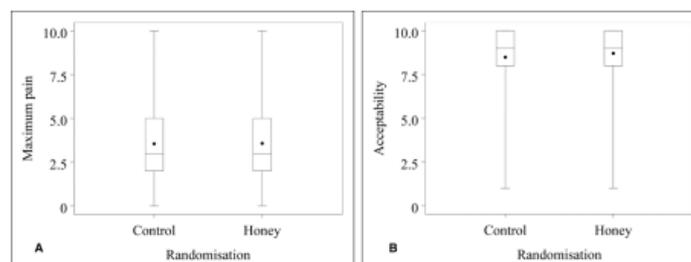


**Figure 4**  Box plots (A) maximum pain recorded over the study period; (B) treatment acceptability.
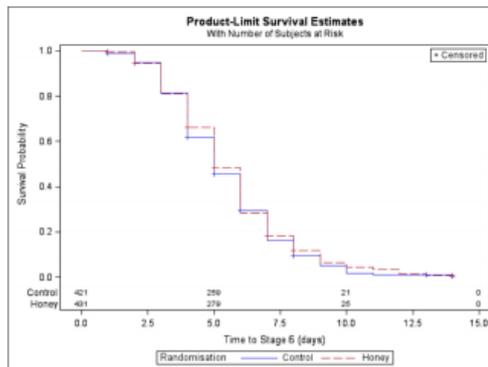
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**Figure 5** Kaplan-Meier survival plots for time to stage 6.

treatments, with an HR of 1.06. The upper 95% confidence limit of 1.22 was within the predefined bound of superiority of 1.25 and as such the study had adequate power. Furthermore, the narrow CIs reported here are within the published risk limits in relation to non-inferiority of HSL interventions against 5% aciclovir.[18] Although this study was designed to test a superiority hypothesis and we did not specify a non-inferiority bound for formal statistical testing, we consider this study is also consistent with non-inferiority of honey compared with aciclovir. There were five participants (one in the honey group and four in the aciclovir group) that received the incorrect treatment. Given the similar time to recovery for the two treatment arms, it was felt that a per-protocol analysis would not add to the assessment of potential bias in the ITT analysis.

In this study, there was no evidence that honey accelerated healing compared with aciclovir in HSL lesions from stage 4 (ulceration). We had anticipated this might have been the case because of the known positive effects of honey on wound healing processes such as angiogenesis, granulation, epithelialisation, reduction in oedema and debridement.[23] We had also anticipated that aciclovir may have provided greater early benefit through inhibiting the viral replication process to abort the natural history of the HSL episode. However, this was not supported by our finding that the proportions of lesions between groups that did not progress to the ulcerative stage were similar and interaction analysis for stage of lesion at study baseline provided no evidence that the effect of randomised treatments were dependent on the stage of the HSL lesion at presentation. The estimated pain severity differences had very narrow CIs and were strongly consistent with non-inferiority.

There are a number of statistical considerations relevant to the interpretation of our data. The outcome variables were of a non-normal distribution and thus underwent non-parametric analysis and medians are reported. For time-to-event outcome variables, data from ongoing episodes were censored and incorporated into

the survival analysis. The definition of time to healing we used was defined as time to return to normal skin, whereas previous studies have defined this as time to loss of crust, allowing for residual erythema (stage 6 in this study).[22] For this reason, we performed a post hoc analysis for time from randomisation to stage 6. This demonstrated a reduction in healing time to this stage, with a median 5 days for both aciclovir and honey.

Our study presents a number of additional strengths in adding to the current evidence base for both topical use of aciclovir and honey for HSL. This appears to be one of the largest single studies of topical aciclovir for HSL[4] and provides sufficient power to conclude that there is no difference between aciclovir and honey in terms of a clinically significant difference of 1 day for healing. This large sample size together with the novel study methodology allowed recruitment of a representative New Zealand cohort from 76 pharmacies both within and outside of traditional, usually urban, research centres, mitigating the jurisdiction effect of traditional research models and capturing the heterogeneity for HSL manifestation and progression in the community.[24] Participants were enrolled at the pharmacist–patient interface when seeking treatment for an active HSL lesion ensuring outcome data represent usual treatment access in the community and avoiding the requirement for pre-randomisation to ensure participants begin study treatment within the prodromal phase. Using smart phone-based diaries containing photographic standards of lesion progression, we were able to both facilitate study participation within usual daily routine, removing the requirement for costly and burdensome clinic visits and maximise chronologically relevant data capture via immediate follow-up of missed entries. These factors provide valuable information as to the generalisability of traditional RCT models assessing HSL which, while providing essential safety, efficacy and regulatory data, are unable to definitively measure drug performance in the highly diverse real-world setting.[25]

There was no provision to mask the allocation of honey to participants; therefore, the consenting pharmacist investigators and participants were unblinded to treatment allocation. This may be considered a potential source of bias however, unavoidable in the context of investigational product and setting, and interestingly, there is no established evidence that participants alter their self-reported data dependent on knowledge of the treatment they are receiving.[26]

Importantly, we have demonstrated the feasibility of conducting a large-scale comparative RCT utilising a cost-effective, regulatory compliant and time-efficient community-based infrastructure, while maintaining robust outcome data. This provides significant opportunity to enhance the evidence base for non-prescribed medications such as CAM by overcoming the limitations of cost-prohibitive traditional models.[27]

These findings suggest that medical grade kanuka honey may be employed as an equivalent therapeutic

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choice to aciclovir cream, for the treatment of HSL, particularly given the emergent issues of drug resistance[5] and the needs of particular patient groups that may be unable to use current pharmacological therapies due to allergy and lack of safety data in pregnancy and breast feeding. While our study does not replicate the apparent superiority of honey over acyclovir cream demonstrated in a clinical pilot study of 2004,[12] there are a number of notable differences and possible explanations for this. Methodologically, the studies are very different, with a very small sample size in the pilot and use of physician-scored lesions compared with patient-reported outcomes in our study. While different honeys share common physicochemical properties, there can be significant variations in their bioactive profiles.[28] The multifloral honey used in the aforementioned pilot study and sourced from the United Arab Emirates will therefore show a very different composition profile to New Zealand kanuka honey, potentially conferring superior antiviral and wound promoting immunostimulatory effects, which led to the decreased time to healing reported. Further understanding of the factors responsible for such effects and improved and standardised analysis techniques will allow for better correlation between composition and the clinical effects of different honeys.

The priority now is to investigate whether honey in combination with either aciclovir or other natural antiviral compounds[29] might have greater efficacy than individual components in the topical management of HSL and whether medical grade honey might have similar efficacy and acceptability in paediatric HSL, a group from which we received much guardian-led interest during the study period. Investigation of the efficacy of medical grade honey in other herpetic indications such as herpes zoster is also warranted.

## CONCLUSIONS

Prior RCTs provide evidence that topical application of aciclovir reduces time to healing and pain resolution in HSL and as such is used as a gold standard front-line topical therapy, globally. The study findings suggest therapeutic equivalence of medical grade Kanuka honey with aciclovir in the treatment of HSL, when used in routine community treatment at the pharmacy interface. This provides rationale for the recommendation of topical honey for HSL management as an acceptable alternative to aciclovir cream.

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