# Feasibility study assessing the association between gut microbiota in healthy adults and antibody response to seasonal influenza vaccination

**Internal Reference Number / Short title:** Gut microbiota and influenza vaccine

**Ethics Ref:** HDEC

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**Chief Investigator Signature:**

There are no conflicts of interest to declare

**Confidentiality Statement**

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## 1. KEY TRIAL CONTACTS

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## 2. SYNOPSIS

1. This is a feasibility study (FS) designed to establish the number needed to participate in a future randomised controlled trial (proposed Main Study (MS)) of a nutrient designed to change gut microbiota in adult humans, with the intention of improving the immune response to a future seasonal influenza vaccine.

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|---|---|---|
| Trial Title | Feasibility study assessing the association between gut microbiota in healthy adults and immune response to seasonal influenza vaccination | |
| Internal ref. no. (or short title) | Gut microbiota and influenza vaccine | |
| Trial Design | Cohort feasibility study | |
| Trial Participants | 18 or older with no allergies to any component of the influenza vaccine | |
| Planned Sample Size | 125 participants with the intention of a minimum of 100 to complete the study | |
| Treatment duration | Single treatment with Medsafe approved annual influenza vaccination for 2016. | |
| Follow up duration | 26 weeks | |
| Planned Trial Period | February 2016 to December 2016 | |
| | Objectives | Outcome Measures |
| Primary | Establish the number of participants required in the control and intervention group, taking into account the likely drop-out and withdrawal rate during the study. | The number needed to participate in the proposed RCT to see a 10% increase in responsiveness (either by proportional increase in antiviral antibody titres jointly or severally, OR by geometric mean titre (GMT) jointly or severally to the Medsafe approved trivalent annual influenza vaccination in the treated group versus the control group with 90% power and an alpha of 5%<br><br>The proportion of participants who fail to complete the feasibility study to Day 28 and at 26 weeks. |
| Secondary | Establish whether our sample can be mapped to Stool Community Types | The proportion of participants who can be successfully mapped to a Stool Community Type |
| | Establish the stability of within-subject Stool Community Type over time | The proportion of participants who have the same Stool Community Type at Day 28 versus Day Zero |
| | Identification of proposed supplemental intervention | Dependent on outcome of FS |

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| | Identify likely inclusion and exclusion criteria for participants | The proportion of participants who take systemic antibiotics or corticosteroids, or who are / become pregnant within 28 days of Day Zero or during the study,<br><br>The proportion of participants who have had exposure to any influenza vaccine in the two years prior to study initiation.<br><br>The proportion of participants who ingest excessive alcohol in the 24 hour period prior to supplying faecal samples<br><br>ANOVA of day 28 antiviral antibody titres against Stool Community Types<br><br>Estimate of the proportions of participants with different stool types who meet the titre-defined criteria for seroconversion. |
| | Measure the likely completion rate of 7-day food diaries and 3-day food diaries in the MS | For the 7-day diary at Day Zero and Day 28: The proportion of participants who complete a minimum of 5 days of the 7-day diary AND whose reported intake for each day is >1.2 x Basal Metabolic Rate (BMR), using predicted BMR based on sex, age, height and weight.<br><br>For the 3-day diary at Day Zero and Day 28: The proportion of participants who complete all of the first 3 days AND whose reported intake for each day is >1.2 x Basal Metabolic Rate (BMR), using predicted BMR based on sex, age, height and weight. |
| | Establish the likelihood of participants providing faecal samples | The proportion of participants who provide a faecal sample at Day Zero and at Day 28<br><br>The proportion of Day Zero and Day 28 faecal samples that are adequately dated and time stamped by (i.e. can be related back to the food diary) |

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| | Establish the likelihood of completing all blood samples at all visits, as outlined in the visit schedule | The proportion of blood samples obtained versus plan, and proportion of analysable samples at Day Zero, Day Three, Day Seven, Day 28 and at 26 weeks |
| | Clarification of the proposed measure for the primary outcome variable | The proportion of participants who seroconvert to ALL arms of the influenza vaccine at Day 28<br><br>The proportion of participants that seroconvert to EACH arm of the influenza vaccine at Day 28<br><br>Calculation and relative ranking of participant GMT to ALL arms of the trivalent influenza vaccine at Day 28<br><br>Calculation and relative ranking of participant GMT to EACH arm of the trivalent influenza vaccine at Day 28 |
| Investigational Medicinal Product(s) | Medsafe approved trivalent influenza vaccine for influenza season 2016 | |
| Formulation, Dose, Route of Administration | As supplied by vendor, 0.5ml, Intra muscular or deep subcutaneous injection | |

## 3. ABBREVIATIONS

| | |
|---|---|
| AE | Adverse event |
| AR | Adverse reaction |
| CI | Chief Investigator |
| CRA | Clinical Research Associate (Monitor) |
| CRF | Case Report Form |
| CRO | Contract Research Organisation |
| CT | Clinical Trials |
| CTA | Clinical Trials Authorisation |
| CTRG | Clinical Trials and Research Governance |
| DMC/DMSC | Data Monitoring Committee / Data Monitoring and Safety Committee |
| FS | Feasibility Study |
| GCP | Good Clinical Practice |
| GMT | Geometric Mean Titre |

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|---|---|
| GP | General Practitioner |
| HAI | Haemagglutination Inhibition |
| IB | Investigators Brochure |
| ICF | Informed Consent Form |
| ICH | International Conference on Harmonisation |
| IMP | Investigational Medicinal Product |
| IRB | Independent Review Board |
| MS | Main Study (Proposed Main Study) |
| PI | Principal Investigator |
| PIS | Participant Information Sheet |
| SAE | Serious Adverse Event |
| SAR | Serious Adverse Reaction |
| SDV | Source Data Verification |
| SMPC | Summary of Medicinal Product Characteristics |
| SOP | Standard Operating Procedure |
| SUSAR | Suspected Unexpected Serious Adverse Reactions |
| TMF | Trial Master File |

## 4. BACKGROUND AND RATIONALE

The key hypothesis for this research is that the restoration of appropriate host-microbiota interactions underpins the beneficial effects for food to build immune defence against influenza. This is supported by recent investigations demonstrating that antibiotic use alters gut microbiota composition, negatively impacting host-microbiota interactions that lead to reduced protective immunity against influenza [1, 2]; and the known influence of nutrition to support ideal host-microbiota interactions [3]. However, there is a lack of randomised controlled trials to substantiate this hypothesis in humans, and seasonal influenza vaccination induces considerable variation in the magnitude of the protective antibody response. The hypothesis underlying the proposed Main Study (MS) is based on the results of a series of trials undertaken in both animals and humans. The MS will be the first time a dietary intervention study will be undertaken solely in humans based on the characterisation of the human gut microbiome, and measures the impact of the intervention on the immune system using the annual influenza vaccine as a proxy for immune defence against respiratory tract infection. Thus, there are a number of feasibility issues that need to be addressed before the proposed MS can be undertaken.

The MS that is the focus of this feasibility study (FS), is a multi-centre two-arm, parallel groups, randomised controlled trial to determine the effect of a high value nutritional supplement versus no supplementation on the immune response of adults to respiratory tract infections. The FS will utilise a Medsafe approved seasonal influenza vaccine for 2016 as a proxy for immune responsiveness at Day 28

and at 26 weeks following vaccination. As no RCTs of this nature have previously been undertaken, the FS is required to inform issues related to the overall study structure, FS issues 1a, 1b, 1c, and 1d; establishing whether all participants can be mapped according to pre-specified Stool Community Types, the proportion of participants who map to each of the Stool Community Types, and the stability of participant Stool Community Type over time (FS issue 1a), identification of the supplemental intervention (FS issue 1b), inclusion and exclusion criteria for participation in the MS (FS issue 1c), and calculating the numbers needed to participate in the trial to detect a 10% increase in influenza vaccine responsiveness in the supplemented group compared to the non-supplemented group with 90% power. We need to establish that this protocol is acceptable to potential participants, and identify issues that will allow for better adherence for the MS. This will be addressed in FS issues 2a, 2b, 2c, and 2d; completion of nutritional diaries (FS issue 2a), provision of faecal samples that are adequately labelled and time-stamped (FS issue 2b), ability to provide complete blood samples (FS issue 2c), and ability to complete a prolonged study (FS issue 2d). The proposed primary outcome variable of the MS is the proportion of participants with a pre-identified Stool Community Type [3] who seroconvert (defined as pre-vaccination titre <1:10 and a post-vaccination titre ≥1:40, OR pre-vaccination titre ≥1:10 AND a minimum four-fold increase on post-vaccination titre) to the three serotypes (2 x influenza A and 1 x influenza B) of the seasonal influenza vaccine in the supplementation arm of the study, compared to the non-supplementation arm at 28 days post-vaccination. FS issue 3a addresses the issue of whether the primary outcome variable should be defined as average seroconversion across ALL three arms of the seasonal influenza vaccine, or whether each arm of the seasonal influenza vaccine should be considered separately. However, there may be utility in assessing the Geometric Mean Titre (GMT) as a possible measure for the primary outcome variable at Day 28, and again whether this should be to all three arms of the influenza vaccine, or whether this should be to EACH arm of the influenza vaccine, and this will also need to be assessed in FS issue 3a. A necessary component of the MS will be the administration of food-diaries to participants immediately prior to faecal samples, to assess whether dietary intake may have caused spurious faecal sample results and / or changes in Stool Community Types, therefore a further feasibility issue (FS issue 4a) is to establish whether it would be best to administer a 7-day or a 3-day food diary to participants.

<u>Summary of feasibility issues:</u>

1a. establishing whether all participants can be mapped according to pre-specified Stool Community Types, the proportion of participants who map to each of the Stool Community Types, and the stability of participant Stool Community Type over time

1b. identification of the proposed supplemental intervention

1c. inclusion and exclusion criteria for participants in the MS

1d. establish the numbers needed to participate in the MS to see a 10% difference in response to the influenza vaccine between the supplemented and non-supplemented groups

2a. completion of nutritional diaries and lifestyle questionnaire

2b. participant provision of faecal samples

2c. participant provision of blood samples

2d. completion of a prolonged study

3. clarification of the proposed measure for the MS primary outcome variable

4. administration of a 3-day or a 7-day food diary

The FS will take place at a small number of Wellington locations, and will NOT involve randomisation to an intervention. ALL participants will receive a Medsafe approved seasonal influenza vaccine. Participants will be recruited from the Wellington Region, with minimal exclusion criteria (allergy / contra-indication to influenza vaccination, immune condition confounding the immune response, inability to complete the study).

After consent, and one to seven days prior to Day Zero, participants will complete a Lifestyle Questionnaire (based on the 'American Gut Project' questionnaire) to identify pre-existing medical conditions that may be related to gut health and / or immune responsiveness (FS issue 1c). The proportion of participants completing the Lifestyle Questionnaire will be calculated and a completion rate of <90% of participants answering 90% or more of the questions will be considered inadequate. Participants will be asked to complete a 7-day food diary prior to vaccination. We will test for an association between macro/micronutrient intake of participants and their Stool Community Type (FS issue 1b). The proportion of participants providing complete 7-day food diaries will inform FS issue 2a and FS issue 4. A completion rate of <90% of the participants completing 7-day food diaries will be considered inadequate. The proportion of participants who complete the first three full days of their 7-day food diary will be calculated. A completion rate of <90% of the 3-day food diaries will be considered inadequate (FS issue 4). Participants will supply a pre-vaccination faecal sample (taken between zero and three days prior to planned vaccine) for analysis and categorisation into one of 4 pre-defined Stool Community Types. The proportion of participants able to supply samples pre-vaccination will inform FS issue 2b and a proportion of <95% will be considered inadequate. The proportion of faecal samples that can be categorised into Stool Community Types will be calculated, and a proportion <90% will be considered inadequate (FS issue 1a).

On Day Zero, participants will have a serum sample taken immediately prior to vaccination for analysis of antiviral antibody titre in serum to each component of the influenza vaccine (FS issues 2c and 3a). Participants will have a full blood count taken to identify any evidence of non-apparent infection or other variation of general health status as this may form an exclusion criteria in the MS, and the proportion for this group will be calculated (FS issue 1c). Further blood samples will be taken to assess gene expression levels and immune profile immediately prior to vaccination to serve as a baseline for subsequent post-vaccination analysis. The proportion of participants from whom each blood test at this time point cannot be taken will be calculated, and a proportion <90% in each instance will be considered inadequate (FS issue 2c). Participants will be asked about any adverse events and any use of systemic antibiotics or corticosteroids since consent (FS issue 1c).

Three days post-vaccination, further samples will be taken to assess gene expression. The proportion of participants from whom each blood test at this time point cannot be taken will be calculated, and a proportion <90% in each instance will be considered inadequate (FS issue 2c). Participants will be asked about any adverse events, and any use of systemic antibiotics or corticosteroids since Day Zero (FS issue 1c).

Seven days post-vaccination blood tests will be taken to assess gene expression and to perform immune profiling. The proportion of participants from whom each blood test at each this point cannot be taken will be calculated, and a proportion <90% in each instance will be considered inadequate (FS issue 2c).

Participants will be asked about any adverse events, and any use of systemic antibiotics or corticosteroids since the last visit (FS issue 1c).

The next scheduled visit is 28 days post-vaccination. Participants will be asked to complete a 7-day food diary (FS issues 2a). Participants will be asked about any adverse events, and any use of systemic antibiotics or corticosteroids since the last visit (FS issue 1c). The proportion of participants providing complete 7-day food diaries will inform FS issue 2a and FS issue 4a. A completion rate of <90% will be considered inadequate. The proportion of participants who complete the first three full days of their 7-day food diary will be calculated. A completion rate of <90% will be considered inadequate (FS issue 4a). Participants will be asked to supply a single faecal sample from between three and zero days prior to the day 28 visit for analysis and categorisation into one of four pre-defined Stool Community Types. The proportion of participants able to supply faecal samples will inform FS issue 2b and a proportion of <95% will be considered inadequate. Day 28 Stool Community Types will be compared to Day Zero Stool Community Types (FS issue 1a). If Stool Community Type has changed, the Day 28 food diary will be compared to the Day Zero food diary to assess whether this can be attributed to change in diet (FS issue 1b). If >10% of participants change Stool Community Type between Day Zero and Day 28 of the study, this will be considered too great a variation for the purposes of undertaking the MS. Serum samples will be taken for antiviral antibody titres in serum to all three arms of the influenza vaccine and compared to Day Zero antiviral antibody titres in serum. Seroconversion, defined as pre-vaccination titre <1:10 and a post-vaccination titre ≥1:40, OR pre-vaccination titre ≥1:10 AND a minimum four-fold increase on post-vaccination titre will be assessed. This analysis will be undertaken by participant and assessed for each arm of the influenza vaccine separately, AND by participant for the mean change in titre to all three arms of the influenza vaccine. The proportion of participants who achieve seroprotection (> 1:40 antiviral antibody titre) at Day 28 will also be calculated. The proportion of participants from whom each blood test at this time point cannot be taken will be calculated, and a proportion <90% in each instance will be considered inadequate (FS issue 2c). We will test for an association between Day Zero Stool Community Type and antiviral antibody titres to each arm of the influenza vaccine, and mean antiviral antibody titres to all 3 arms of the influenza vaccine (FS issue 3a). The number of participants needed to enrol in the MS to detect a 10% increase in immune response in the supplemented group compared to the non-supplemented groups with 80% or 90% power, and an alpha of 5% will be calculated (FS issue 3).

The final study visit will take place at 26 weeks post-vaccination. Serum samples will be taken (FS issue 2c) for antiviral antibody titres to all three arms of the influenza vaccine and compared to Day Zero and Day 28 titres. We will test for an association between the mean change in antiviral antibody titres to ALL three arms of the influenza vaccine between Day Zero, Day 28 and 26 weeks, and between EACH arm of the influenza vaccine and Stool Community Type (FA issue 3). Further blood samples will be taken for immune profiling. The proportion of participants from whom each blood test at this time point cannot be taken will be calculated, and a proportion <90% in each instance will be considered inadequate (FS issue 2c). The drop-out rate (those who presented at 26 weeks compared to those who presented at Day Zero and received the influenza vaccine) will be calculated with appropriate confidence intervals. The proportion of participants who meet possible exclusion and / or withdrawal criteria for the Proposed MS, including, but not limited to exposure to influenza vaccine in the two years prior to study initiation, pregnancy during the study, use of systemic antibiotics or corticosteroids within the 28 days prior to study initiation, use of systemic antibiotics or corticosteroids during the period of the study will be calculated (FS issue 1d).

**Additional testing for participants who have a 'low' and 'high' response to the influenza vaccine (FS issue 1b and 1c):**

Due to budget and potential time and sample constraints, the testing of Day Zero, Day Three, Day Seven and Week 26 samples for gene expression and immune profiling will be limited to approximately 40 individuals. It is not possible to identify the specific 40 individuals until the Day 28 antiviral antibody titres have been taken and analysed. Hence all participants will have tissue samples taken at Day Zero, Day Three, Day Seven and Week 26 for gene expression and immune profiling, but only 40 individuals will be analysed.

The low and high responders to the influenza vaccine will be identified using a number of procedures including ranking according to proportional increase in Day 28 versus Day Zero antiviral antibody titres, and proportional increase in Day 28 versus Day Zero GMT. The bottom 20 responders will be age (±5 years) and sex-matched to a participant within the high responding group.

The samples of the low and high responding participants will undergo additional testing:

- metabolomic analysis of plasma on Day Zero
- cytomegalovirus antibody testing on Day Zero
- RNA sequencing gene expression analysis (including the identification of HLA type) on Day Zero, Day Three and Day Seven samples
- flow cytometry immune profiling on Day Zero and Day Seven samples
- ELISpot immune profiling on Day Zero, Day Seven and Week 26 samples
- metagenomic analysis of faecal microbiota at Day Zero and Day 28 samples

## 5. OBJECTIVES AND OUTCOME MEASURES

| **Primary Objective** | **Outcome measures** |
|---|---|
| Establish the number of participants required in the control and intervention group, taking into account the likely drop-out and withdrawal rate during the study. | The number needed to participate in the proposed RCT to see a 10% increase in responsiveness to the Medsafe approved annual influenza vaccine in the treated group versus the control group with 90% power and an alpha of 5%<br><br>The proportion of participants (95% CI) who fail to complete the feasibility study to Day 28<br><br>The proportion of participants (95% CI) who fail to complete the feasibility study in its entirety |
| **Secondary Objectives** | **Outcome Measures** |
| Establish the stability of within-subject Stool Community Type over time | The proportion of participants (95% CI) who can be mapped to any of the four pre-specified Stool Community Type at Day Zero<br><br>The proportion of participants (95% CI) who map to Stool |

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| | Community Types A, B, C and D respectively at Day Zero<br><br>The proportion of participants (95% CI) who can be mapped to any of the four pre-specified Stool Community Type at Day 28<br><br>The proportion of participants (95% CI) who map to Stool Community Types A, B, C and D respectively at Day 28<br><br>The proportion of participants (95% CI) who have the same Stool Community Type at Day 28 versus Day Zero |
| Identification of proposed supplemental intervention | Will be dependent on FS results |
| Identify likely inclusion and exclusion criteria for participants | The proportion of participants (95% CI) who take systemic antibiotics within 28 days of Day Zero<br><br>The proportion of participants (95% CI) who take systemic antibiotics between Day Zero and the 26 week visit<br><br>The proportion of participants (95% CI) who take systemic corticosteroids within 28 days of Day Zero<br><br>The proportion of participants (95% CI) who take systemic corticosteroids between Day Zero and the 26 week visit<br><br>The proportion of participants (95% CI) who ingest alcohol (in any quantity – as documented in the 7-day food diary) in the 24 hour period prior to supplying faecal samples<br><br>The proportion of participants (95% CI) who have had an influenza vaccine in the previous 2 years<br><br>The proportion of participants (95% CI) who are pregnant at Day Zero, or become pregnant during the study |
| Measure the likely completion rate of 7-day food diaries and 3-day food diaries in the MS | The proportion (95% CI) of participants who complete a minimum of 5 days of the 7-day diary AND whose reported intake for each day is >1.2 x Basal Metabolic Rate (BMR), using predicted BMR based on sex, age, height and weight at Day Zero<br><br>The proportion (95% CI) of participants who complete a minimum of 5 days of the 7-day diary AND whose reported intake for each day is >1.2 x Basal Metabolic Rate (BMR), using predicted BMR based on sex, age, height and weight at Day 28<br><br>The proportion (95% CI) of participants who complete all of the first 3 days of the 7-day diary AND whose reported intake for each day is >1.2 x Basal Metabolic Rate (BMR), using predicted BMR based on sex, age, height and weight at Day Zero |

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| | The proportion (95% CI) of participants who complete all of the first 3 days of the 7-day diary AND whose reported intake for each day is >1.2 x Basal Metabolic Rate (BMR), using predicted BMR based on sex, age, height and weight at Day 28 |
| Measure the likely completion rate of lifestyle questionnaires in the MS | The proportion (95% CI) of participants who complete a minimum of 90% of the questions in the Lifestyle Questionnaire |
| Establish the likelihood of participants providing faecal samples | The proportion of participants (95% CI) who provide a faecal sample at Day Zero<br><br>The proportion (95% CI) of Day Zero faecal samples that are adequately dated and time stamped by participants (i.e. can be related back to the food diary)<br><br>The proportion of participants (95% CI) who provide a faecal sample at Day 28<br><br>The proportion (95% CI) of Day 28 faecal samples that are adequately dated and time stamped by participants (i.e. can be related back to the food diary) |
| Establish the likelihood of completing all blood samples at all visits, as outlined in the visit schedule | The proportion (95% CI) of blood samples obtained versus plan, and proportion (95% CI) of analysable samples at Day Zero<br><br>The proportion (95% CI) of blood samples obtained versus plan, and proportion (95% CI) of analysable samples at Day Three<br><br>The proportion (95% CI) of blood samples obtained versus plan, and proportion (95% CI) of analysable samples at Day Seven<br><br>The proportion (95% CI) of blood samples obtained versus plan, and proportion (95% CI) of analysable samples at Day 28<br><br>The proportion (95% CI) of blood samples obtained versus plan, and proportion (95% CI) of analysable samples at 26 weeks |
| Clarification of the proposed measure for the primary outcome variable in the MS | The proportion (95% CI) of participants who seroconvert (pre-vaccination antiviral antibody titre <1:10 and a post-vaccination antiviral antibody titre ≥1:40, OR pre-vaccination antiviral antibody titre ≥1:10 AND a minimum four-fold increase on post-vaccination antiviral antibody titre) to ALL arms of the influenza vaccine at Day 28<br><br>The proportion (95% CI) of participants that seroconvert (pre-vaccination antiviral antibody titre <1:10 and a post-vaccination antiviral antibody titre ≥1:40, OR pre-vaccination antiviral antibody titre ≥1:10 AND a minimum four-fold increase on post-vaccination antiviral antibody titre) to EACH arm of the influenza vaccine at Day 28<br><br>The proportion (95% CI) of participants who undergo a 2.5-fold |

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| | increase in GMT to ALL arms of the influenza vaccine at Day 28<br><br>The proportion (95% CI) of participants who undergo a 2.5-fold increase in GMT to EACH arm of the influenza vaccine at Day 28<br><br>The proportion (95% CI) of participants who achieve seroprotection (antiviral antibody titre >1:10) to ALL arms of the influenza vaccine at Day 28<br><br>The proportion (95% CI) of participants who achieve seroprotection (antiviral antibody titre >1:10) to EACH arm of the influenza vaccine at Day 28 |

## 6. TRIAL DESIGN

A feasibility study comprising an interventional cohort trial that will compare the pre-vaccination gut microbiota of 100 participants and seroconversion rates to a Medsafe approved seasonal influenza vaccine for 2016.

Individual participation is expected to last for 26 weeks. During that 26 weeks there will be 6 study visits as well as a study information seminar that will be used to explain the study. Visit 1 will occur immediately after the seminar if informed consent is given.

Potential participants will be given a participant information sheet before attending a study seminar that will outline the reasons for the study and what participation will entail. Potential participants will also be taken through the inclusion and exclusion criteria at this seminar. The seminars will have up to 50 participants attending.

Visit 1 (Day -14 to -7)

- Seminar explaining reasons for study and what participation will entail
- Dietician explanation of how to fill in food diary
- Informed consent
- Brief medical history
- Participant given lifestyle questionnaire, paper copy of food diary and faecal collection kit
- Participant instructed on how to use faecal collection kit
- Participant given visit schedule

Visit 2 (Day 0)

- Return paper copy of food diary
- Return faecal collection kit
- Review of Adverse Events
- Brief physical (temperature & general wellness)
- Blood test (1 x 6ml Serum tube, 7 x 10 ml and 1 x 6ml Heparin tubes and 1 x 3ml CBC tube)
- Vaccination with influenza vaccine
- Observation period (20 minutes)

Where a participant has not been able to supply a faecal sample pre-vaccine, they will be able to supply a faecal sample within 48 hours of vaccination, and will be asked to continue with their food diary to capture nutritional intake prior to sampling.

Where a participant is not vaccinated for any reason, they will be able to be 're-screened' but will be required to complete a further 7-day food diary and supply a faecal sample no more than 7 days prior to their new Day Zero.

Visit 3 (Day 3 (+2 day window))

- Review of Adverse Events
- Blood test (1 x 10mL and 1 x 3mL Heparin tube)

Visit 4 (Day 7 (+3 day window)

- Review of Adverse Events
- Blood test (5 x 10 mL Heparin tubes)
- Participant given second faecal collection kit and second copy of paper food diary

Visit 5 (Day 28 (window: -3 to +7 days))

- Return paper copy of food diary
- Return faecal collection kit
- Review of Adverse Events
- Blood test (1 x 6mL Serum tube)

Visit 6 (180 days (+/- 14 days))

- Blood test (1 x 6mL Serum tube and 2 x 10mL Heparin tubes)

## 7. PARTICIPANT IDENTIFICATION

### 7.1. Trial Participants

125 healthy adult participants will be enrolled, to allow for a 20% drop-out rate, ensuring completion of 100 participants. Trial participants will be enrolled through media-advertising and by word of mouth.

### 7.2. Inclusion Criteria

- Participant is willing and able to give informed consent for participation in the trial
- Male or Female, aged 18 to 64 years
- In the Investigator's opinion, is able and willing to comply with all trial requirements

### 7.3. Exclusion Criteria

The participant may not enter the trial if ANY of the following apply:

- They have had a known severe reaction or allergy to any components of the influenza vaccine
- They have any contra-indications to vaccination per recommendations of vaccine manufacturer
- They have a history of Guillain-Barre Syndrome within 6 weeks of receiving a previous influenza vaccine
- They have an impaired immune system that may confound immune response testing; i.e. any condition that impairs participant immune response through either the condition itself or through the treatment of the condition
- They have already received the 2016 seasonal influenza vaccine
- They have any other clinical condition which the investigator deems relevant for exclusion from the study

## 8. TRIAL PROCEDURES

### 8.1. Recruitment

Participants will be recruited through advertising and word of mouth. Participants will be encouraged to make contact with investigators, and will be sent an information pack including the Participants Information Sheet electronically or by mail. Potential participants will be invited to attend recruitment seminars that will be held at centralised locations. They will be informed of the study requirements. At this point, informed consent will be obtained.

### 8.2. Informed Consent

Informed consent will be obtained according to ethical and GCP guidelines. Each participant will sign an informed consent form at the first visit, prior to conducting the procedures listed in Section 6: Trial Design.

### 8.3. Baseline Assessments

Day -14 to -1

1. Participants will be given a schedule outlining the planned study visits, dates will be calculated from their intended Day Zero date
2. Participants will complete the lifestyle questionnaire prior to attending their Day Zero visit.
3. Participants will be advised to start their 7-day food diary, so that the morning of the seventh day of the diary falls on the morning of Day Zero.
4. A brief medical history will be done
5. A faecal sample collection kit will be distributed and participants will be advised to take a faecal sample as close to Day Zero as possible, and no earlier than minus 3 days to ensure wherever possible, that three days of the food diary are completed prior to faecal sampling

On Day Zero:

- Participants will supply a faecal sample that has been taken between zero to three days before the Day Zero Visit
- Participants will return the completed 7-day food-diary.
- Participants will under-go a pre-vaccine blood sample for antiviral antibody titres to the influenza vaccine, additional blood sampling for gene expression analysis and immune profiling, and full blood count and differential.
- Participants will undergo a brief physical (including temperature) to ensure they are well enough to receive the influenza vaccine.
- Participants will receive the Medsafe approved influenza vaccine.

### 8.4. Subsequent Visits

Visit 3

- Review of Adverse Events
- Blood test for gene expression analysis

Visit 4

- Review of Adverse Events
- Blood test for gene expression analysis and immune-profiling
- Participant given second faecal collection kit and second copy of paper food diary.

Visit 5

- Return paper copy of food diary
- Return faecal collection kit
- Review of Adverse Events
- Blood test for antiviral antibody titres in serum

Visit 6

- Blood test for gene expression analysis and immune-profiling

### 8.5. Sample Handling

All samples will be processed according to Standard Operating Procedures of the investigating sites.
Samples, questionnaires and diaries will be labelled with allocated participant numbers only, so that personal information remains confidential.

For those participants who have given consent to 'Future Unspecified Research', the study samples obtained under this protocol will be retained for their future life at MIMR, and accessed by MIMR in accordance with the prevailing ethical requirements of the day.

For those participants who have NOT given consent to 'Future Unspecified Research', the study samples obtained under this protocol will be retained until completion of the study (including publication of the results where relevant), and will then be destroyed MIMR.

### 8.6. Discontinuation/Withdrawal of Participants from Trial Treatment

Each participant has the right to withdraw from the trial at any time. In addition, the Investigator may discontinue a participant from the trial at any time if the Investigator considers it necessary for any reason including:

- Ineligibility (either arising during the trial or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with treatment regimen or trial requirements
- An adverse event which results in inability to continue to comply with trial procedures
- Withdrawal of consent
- Loss to follow up

Withdrawn participants will not be replaced.

The reason for withdrawal will be recorded in the CRF.

If the participant is withdrawn due to an adverse event, the Investigator will arrange for follow-up visits or telephone calls until the adverse event has resolved or stabilised.

Where possible, despite withdrawal from the trial, all data collected up until the time of withdrawal will be included in the analysis.

### 8.7. Definition of End of Trial

The end of trial is the date of the last visit of the last participant.

## 9. INFLUENZA VACCINE

### 9.1. Influenza Vaccine Description

Pending Medsafe approval of 2016 seasonal influenza vaccine.

### 9.2. Storage of Influenza Vaccine

Storage of the vaccine will be in accordance with the requirements of the manufacturer and cold chain storage records will be kept at all participating sites.

### 9.3. Compliance with Trial Treatment

As there is only a single vaccine administration, 100% compliance with trial treatment is expected.

### 9.4. Accountability of the Trial Treatment

Only subjects enrolled in the FS will receive the vaccine. The vaccine will only be administered by authorized site staff. The vaccine will be administered on Day Zero. Used vaccine syringes will be disposed of immediately after administration.

The CI is responsible for study treatment accountability, reconciliation and record maintenance. The investigator, or designated site staff must maintain study treatment accountability records throughout the course of the study. The responsible person(s) will document the amount of study treatment received from the supplier and the amount administered to participants. The required accountability unit for this study will be one standard vaccine dose of 0.5mL. Discrepancies are to be reconciled or resolved.

### 9.5. Concomitant Medication

At consent, participants will be asked what their medications are, and any changes between each visit will be recorded and will inform the inclusion and exclusion criteria to the MS.

## 10. SAFETY REPORTING

### 10.1. Definitions

| | |
|---|---|
| Adverse Event (AE) | Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences, which are not necessarily caused by or related to that product. |
| Adverse Reaction (AR) | An untoward and unintended response in a participant to an investigational medicinal product, which is related to any dose administered to that participant.<br>The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out.<br>All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to |

| | |
|---|---|
| | the trial medication qualify as adverse reactions. |
| Serious Adverse Event (SAE) | A serious adverse event is any untoward medical occurrence that:<br>• results in death<br>• is life-threatening<br>• requires inpatient hospitalisation or prolongation of existing hospitalisation<br>• results in persistent or significant disability/incapacity<br>• consists of a congenital anomaly or birth defect.<br>Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.<br>NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. |
| Serious Adverse Reaction (SAR) | An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided. |
| Suspected Unexpected Serious Adverse Reaction (SUSAR) | A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out:<br>• in the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product<br>• in the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question. |

NB: To avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above.

Any pregnancy occurring during the clinical trial and the outcome of the pregnancy should be recorded and followed up for congenital abnormality or birth defect, at which point it would fall within the definition of "serious".

### 10.2. Causality

The relationship of each adverse event to the trial medication must be determined by a medically qualified individual according to the following definitions:

**Related**: The adverse event follows a reasonable temporal sequence from trial medication administration. It cannot reasonably be attributed to any other cause.

**Not Related**: The adverse event is probably produced by the participant's clinical state or by other modes of therapy administered to the participant.

## 10.3. Procedures for Recording Adverse Events

All AEs occurring until Day 28 of the trial that are observed by the Investigator or reported by the participant will be recorded on the CRF, whether or not attributed to trial medication.

The following information will be recorded: description, date of onset and end date, severity, assessment of relatedness to trial medication, other suspect drug or device and action taken. Follow-up information should be provided as necessary.

The severity of events will be assessed on the following scale: 1 = mild, 2 = moderate, 3 = severe.

AEs considered related to the trial medication as judged by a medically qualified investigator or the Sponsor will be followed either until resolution, or the event is considered stable.

It will be left to the Investigator's clinical judgment to decide whether or not an AE is of sufficient severity to require the participant's removal from treatment. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must undergo an end of trial assessment and be given appropriate care under medical supervision until symptoms cease, or the condition becomes stable.

## 10.4. Reporting Procedures for Serious Adverse Events

All SAEs (other than those defined in the protocol as not requiring reporting) must be reported on the SAE reporting form to the MRINZ / Sponsor Safety Monitoring Committee within 24 hours of the Site Study Team becoming aware of the event. The MRINZ / Sponsor Safety Monitoring Committee will perform an initial check of the report, request any additional information, and ensure it is reviewed by the Medical Monitor on a weekly basis. All SAE information must be recorded on an SAE form and retained at the MRINZ site. Additional and further requested information (follow-up or corrections to the original case) will be detailed on a new SAE Report Form and supplied to the MRINZ / Sponsor Safety Monitoring Committee and the sponsor.

## 10.5. Expectedness

Expectedness will be determined according to the Investigators' Brochure.

## 10.6. SUSAR Reporting

All SUSARs will be reported by the CI to the Centre for Adverse Reactions Monitoring (CARM) and to the HDEC as applicable. For fatal and life-threatening SUSARS, this will be done no later than 7 calendar days after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days.

This is effectively an open label trial and no un-blinding will be required.

Principal Investigators will be informed of all SUSARs for the relevant IMP for all studies with the same Sponsor, whether or not the event occurred in the current trial.

### 10.7. MRINZ / Sponsor Safety Monitoring Committee

The MRINZ and the Study Sponsor will appoint an independent monitoring committee to conduct a review of all SAEs for the trial reported during the quarter and cumulatively. The aims of this committee include:

- To pick up any trends, such as increases in un/expected events, and take appropriate action
- To seek additional advice or information from investigators where required
- To evaluate the risk of the trial continuing and take appropriate action where necessary

## 11. STATISTICS

### 11.1. Description of Statistical Methods

The following table shows the sample size required in the MS to test for the difference between responsiveness to the influenza vaccine between the treated and the untreated group, exclusive of drop-out rates:

| Proportion seroconverted | | N total (divide by 2 for each group) | |
|---|---|---|---|
| In FS | To detect in MS: | 80% power | 90% power |
| 0.5 | 0.6 | 776 | 1038 |
| 0.6 | 0.7 | 712 | 954 |
| 0.7 | 0.8 | 588 | 794 |
| 0.8 | 0.9 | 398 | 532 |

The sample size will then be adjusted to account for the drop-out rate calculated at Day 28.

For this feasibility study the distribution of the rise of titre of antiviral antibodies against influenza vaccine is not well reported in the literature although we anticipate that it might be logarithm normal distributed. We will explore the distribution of this outcome variable in the feasibility study by ANOVA (a general linear model) of titre against community stool type with residual analysis with an appropriate

transformation. If the distributional assumptions are best met on the logarithm normal scale then the difference in titres will be reported as ratio of geometric means.

We will also estimate the proportions of participants with different stool types who have a four-fold titre rise as this is the accepted way of reporting whether seroconversion as has occurred.

For the sample size for the feasibility study having 15 to 20 participants with each stool type should be appropriate to have reasonable precision in the regression estimates.

A sample size of 100 in the FS gives a margin of error for a proportion of +/- 10%, thus a seroconversion rate in the FS of 50% will be estimated at 40 to 60%.

SAS 9.3 will be used.

### 11.2. The Number of Participants

125 participants will be recruited to allow for a drop-out and withdrawal rate of 20%, and thus we anticipate that a minimum of 100 participants will complete the 6-month trial.

### 11.3. Intention to treat Analysis

Participants who give informed consent, but fail to receive the vaccine will be included in the drop-out calculation for the FS, but no other data will be analysed

All participants who receive the vaccination on Day Zero will be included in the analysis. All data collected from participants until their withdrawal from the trial will be included in the analysis.

### 11.4. Procedures for Reporting any Deviation(s) from the Original Statistical Plan

Deviations from the original statistical plan will be reported to the relevant HDEC, and to ANZCTR prior to the revised statistical analysis being undertaken.

## 12. DATA MANAGEMENT

### 12.1. Source Data

Source data is defined as follows:

1. Participant and investigator completed Informed Consent Form (ICF) for this study 'Gut Microbiota and Influenza Health'

2. Participant and investigator completed ICF for Future Unspecified Research (if applicable)
3. Participant completed Lifestyle Questionnaire, either in paper format, or as completed in Wufoo™ Online Forms
4. Participant completed 7-day food diaries at Day Zero and Day 28
5. Nutritional analysis of participant's 7-day food diary, as developed by the Human Nutrition Unit, University of Auckland, in a Microsoft Excel or Access file.
6. Laboratory analyses:
    a. Full Blood Count reports
    b. Immunological and gene expression test results
    c. Faecal Stool Community Type Results and Metagenomics

### 12.2. Access to Data

In accordance with GCP MRINZ's SOPs, monitors will review the data in accordance with section 13.0. The CI agrees to allow MIMR access to all relevant documents for the purposes of monitoring.

At the end of the study, a de-identified dataset will be made available to MIMR.

### 12.3. Data Recording and Record Keeping

The only document with the participant name and contact details within it will be the ICF's for this FS and for Future Unspecified Research. At consent, all participants will be allocated a unique trial specific number which will be used for all diaries, questionnaires and tissue samples. Thus all remaining documentation will be de-identified. This unique trial specific number will be used in any databases. The name and any other identifying details will NOT be included in any trial data electronic file.

Following closure of the study, the CI will maintain all site study records, except for those required by local regulations to be maintained by someone else, in a safe and secure location. The records will be maintained to allow easy and timely retrieval, when needed (e.g., audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by local laws/regulations or institutional policy, some or all of these records can be maintained in a format other than hard copy. The CI must assure that all reproductions are legible and are a true and accurate copy of the original, and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the CI must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions. The minimum retention period will be 15 years.

## 13. QUALITY ASSURANCE PROCEDURES

The trial will be conducted in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures.

Regular monitoring will be performed according to GCP. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

## 14. SERIOUS BREACHES

A serious breach is defined as "A breach of GCP or the trial protocol which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or

(b) the scientific value of the trial".

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the C.I., the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to

## 15. ETHICAL AND REGULATORY CONSIDERATIONS

### 15.1. Declaration of Helsinki

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

### 15.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice.

### 15.3. Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to a Health and Disability Ethics Committee (HDEC), and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

### 15.4. Reporting

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the HDEC, host organisation and Sponsor. In addition, an End of Trial notification and final report will be submitted to HDEC, host organisation and Sponsor.

### 15.5. Participant Confidentiality

The trial staff will ensure that the participants' anonymity is maintained. The participants will be identified only by a participant ID number on all trial documents and any electronic database, with the exception of the CRF, where participant initials may be added. All documents will be stored securely and only accessible by trial staff and authorised personnel. The trial will comply with current and future New Zealand legislation.

### 15.6. Expenses and Benefits

Participants will be compensated a total of $250 to cover their time and travel for study procedures. They will also receive a free influenza vaccination as a part of the study procedures.

### 15.7. Other Ethical Considerations

## 16. FINANCE AND INSURANCE

### 16.1. Funding

This study is funded by The High Value Nutrition National Science Challenge Priority Research Programme. The financial relationship between MRINZ and MIMR is covered by contract.

### 16.2. Insurance

Aspects relating to insurance of the investigators and MIMR are covered in the financial contract between MRINZ and MIMR. It is anticipated that this study will receive approval form the appropriate ethics review board, and as such participants may be eligible for compensation form the Accident Compensation Corporation (ACC) in the event they suffer harm as a result of participating in the study.

## 17. PUBLICATION POLICY

The study findings will be published by MRINZ, in a scientific peer reviewed journal, according to the International Committee of Medical Journal Editors recommendations. The Investigators listed on page 1 will be listed as authors, in recognition of their contribution to the design, implementation and oversight of the study.

Results of the study will be sent to participants on request (once available) and will be made available on a publicly available trial registry website, recognised by the World Health Organisation International Clinical Trials Registry Platform (WHO ICTRP) as a Primary Registry.

## 18. REFERENCES


1. Ichinohe, T., et al., *Microbiota regulates immune defense against respiratory tract influenza A virus infection.* Proceedings of the National Academy of Sciences of the United States of America, 2011. **108**(13): p. 5354-9.
2. Oh, J.Z., et al., *TLR5-Mediated Sensing of Gut Microbiota Is Necessary for Antibody Responses to Seasonal Influenza Vaccination.* Immunity, 2014. **41**(3): p. 478-92.
3. Ding, T. and P.D. Schloss, *Dynamics and associations of microbial community types across the human body.* Nature, 2014. **509**(7500): p. 357-60.
4. Furman, D., et al., *Cytomegalovirus infection enhances the immune response to influenza.* Science translational medicine, 2015. **7**(281): p. 281ra43.

## 19. APPENDIX A: TRIAL FLOW CHART

| | Visit One -14 to -7 days | -7 days | -3 to 0 days | Visit 2 Day 0 | Visit 3 Day 3 | Visit 4 Day 7 | Day 22 | Day 25 to Day 28 | Visit 5 Day 28 | Visit 6 Week 26 |
|---|---|---|---|---|---|---|---|---|---|---|
| Informed consent | **X** | | | | | | | | | |
| Informed consent future research | **X** | | | | | | | | | |
| Conmed documentation | X | | | | | | | | | |
| Start food diary | | **X** | | | | | **X** | | | |
| Take stool sample | | | **X** | | | | | **X** | | |
| Complete Lifestyle questionnaire | | **X** | | | | | | | | |
| Return food diary | | | | **X** | | | | | **X** | |
| Return lifestyle questionnaire | | | | **X** | | | | | | |
| Return stool sample | | | | **X** | | | | | **X** | |
| Review adverse events | | | | **X** | **X** | **X** | | | **X** | |
| Review Conmed | | | | **X** | X | X | | | X | X |
| Temperature | | | | **X** | | | | | | |
| Serum sample | | | | **X** | | | | | **X** | **X** |
| Full Blood Count | | | | **X** | | | | | | |
| Immune -profile sampling | | | | **X** | | **X** | | | | **X** |
| Gene expression sampling | | | | **X** | **X** | **X** | | | | |
| Vaccination | | | | **X** | | | | | | |

**APPENDIX B: AMENDMENT HISTORY**