STATISTICAL ANALYSIS PLAN

**SAP Title:** NICEGUT SAP: Analysis plan for The NICE-GUT trial: A randomised, placebo-controlled trial of oral nitazoxanide for the empiric treatment of acute gastroenteritis among Australian Indigenous children

**Trial Registration Number:** ACTRN12614000381684

**SAP Version:** 0.5

**SAP Date:** 29 August 2019

**Signatures:**

**SAP Author &**

**Trial Statistician:** Dr Julie Marsh ………………………………….. Date: ………………

**Chief Investigator:** Dr Tom Snelling ………………………………….. Date: ………………



# **Glossary**

|  |  |
| --- | --- |
| Abbreviation | Explanation |
| ITT | Intention to treat |
| FSR | Final Statistical Report |
| OR | Odds ratio |
| ICH | International Conference on Harmonisation |
| SAP | Statistical Analysis plan |
| CRF | Clinical record form |
| SD | Standard deviation |
| CV | Coefficient of variation (i.e. SD / mean) |
| ASA | American Statistical Society (USA) |
| RSS | Royal Statistical Society (UK) |
| CPI | Coordinating Principle Investigator |
| PI | Principle Investigator |
| TSC | Trial Steering Committee |

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# Preface

This Statistical Analysis Plan (SAP) describes the planned analyses and reporting for The Nice-Gut trial: The NICE-GUT trial: A randomised, placebo-controlled trial of oral nitazoxanide for the empiric treatment of acute gastroenteritis among Australian Indigenous children.

The structure and content of this SAP provides enough detail to meet the requirements identified by the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use: Guidance on Statistical Principles in Clinical Trials. All work planned and reported for this SAP will follow international guidelines (ICH Harmonised Tripartite Guideline: Statistical Principles for Clinical Trials E9,1998) and national guidelines ((i) ASA: Ethical Guidelines for Statistical Practice. Prepared by the Committee on Professional Ethics, 1999; (ii) RSS: The Royal Statistical Society: Code of Conduct, 1993; (iii) SSAI: The Statistical Society of Australia Inc. Code of Conduct) for statistical practice.

The planned analyses identified in this SAP will be included in future manuscripts and trial report(s). Exploratory analyses not necessarily identified in this SAP may be performed to further address the objectives of the trial. Any post-hoc or unplanned analyses not specified in this SAP will be clearly identified as such in the Final Statistical Report (FSR) and any resulting manuscripts for publication.

This SAP was written by a qualified statistician without access to the trial database and was reviewed by clinical investigators who were blinded to individual treatment allocation and treatment-related study results. Access to the trial database is restricted until data collection and cleaning is completed and the database will be locked prior to final data extraction.

Revision history is located at the back of this document.



Section 1: Administrative Information

**Protocol Title:** The NICE-GUT trial: A randomised, placebo-controlled trial of oral nitazoxanide for the empiric treatment of acute gastroenteritis among Australian Indigenous children

**Trial registration number:** ACTRN12614000381684

**Protocol Version:** NICE GUT Protocol V8\_20170606

**Protocol Date:** 2017-06-06

**SAP Revision History:**

|  |  |  |  |
| --- | --- | --- | --- |
| **SAP Version/Date** | **Protocol Version/Date** | **Justification for SAP revision** | **Implementation stage relative to interim analyses** |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

Implementation stages: prior to interim 1, prior to interim 2, ……, prior to interim n.

**Additional Documentation:**

This SAP has been written based on information contained in the following addition documents:

1. CRF: Version 2, dated 21 January 2015

2. Data Dictionary: Version 1, dated 27 July 2016

3. Data Safety Monitoring Board (DSMB) Nice-Gut Terms of Reference: Version 4, dated 25 January 2016

5. ICH Guidance on Statistical Principles for Clinical Trials.

6. ICH Guidance on Structure and Content of Clinical Study Reports

**Roles & Responsibility** (Names, affiliations, and roles of SAP contributors)

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# **Background and Rationale**

Acute gastroenteritis was estimated to cause around 1.3 million of the 8.8 million annual deaths of children younger than 5 years in 2008, with around one-third of these deaths (453,000) attributable to rotavirus and most occurring in resource-poor settings. Frequent and severe enteric infections have been associated with growth-faltering and cognitive impairment. Within Australia, Aboriginal and Torres Strait Islander children suffer a heavy burden of diarrhoeal and intestinal infectious disease, with both the rates and severity of disease being significantly higher than in non-Aboriginal infants.

A number of small, randomised trials have suggested that compared with no treatment or placebo, recovery is faster among children treated with NTZ for giardia, amoebiasis, cryptosporidium, rotavirus, and norovirus gastroenteritis, and for diarrhoea where no enteropathogens are found. The seemingly broad range of enteropathogens for which efficacy has been shown or asserted based on in vitro data, raises the prospect of empiric use of NTZ for the syndromic treatment of paediatric infectious diarrhoea. This trial examines the effect of oral NTZ treatment compared to placebo on the duration of significant illness amongst Australian Indigenous children with acute gastroenteritis.

We hypothesise that empiric NTZ therapy for acute gastroenteritis in Australian Indigenous children under 5 years of age will reduce the symptoms of gastroenteritis and time spent in hospital.

This statistical analysis plan (SAP) is written to be consistent with the CONSORT 2010 Statement (Moher et al., 2010). The SAP provides a guide for the Trial Statistician during the statistical analysis of all quantitative outcomes and supports transparent and repeatable research.

# 

# **2.2. Objectives**

## Primary

To determine whether empiric treatment with oral NTZ, compared to placebo, reduces the time period of significant illness in Australian Indigenous children hospitalised for acute gastroenteritis.

## Primary Outcome

The primary objective is quantified through the time-period of significant illness. The outcome is designed to measure effectiveness of NTZ.

### The “time period of significant illness”, defined as the time from randomisation to either:

### (i) the time at which a score of two on the ‘medical readiness for discharge’ scoring system [the time difference between variables RANDDT & RANDTM compare to either STUDDT, ASS1TM/ASS2TM/ASS3TM & ASS1READCAT/ASS2READCAT/ASS3READCAT or DISCHDT, DISCHTM & DICHREAS] is first given by a health care professional, study doctor or study nurse,

### OR

### (ii) time of actual “discharge from hospital” [the difference between variables RANDDT & RANDTM compare to DISCHDT, DISCHTM & DICHREAS], where discharge from hospital refers to discharge by a doctor, but not absconded, transferred to another health care facility or deceased, whichever is sooner.

### Statistical Hypotheses

While the protocol hypothesis is a reduction in the time of significant illness (ie. *time to cure*) for individuals receiving NTZ compared to placebo, the statistical null hypothesis is no difference between the treatment arms. Using a Cox proportional hazards model, we construct the primary hypothesis based on the posterior distribution of the hazard ratio () for the NTZ arm compared to placebo (an exponentiated parameter estimate).

Null hypothesis, Alternate hypothesis,

A hazard ratio of one () indicates no difference between the NTZ and placebo arms. A hazard ratio in excess of one (>1) implies a shorter median time of significant illness in the NTZ arm relative to placebo, whereas a hazard rate less than one (<1) implies a greater median time of significant illness in the NTZ arm.

In the Bayesian Cox proportional hazard model, the treatment effect (ie. log hazard ratio, log()) is given a vague prior (log()~N[0,10], ie. the prior is approximately Normal distributed with a mean of zero and a variance of 10) such that the prior probability that nitazoxanide is beneficial is the same as the prior probability that it is futile. In the final analysis, if the probability of the alternate hypothesis is greater than 0.97 then NTZ is declared to be efficacious, i.e. when the posterior probability that the treatment effect is beneficial is greater than 0.97 or .

## Secondary

The secondary objectives are explorative and examine other aspects of nitazoxanide efficacy and safety.

Originally the following secondary objectives were defined to determine the effect of empiric treatment with nitazoxanide compared to placebo on:

1. the total length of stay in hospital;
2. the frequency of (i) stools and (ii) vomiting episodes during the period of significant illness;
3. the presence and severity of solicited symptoms associated with acute gastroenteritis during and post treatment;
4. the presence and severity of dehydration during the period of significant illness;
5. the time period for which IV, IO or NG rehydration is administered during the period of significant illness.

And to compare the proportion of the following safety and tolerability parameters in participants treated with nitazoxanide compared to placebo:

1. The occurrence of adverse events attributed to the study treatment
2. Mortality at 60 days
3. Recurrent gastroenteritis requiring health care assessment and/or intervention within 60 days of enrolment
4. Malnutrition (as diagnosed by a medical doctor) requiring health care assessment and/or intervention within 60 days of enrolment
5. Prolongation of acute gastroenteritis beyond day 7 after enrolment

However, having established the safety of NTZ in around 100 individuals (ie. over 200 recruited overall),

## Secondary Outcomes

There are several secondary endpoints that fall under clinical and safety domains, which are summarised below.

Clinical

1. Total length of stay in hospital

The period of time for which hospitalisation is required for any medical reason, defined as the time from randomisation to the time of “actual discharge from hospital” [the time difference between variables RANDDT & RANDTM compared to DISCHDT & DISCHTM].

1. Total number of stools and vomiting episodes during significant illness

During the “time period of significant illness” [identified in the primary outcome], the count of the total number of (i) stools [variable DIARRHNO] and (ii) vomiting episodes [variable VOMITNO] from combined CRF and diary card.

1. Disease signs and symptoms between study days 1-7

The presence and maximum severity of solicited symptoms associated with acute gastroenteritis (vomiting, diarrhoea, overall symptoms and activity level) from the calendar day of randomisation (study day 1) to the end of study day 7

1. Dehydration

4.1 The presence and severity of dehydration from randomisation to either discharge or ready for discharge, whichever is sooner.

4.2 The time interval between either starting rehydration (IV, IO or NG) or randomisation (whichever is the later), and ceasing rehydration. The time of cessation is defined as the time of completion of the last IV, IO or NG rehydration that is followed by actual discharge, or by a period of at least 24 hours before any further IV, IO or NG fluid rehydration is given.

Safety

### Adverse events attributed to the study treatment

### The total number of adverse events, stratified by body system, that have been attributed to the study treatment (placebo or NTZ) by the investigator, as indicated by a coding of “yes” for variable AEREL.

### Serious Adverse events

### The total number of serious adverse events, stratified by body system, that have been classified as “serious” by the investigator, as indicated by a coding of “yes” for variable AESER.

### Adverse events

### Mortality within 60 days of randomisation, as indicated by variables DECYN and DECDT.

### Recurrent gastroenteritis requiring health care assessment and/or intervention within 60 days of randomisation, as indicated by: <identification needs further discussion>

### Malnutrition (as diagnosed by a medical doctor), not present on index admission, requiring health care assessment and/or intervention within 60 days of randomisation, as indicated by: <identification from AE reporting – may require further discussion due to potential for bias>

### Prolongation of acute gastroenteritis beyond day 7 after randomisation, as indicated by:

### For those participants who remain in hospital after study day 7 (variable DAY7YN): Date of gastroenteritis resolution (from Participant Discharge page) more than 7 days after randomisation based on variable GASTDT;

### For those participants who are discharged ≤ study day 7 but excluding participants transferred to another hospital or those who died (variable DISCHREAS): any occurrence of gastroenteritis recorded in the Study Day 30 or Day 60 reviews (variable MSEVENT) which commenced prior to date of last treatment dose (based on variables LSTDSDT & MSEVENTSTDT)

### Hypotheses

The hypotheses for the secondary outcomes are analogous to those of the primary. Specifically, the clinical outcomes are all framed in terms of time to events and we therefore test the null hypothesis that the null hypotheses are that the acceleration factor equates to unity. The laboratory outcome tests the null that the odds ratio of the treatment relative to the control is unity and similarly for the safety endpoints.

# Study Methods

## Trial Design

A multi-centre, double-blind, randomised, placebo-controlled, parallel group trial of oral nitazoxanide for the treatment of acute gastroenteritis. Patients were randomised in a 1:1 allocation to one of two treatment arms:

• Nitazoxanide 7.5mg/kg/dose for infants < 1 year old or 100mg for children 1 to 3 years old or 200mg for children 4 years of age, 12 hourly on six occasions orally or by nasogastric/enteric tube

• Placebo, 12 hourly on six occasions orally or by nasogastric/enteric tube

The trial has four strata that represent the place of residence (remote/urban) and age (<1y or ≥1y). The treatment allocation sequence was generated separately for each strata and the statistician is unblinded to treatment group. The maximum planned sample size is 300 children. Due to the pragmatic design of the study no further adjustments were necessary for participants that are non-compliant, withdraw from the study or are lost to follow up. The trial has the following key features:

1. Double-blind, randomised, placebo-controlled trial;
2. The procedures for enrolment, intervention, end-point and analysis are based on the principles of pragmatic trial design;
3. Non-fixed sample size up to 300 participants based on Bayesian stopping rules;
4. Fixed 1:1 enrolment into the active and control arm throughout the trial;
5. Frequent interim analyses that can result in the trial stopping early for futility or expected success.

Given the potential of early stopping due to superiority or futility, it is not possible to explicitly state at the start of the RCT how many participants will be recruited, only the maximum number of participants who may be recruited. Recruitment continues up to the maximum number of participants unless the criteria for pre-specified statistical triggers (i.e. threshold for stopping rules) are met at an earlier time point based on frequent interim analyses. This trial is multifactorial, simultaneously evaluating intervention effects in the urban compared to remote regions and in children <1y compared to children ≥1y. Study participation is from enrolment until 36 months of age (see Trial Flow Chart, Appendix C of protocol).

## Randomisation

The stratified (urban vs remote), random allocation of two treatment arms called “A” and “B” to continuous randomisation numbers (1 to 300) has been generated by the trial statistician. The allocation was computer-generated using an appropriate block size known only to the trial statistician. The allocation ratio within these strata will be 1:1 “A” to “B” and the trial statistician will maintain the password protected file containing the allocation sequences.

Note – the trial statistician may be asked to provide treatment allocation details should medical or safety reasons warrant it. Authorisation for unblinding will be given to the trial statistician by the CI or delegate. See section 9.5 of protocol for further details.

## Sample Size

There is no fixed sample size only a maximum sample size of 300 participants. The simulations section details the simulations run to quantify the operating characteristics of the trial, including type I error rates and statistical power.

## Framework

This is a superiority trial - our goal is to detect the difference between the NZT and placebo in the defined environmental setting. We will present comparisons of the NZT against placebo with the aim of first rejecting the null hypothesis of no-difference and then estimating the size of the difference (in terms of a difference in proportions) between treatments.

## Interim Analyses and Stopping Rules

The first and second interim analyses are scheduled to occur when 126 and 150 participants have been enrolled, respectively. Thereafter, interim analyses will occur every 20 participants, up to a maximum number of 8 interims when enrolment is 270 participants, unless the decision threshold is met (a statistical trigger) at a previous interim. The stopping rule decision algorithm is outlined below and should be read in conjunction with the analysis methods contained in this document.

At all interims there will be a small percentage of participants who have not been enrolled in the trial long enough to assess the criteria for the primary outcome (time of significance illness). These participants will be right censored with the censored time being the duration of time from the randomisation to the time of the current interim.

1. Based on the data we have at the time of the interim, we compute the posterior distribution of the treatment effect (odds ratio) for the immunological outcome. We then draw from the posterior predictive distribution to simulate both the immunological results that are not yet available from the enrolled participants and the results from future participants up to the maximal number of participants (250). This simulated dataset of the immunological results conditional on our current data and parameter estimates that we might observe if we utilised all resources for seroconversion testing. Using this data, we compute the treatment effect () and assess , which, if is an odds ratio, would indicate a high probability of increased seroconversion in the treatment arm. We then repeat this process many () times, making draws from the posterior predictive distribution to create other simulated datasets on which we perform the same test. We then compute the predicted probability of success (PPoS) as where represents an indicator function that equals 1 if the expression it contains evaluates to true and zero otherwise and is a threshold probability that we define.

We conduct an analogous procedure using the clinical outcome measure – that is, we compute the posterior distribution of the parameter estimates from an event analysis and use these estimates to form a posterior predictive distribution of event times ultimately allowing us to produce .

If or we declare the trial to be futile due to its very low chance of success and we would cease enrolment. The final analysis will then occur once all outstanding venous sampling test results have been completed.

1. The data we have at the time of the interim on the clinical outcome (time to medical attendance) will contain some events but also censored values (the censor time will be the duration between randomisation and the time of the interim). We will compute the posterior of the parameter estimates from an event analysis and assess where is the acceleration factor from an accelerated failure time survival model. Note – assuming the occurrence of the event is negative for the individual, implies that exposure beneficial as the event is delayed relative to individuals in the control group.

If we find that we cease enrolment and declare success. In this scenario the final analysis will occur once all venous sampling results have been obtained. However, individuals who have not attended hospital by the time that all venous samples time are available will be censored.

1. Using the posterior distribution of the treatment effect (odds ratio) for the immunological outcome at the time of the interim analysis, we assess and we assess the posterior obtained from the survival analysis for the clinical outcome.

If we find that but then we continue enrolment but cease venous sampling.

1. If none of the above rules are met, we continue enrolling and venous sampling.

## Timing of Final Analysis

All outcomes will be analysed collectively at the conclusion of the trial. As of the time of writing (Dec 2018) data collection has started and approximately 50 participants have been enrolled. The protocol specifies that trial is currently planned to run until (late?) 2020. However, assuming that we expend all resources and reach the maximum sample size at the accrual rate of approximately 50 participants per 3-month period, then the data will not be available for the final analysis until Sep 2023.

Predefined subgroup analyses: Of the enteric pathogens which commonly cause gastroenteritis in this population, the strongest evidence is for nitazoxanide efficacy against the viral pathogens rotavirus and norovirus. Rotavirus and norovirus infection is most common in rotavirus unvaccinated children, in children younger than 12 months, and in children with moderate to severe illness (Snelling, NHMRC Project Grant 545233). We will therefore test the hypothesis that the effect of nitazoxanide treatment is modified by infecting pathogen type, and also by baseline predictors of viral infection which are identifiable at presentation. Specifically, subgroup analyses to determine the effect of empiric treatment with nitazoxanide compared to placebo on the duration of significant illness in participants will be performed according to the following if numbers permit

1. causative pathogen group (bacteria, virus, parasite)
2. 2. prior rotavirus vaccine receipt (never vaccinated, partially vaccinated, fully vaccinated)
3. 3. age as <1 year of age or ≥1 year
4. 4. severity of illness at presentation in those with no, mild, moderate or severe acidosis or dehydration at admission

## Timing of Outcome Assessments

Data will be collected and analysed as per Table 1.

|  |  |  |  |
| --- | --- | --- | --- |
| Interim | Date | Approximate  Sample Size | Comment |
| 1 | Jan 2017 | 126 | Performed after the protocol amendment which detailed the change in analysis from Frequentist to Bayesian methods. |
| 2 | Jul 2017 | 150 | Outbreak of gastroenteritis in Alice Springs in May-Jun 2017 |
| 3 | Aug 2017 | 170 | Outbreak of gastroenteritis in Alice Springs in May-Jun 2017 |
| 4 | Mar 2018 | 190 | Slow recruitment following the outbreak |
| 5 | *Unknown* | 210 | Recruitment suspended until a site outbreak of gastroenteritis (agreed with DSMB) |
| 6 | *Unknown* | 230 | Recruitment suspended until a site outbreak of gastroenteritis (agreed with DSMB) |
| 7 | *Unknown* | 250 | Recruitment suspended until a site outbreak of gastroenteritis (agreed with DSMB) |
| 8 | *Unknown* | 270 | Recruitment suspended until a site outbreak of gastroenteritis (agreed with DSMB) |
| Final | *Unknown* | 300 | Decision to suspend recruitment until a site outbreak of gastroenteritis agreed with DSMB |

Table 1: Planned timings for interim analyses. Future dates of interim analyses are dependent on recruitment, which can only occur during an outbreak of gastroenteritis at any of the sites

# Statistical Principals

## Type I Error Rate/Level of Significance

The sample size calculations for the primary analysis assumed a significance level of 0.05. The primary analysis will use a two-sided type 1 error rate (significance level) of 0.05. All a priori analyses will be reported in the main manuscript that documents the results from the trial. Appropriate corrections for multiple testing will be applied to secondary outcomes using the false discovery rate (FDR). 95% confidence intervals will be reported on treatment effect.

## Rationale for any deviation from Pre-specified Analysis Plan

As per the protocol, significant deviation from the SAP will be discussed with the study statistician (and reported to the DSMB) and reflected in any publications that arise from this study.

## Quality Control

Detail of quality control procedures, including monitoring for protocol compliance, is detailed the protocol section 15.

Additionally, the SAP and all statistical analyses will be subject to review from a statistician from AHI at the Telethon Kids Institute. An independent statistician will review all code.

Study personnel will perform quality control checks on the final tables, listings and figures to ensure accurate reporting in terms of titles, labels and frequencies or totals.

## Analysis Population

The primary analysis will be conducted on an intent-to-treat (ITT) basis. All randomised participants will contribute to the primary analysis of the primary endpoint and participants will be analysed in the group they were randomised to, irrespective of subsequent receipt of the correct study drug and adherence to protocol. The ITT population will be described, including a justification of any participants randomised but excluded from the analysis.

All randomised participants who adhere to the study procedures will form the Completers Population (CP) and be analysed in a secondary data analysis. The eligibility for the CP is defined below and excludes participants who receive rescue treatment with NTZ.

• have signed the requisite consent forms and successfully completed the screening assessments; and

• have been adherent (see protocol section 11.7) to study treatment (or the actual treatment received in case of randomisation or drug administration error); and

• have not discharged against medical advice or absconded; and

• have received no bias or interference that may interfere with potential study treatment effect, either according to the protocol or in the view of the study investigators; and

• have not received rescue treatment with NTZ.

# Trial Population and Processes

In this trial there are two subgroups representing participants normally residing in a major city, inner regional or outer regional area and participants residing in remote or very remote areas according to the Australian Government Department of Health Australian Standard Geographical Classification – Remoteness Area (ASGC-RA) system. All recruited participants will contribute to the primary and secondary analyses.

The data will be analysed on an intention to treat (ITT) basis with all randomised participants contributing to the primary analysis of the primary endpoint. The population to be analysed for the primary endpoint, the full analysis set, will be described including a justification of any participants that were excluded.

## Overview of Trial Processes

Prospective participants (newborn infants) will be identified by a member of the study team by screening admission lists of maternity wards at participating hospitals or by referral by the treating team. Prospective participants may also be recruited from other settings.

After participants provide consent and inclusion criteria have been met, we undertake baseline assessment, serum sampling (blood sample), randomisation and vaccination and (optionally) saliva genetic sampling. Note - once they have been randomised, the infant is enrolled in the study. If blood sampling is refused or unsuccessful the infant can still be enrolled. As noted earlier, blood will be collected from a maximum of 250 infants. The baseline data collected includes demographics, prior vaccination history, current illnesses, previous confirmed RV infection, breast feeding status and anthropometric indices (see protocol section 9.4 for further detail).

The above procedures (consent through to vaccination) should all occur within 12 hours of each other (protocol section 9.4.1.5). Study day 1 is defined as the day (starting from midnight) that vaccination (ROTARIX – a human monovalent oral live-attenuated vaccine that contains the most common human disease serotype, syn. RV1) occurs regardless of the time vaccination was administered.

The first follow up visit (follow-up 1) is intended to occur between day 28 and 55 (inclusive) and involves checking for elimination criteria and collecting a follow-up blood sample (if an initial sample was collected) (protocol section 9.4.2). From the baseline sample and follow up sample results we will assess the seroconversion status associated with the second dose and the third dose respectively. However, the reality is that the first scheduled vaccination may have been missed and so we might really know is the seroconversion status associated with the first and second dose.

On-going surveillance of all participants occurs until 36 months (and within a window of up to 36 months and 4 weeks). The first check is on the participants medical records within 30 to 40 days of vaccination to determine whether any hospitalisations have occurred. Thereafter, more detailed medical record reviews will occur every 6 months after vaccination to determine whether medical attendance occurred for diarrhoea/gastro or any adverse events. The surveillance period is ongoing until the end of the study, defined as the date of the last child’s third birthday (protocol section 9.4.3). However, the trial may also cease for other reasons.

## Eligibility

Full participant eligibility/inclusion criteria are documented in the protocol section 8.2. In brief, participants must be between 6 months and less than 12 months, identify as ATSI, have received 1 or 2 prior doses of RV1 and be willing to participate (as informed by legal care giver).

Exclusion and elimination criteria are also detailed in section 8 of the protocol.

Protocol section 9.1 details the reasons that should be recorded in the event of participant non-eligibility.

## Participant flowchart

All participants who provide informed consent will be accounted for in the final study report and their progress in the study will be accounted for using a CONSORT-style flow diagram (see Figure 1). The number (percentage) of participants who provide informed consent, are allocated to each treatment group and loss to follow-up, along with reasons for study withdrawal, and major protocol deviations and violations will be documented.

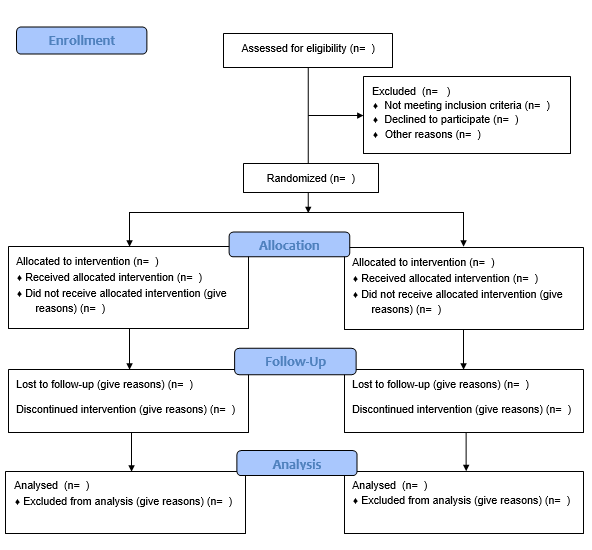


Figure 1 Consort 2010 flowchart (participants are defined as enrolled once they have been randomised).

## Level of withdrawal

We will document the reasons and details relating to loss to follow up and withdrawal. Withdrawal may occur due to elimination criteria being met or the by choice of the legal care giver.

## Baseline characteristics to be summarised

Demographic and other baseline characteristics will be summarised by assigned treatment group for the ITT population. These include:

* Demographics: age [*RANDDT-Birthdate*], gender [*Gender*], Indigenous status [*INDIGSTAT*], usual place of residence [*RESCLASS*] and place of residence the night before hospital admission [*ADMITCLASS*].
* Anthropometric indices: height [*RANDHGT*], weight [*RANDWT*] and mid upper arm circumference [*ARMCIRC*].
* History of presenting illness: duration of diarrhoea [*DIARRDUR*], grade of diarrhoea [*DIARRHGD*], temperature [*RANDTEMP*], pulse [*RANDPULSE*], respiration rate [*RANDRESPS*] and hydration status [HydrStat].
* Prior vaccination history obtained from parent/vaccine record/AIR/NTIR database: number of doses of rotavirus vaccine received [*derived from ROTAVACCDT & ROTAVACCTYP*]
* Medical history prior to enrolment: including condition [MHCAT], whether start date prior to randomisation [RANDDT-MHSTDAT], duration [MHSTDAT-MHENDAT] and whether ongoing at enrolment [MHONGO]).
* Medications prior to enrolment: (including medication generic name [Medication], indication [Indication], whether start date prior to randomisation (RANDDT-MHSTDAT), duration [MHSTDAT-MHENDAT] and whether ongoing at start of study period [MHONGO])

Categorical variables will be summarised by frequencies and percentages. Percentages will be calculated according to the number of patients for whom data are available (observed). Where values are missing, the denominator, which will be less than the number of patients assigned to the treatment group, will be reported either in the body or a footnote in the summary table. Continuous variables with symmetric distributions will be summarised by treatment group using mean and standard deviation, whereas continuous variables with asymmetric distributions will be summarised using median and interquartile range (25% and 75% percentiles).

## Data Source

Data will be sourced from a specifically designed clinical record form (CRF) comprising consent forms, eligibility assessment, visit record, adverse event details and protocol deviations. All data will be entered into a online database build in Medrio with data queries raised by the data manager and trial statistician, who are also responsible for data collection.

Data is sourced from a specifically designed clinical record form (CRF) and parent-reported diary card and further supplemented by data from laboratory and pharmacy records and medical records based on reviews performed at Study Day 30 and Day 60. CRF entries will be considered source data if the CRF is the site of the original recording (e.g. there is no other written or electronic record of data). All data will be entered into an online database build in Medrio by study personnel who remain blinded to the treatment allocation. Data queries are raised by the data manager. The final planned analysis, outlined in the protocol and detailed in this SAP, will be performed only after the last participant has completed Study Day 60 and the database has been cleaned and locked.

SAEs and measures of study implementation and conduct have been monitored on a regular basis by a Data and Safety Monitoring Board (DSMB). Only DSMB members and statisticians compiling closed-session reports for DSMB meetings will have access to un-blinded interim data and results.

# Configuration and Formatting Guidelines

Portrait orientation is the default for all tables, figures and data listings unless indicated otherwise.

All R script files developed for a table, figure or data listing will be self-contained to facilitate transfer of programs to multiple computing environments. A separate R script file will be written to produce each table, figure or listing.

## Tables & Listings (raw data)

The first title line will be the number of the table or data listing. The second (and if required, third) title line will be the description of the table or data listing as indicated in the Planned Tables and Planned Listings sections below. All tables and data listings will have the name of the relevant R script file and a date-time stamp on the bottom of each output. Additionally:

* Sample sizes shown with summary statistics are the number of participants with non-missing values.
* Summaries for categorical variables will include only levels with observed data. Percentages corresponding to null categories (cells) will be suppressed.
* All summaries for continuous variables with symmetric distributions will include: N, mean and SD.
* All summaries for continuous variables with asymmetric distributions will include: N, median and quartiles 25% and 75% (interquartile range). 95% confidence intervals, coefficient of variation (CV) or %CV may be used as appropriate.
* All percentages will be rounded and reported to a single decimal place (xx.x%). If percentages are reported as integers, percentages greater than 0% but <1% will be reported as <1%, whereas percentages greater than 99% but <100% will be reported as >99%. A percentage of exactly 100% will be reported as 100%.
* Summaries that include p-values will report the p-value to two decimal places if greater than 0.05 (i.e. non-significant) or to either three decimal places with a leading zero (e.g. p-value=0.001) or scientific notation for p-values <0.001 (e.g. p-value=2x10-5).

## Figures

Legends will be included in all figures with more than one level or category for an explanatory variable (e.g. separate lines for males and females). Figure lines, including axes, should be wide enough to see the line even after rescaling. No figures will be created with titles to facilitate flexible use of the figures in reports, manuscripts and presentations; figure titles will be added after the file has been inserted into a WORD, PowerPoint or other such document.

## Planned Tables

Table 1. Enrolment, age [*RANDDT-Birthdate*] and remote status [*ADMITCLASS*] classification and randomisation strata [*STRATA*] by treatment arm [*TMTDSC*] and site [*Site*]

Table 2. Consent [absent/present *CONSDT*], randomisation [absent/present *RANDDT*], withdrawals [derived variable ….], protocol deviations and violations [derived variable …] by treatment arm [*TMTDSC*] and site [*Site*]

Table 3. Eligibility criteria [*INC1-INC7,EXC1-EXC11*] by treatment arm [*TMTDSC*] and site [*Site*]

Table 4. Demographic and baseline characteristics [see *Baseline Characteristics* on page 15, excluding medical history and medications] by treatment arm [*TMTDSC*] and site [*Site*]

Table 5. Microbiology at admission to hospital by treatment group [*TMTDSC*] and strata [*STRATA*]

1. blood culture growth detected [*BLCLORGYN*] and significance of organism [*BLCLORGSIG*]
2. urine culture organism detected [URINORGYN], significance of organism [URIN*ORGSIGYN*], white cell count [URINWCC] and red cell count [URINRCC])
3. stool sample rotavirus, norovirus and adenovirus test results

Table 6. Primary outcome by treatment group [*TMTDSC*] and strata [*STRATA*]

Table 7. Secondary outcomes by treatment group (to be further defined)

Table 8. SAEs by treatment group

Table 9. Relationship of SAE to study medication by treatment group

## Planned Figures

Figure 1. Flowchart of participant progression through the study (CONSORT)

Figure 2. <further figures to be defined>

Figure 3. <further figures to be defined>

Figure 4. <further figures to be defined>

## Planned Listings (raw data)

Listing 1. Medical history prior to enrolment (including condition [*MHCAT]*, whether start date prior to randomisation [*RANDDT-MHSTDAT*], duration [*MHSTDAT-MHENDAT*] and whether ongoing at enrolment [*MHONGO*])

Listing 2. Medications prior to enrolment (including medication generic name [*Medication*], indication [*Indication*], whether start date prior to randomisation (*RANDDT-MHSTDAT*), duration [*MHSTDAT-MHENDAT*] and whether ongoing at start of study period [*MHONGO*])

Listing 3. Microbiology at admission to hospital (including (i) blood culture growth detected [*BLCLORGYN*], name of organism [*BLCLORG*] and significance of organism [*BLCLORGSIG*]; (ii) urine culture organism detected [URINORGYN], name of organism [URINORG], significance of organism [URIN*ORGSIGYN*], white cell count [URINWCC] and red cell count [URINRCC])

# Analyses

Notes to self –

tte

to be summarised as the median and inter-quartile range for each group

seroconversion

, to be summarised as the proportion of all participants per group

## Analysis Methods

Bayesian predictive approach to interim monitoring in clinical trials, pg 2180, section 4,

https://onlinelibrary.wiley.com/doi/pdf/10.1002/sim.2204

All analyses sit within a Bayesian framework.

### Analysis of co-primary outcomes

For the clinical outcome we use time to event analyses, specifically, accelerated failure models or proportional hazard models to model with distributional assumptions adopted based on goodness-of-fit. Our initial model is a lognormal (maybe log-logistic) AFT that models time to event (in weeks) directly (rather than model the hazard as is done in PH models). The model will include covariates:

1. Dichotomous indicator variable for treatment arm (coded as 0/1)
2. Regional strata (categorial variable)
3. Baseline seroconversion status (continuous variable serum anti-rotavirus IgA)

We will evaluate the probability of a treatment effect using a credible interval constructed from the posterior distribution for the acceleration factor. If we find where is defined as the acceleration factor of the treatment arm relative to the control arm, then we will reject the null hypothesis of no-difference.

Our analyses will use uninformative priors. For a lognormal AFT model, this implies that we will use an inverse gamma distribution for the scale parameter and

For the immunological outcome we will use a Bayesian hierarchical model (syn. Generalised linear mixed-effects regression (GLMM)) with binomial family and logit link. The model will include covariates:

1. Dichotomous indicator variable for treatment arm
2. Random effect for regional strata
3. Baseline seroconversion status

Our analyses will use uninformative priors. For the above model, this implies that we will use an ? distribution for the. Hyperparameters.

Model assumptions will be assessed using appropriate diagnostic measures such as posterior predictive checks.

### Analyses of secondary outcomes

Table 2 summarises secondary outcome analyses covered by this SAP.

|  |  |  |  |
| --- | --- | --- | --- |
| **Secondary analysis ID** | **Outcome Description** | **Notes** | **Statistical Model** |
| 1-CLIN | time to hospitalisation for acute gastroenteritis or acute diarrhoea illness. | Time from randomisation to hospitalisation (in weeks). Measure of interest is the treatment effect – acceleration factor of treatment. | Methods analogous to those used in primary analysis. |
| 2-CLIN | time to hospitalisation for rotavirus-confirmed  diarrhoea illness. | Analogous to above. | As above. |
| 3-CLIN | proportion of participants meeting the jurisdictional case definition of rotavirus infection | Dichotomous dependent variable characterising meeting case definition or not. Binomial logistic regression analogous to primary analysis. |  |
| 4-CLIN | change in IgA log titre | Continuous dependent variable |  |
| 1-SAF | proportion of participants meeting the case definition of intussusception (Brighton criteria) | As per 3-CLIN. Dichotomous dependent variable. |  |
| 2-SAF | the proportion of participants meeting the case  definition of a serious adverse event | As per 3-CLIN. Dichotomous dependent variable. |  |

Table 2 Summary of secondary analyses – CLIN – clinical endpoint looking at effectiveness, SAF – safety and tolerability endpoint,

The following analyses have been dropped from the protocol and the specified analyses will be detailed elsewhere:

### Analyses of safety outcomes

See above.

### Participant characteristics and baseline comparisons

Demographics and baseline prevalence of impetigo and scabies will be presented using appropriate summary statistics to assess balance.

Categorical variables will be summarised by frequencies and percentages.

Percentages will be calculated according to the number of participants for whom data are available (observed).

Where values are missing, the denominator, which will be less than the number of participants assigned to the treatment group, will be reported either in the body or a footnote in the summary table. Continuous variables with symmetric distributions will be summarised by treatment group using mean and standard deviation, whereas continuous variables with asymmetric distributions will be summarised using median and interquartile range (25% AND 75% percentiles).

### Subgroup Analyses

Subgroup – primary and secondary endpoints among participants who received 1 dose and 2 dose of rv1 prior to the study intervention.

## Simulating operating characteristics

## Sensitivity Analyses

Adjust for covariates that may be confounding?

Baseline seroconversion?

### Outliers

Outliers will be identified ]using applicable diagnostics. All outliers will be assessed to determine the potential influence on the statistical results and, if deemed appropriate, sensitivity analyses may be performed both including and excluding the outlier(s). Discrepant results from these two analyses will be explained in the text of the final statistical report and any associated manuscripts and details of the analyses performed and results will be included in an appendix.

## Missing Values

Missingness is classified into Missing completely at random (MCAR), Missing at random (MAR) and Missing not at random (MNAR). MCAR characterises data where missing values are independent of observed and unobserved measures and a complete-case analysis can be unbiased. MAR characterises data where the missingness is dependent on observed measures and therefore can be reasonably predicted using imputation methods. MNAR characterises data where missing values are dependent on unobserved measures and thus cannot be predicted without bias and thus sensitivity analyses are necessary.

Reasons for missing data pertaining to the primary endpoint and safety endpoints (including withdrawal of consent, loss to follow-up, removal from study due to serious side effects, death, or inability to obtain any laboratory results) will be indicated. The quantity of this missing data for each treatment arm and strata will be compared.

1. Missing completely at random (MCAR)

Data that is missing independent of observed or unobserved measurements will not be included in the statistical analysis as these may be considered a random and representative sample from the total study population. For example: a non-health related move to another state results in missing data after study day 5. These data occurrences are anticipated to be rare in the study.

2. Missing at random (MAR)

When the probability of an observation being missing is dependent on observed measurements, then the missing observations can be reasonably predicted, with minimal bias, using multiple imputation techniques. For example: (i) when a participant drops out due to lack of efficacy reflected by a series of poor efficacy outcomes that have been observed; or (ii) when a participant absconds due to a series of good efficacy outcomes that have been observed. In either case, it would be appropriate to impute subsequent efficacy outcomes for this participant based on the observed measurements & covariates.

3. Missing not at random (MNAR)

When the probability of an observation being missing is dependent on unobserved measurements then future observations cannot be predicted without bias. In this case sensitivity analyses will need to be performed replacing the missing outcome data with the extremes of the scale. For example: missing time of discharge from hospital may be set to the minimum value across all participants for the first sensitivity analysis and then set to the maximum value for the second sensitivity analysis.

## Additional Analyses Beyond Scope of SAP

None identified.

### Harms

Section 12 of protocol.

The investigational medicinal product, ivermectin, is generally well tolerated. However, reported adverse effects include asthenia/fatigue, abdominal pain, anorexia, constipation, diarrhoea, vomiting, nausea, somnolence, dizziness, vertigo, tremor, pruritus, rash and urticaria. Ivermectin has also been associated with laboratory abnormalities.

Additional products to be used during the trial are documented in Section 7.5 of the protocol.

SAEs and measures of study conduct and implementation by treatment group will be monitored on a regular basis by a Data and Safety Monitoring Board (DSMB). Only DSMB members and statisticians compiling closed-session reports for DSMB meetings will have access to un-blinded interim data and results.

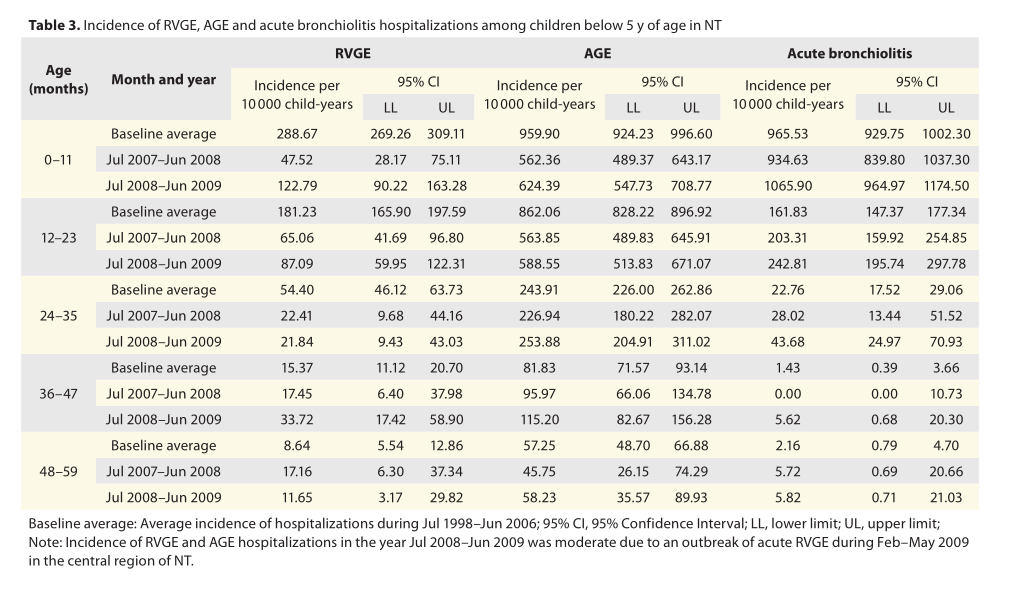
## Software Used in the Trial Analyses

All data manipulations, calculation of summary statistics, tabulations, listings, analyses and graphics will be documented in script files and performed using R (version 3.5.1, 2018 or higher), Julia (version 1.0.2) and Stata (version 15 or higher).

# Revision History

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Version 1 to Version 2 Revisions (Date: ) | | | | |
| ID | Section/Page | Original Text | Revised Text | Comment |
| V0.3 |  |  |  | First draft |
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# Appendix 1



# 

# Appendix 2: Trial Definitions

|  |  |
| --- | --- |
| Acute gastroenteritis | Any episode of illness in which the predominant feature is the onset (or abrupt worsening) of looser-than-normal stools and/or forceful vomiting and for which an infectious cause is confirmed or suspected. |
| Diarrhoea | Any watery or looser-than-normal stools. |
| Discharge from hospital | Discharged from hospital by a doctor, but not absconded, transferred to another health care facility or deceased |
| Gastroenteritis episode | Gastroenteritis episodes begin on the first day that vomiting and / or diarrhoea is reported (day 1) and continues until symptoms return to baseline. |
| Gastroenteritis episode resolution | An episode is considered resolved when a study participant’s symptoms have returned to baseline for ≥4 days. Episodes which stop and have additional symptoms occurring less than 4 days following the last symptom are considered to be a continuation of the original episode. |
| Calendar day | A calendar day is the 24-hour period from midnight of one day to midnight the next. A different 24-hour period may be defined by the legal carer (e.g., sunrise of one day to sunrise the next day) for the purpose of recalling maximum severity; however, calendar days should be counted for all “duration” fields. |
| Hospitalisation | Any overnight stay in any health facility |
| Indigenous | Any child who is identified by his or her parent or legally responsible care-giver as being of Aboriginal, Torres Strait Islander and / or South Sea Islander descent. |
| Legally responsible care-giver / parent | A parent of an Aboriginal child includes a person who is regarded  as a parent of the child under Aboriginal customary law or  Aboriginal tradition. |
| Period of Significant illness | The period for which hospitalisation is required for medical reasons, defined as the time from randomisation to  (i) the time at which a score of two on the ‘medical readiness for discharge’ scoring system (see appendix G) is first given by a health care professional, study doctor or study nurse, OR  (ii) the time of actual “discharge from hospital” (see definition below), whichever is sooner. |
| Rehydration | An attempt to replace or maintain bodily fluids lost from expulsion during diarrhoea and/or vomiting episodes using either nasogastric therapy (NGT), intravenous therapy (IVT) or intra-osseous therapy (IOT). This does not include caloric therapy (feeding), or use of low volume IVT (<10mL/hr) solely to maintain IV cannula patency.). |
| Remote | Those areas described as very remote in the ASGS remoteness scale |
| Study day | A full calendar day starting with the day that randomisation occurred, study day 1, and each subsequent calendar day until study day 7. Note that study day 1 commences from midnight on the day that randomisation occurs regardless of when the first dose of study drug is administered. |
| Urban | Those areas defined as outer regional or remote in the ASGS remoteness scale |
| Vomiting | One or more episodes of forceful emptying of stomach contents. |

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