CLINICAL PROTOCOL COVER PAGE

Protocol Title:	A randomized, double-blind, placebo controlled, parallel clinical trial to investigate the safety and efficacy of *Bacillus coagulans* SNZ 1969 on immune health in healthy school-aged children
Protocol Number:	25SACCP01
Protocol Date:	August 28, 2025
Version:	2
Study Design:	Randomized, double-blind, placebo controlled, parallel clinical trial
Sponsor:	SANZYME BIOLOGICS PVT LTD 2nd Floor, Sattva Signature Tower, No 8-2-472/1/A/B/SF-3, Road No. 1 Banjara Hills, Hyderabad – 500 034 India
Sponsor Contact:	Dr. Dinesh Nair PhD Vice President, Scientific Affairs SANZYME BIOLOGICS PVT LTD 2nd Floor, Sattva Signature Tower, No 8-2-472/1/A/B/SF-3, Road No. 1 Banjara Hills, Hyderabad – 500 034 India 91 40485 89999
CRO:	KGK Science Inc. 275 Dundas Street, Tower A Suite G025 London, ON N6B 3L1 Canada
Medical Director	

Protocol 25SACCP01 A randomized, double-blind, placebo controlled, parallel clinical trial to investigate the safety and efficacy of Bacillus coagulans SNZ 1969 on immune health in healthy school-aged children.

(Qualified Investigator): David Crowley, M.D
KGK Science Inc.
519-438-9374

Protocol 25SACCP01 A randomized, double-blind, placebo controlled, parallel clinical trial to investigate the safety and efficacy of Bacillus coagulans SNZ 1969 on immune health in healthy school-aged children.

PROTOCOL SIGNATURE SHEET

The sponsor and the investigator agree to conduct the study in compliance with the clinical study protocol (and amendments), International Conference on Harmonization (ICH) guidelines for current Good Clinical Practice (GCP) and applicable regulatory requirements.

Name	Signature	Date
Sponsor: Dr. Dinesh Nair PhD Vice President of Scientific Affairs SANZYME BIOLOGICS PVT LTD 2nd Floor, Sattva Signature Tower, No 8-2-472/1/A/B/SF-3, Road No. 1 Banjara Hills, Hyderabad – 500 034 India		24/09/2025
Contract Research Organization: Marc Moulin, PhD Director of Clinical Services KGK Science Inc. 275 Dundas Street, Tower A Suite G02 London, ON N6B 3L1 Canada	Marc Moulin	Oct. 3, 2025

1 SCHEDULE OF ASSESSMENTS [1]

PROCEDURES/ASSESSMENTS	V1 Screening	V2 Baseline Day 0	V3 Day 28 ± 2 (Remote)	V4 Day 56 ± 2 (Remote)	V5 Day 84 ± 2 (EOS)
Informed consent & Assent (*if applicable*)	●				
Review inclusion/exclusion criteria	●	●			
Review medical history	●				
Review concomitant therapies	●	●	●	●	●
Vitals: Height, weight, heart rate, blood pressure	●	●			●
Urine Pregnancy Test	●	●			●
Randomization		●			
Quantibody® Human Immune Response Array		●			●
Immunoglobulin A (IgA), G (IgG), E (IgE), and M (IgM)		●			●
Saliva sIgA		●			●
Primary Endpoint Assessment (URTI & GITI symptoms via study diary)		●	●	●	●
IP Dispensed		●			
IP Returned					●
Stool Collection Kit Dispensed	●	●			
Stool Collection Kit Returned (for Fecal Microbiome Analysis)		●			●
Study Diary Dispensed (including CARIFS, Additional Respiratory Tract Symptoms, and GITI symptom questionnaires)	● [1]	●	●	●	
Study Diary Reviewed (including CARIFS, Additional Respiratory Tract Symptoms, and GITI symptom questionnaires)		●	●	●	●
Compliance Reviewed			●	●	
Compliance Calculated					●
Adverse Events		●	●	●	●

[1] Participants will complete the study diary inclusive of the CARIFS questionnaire, Additional Respiratory Tract Symptoms, and GITI symptoms, during the run-in period between screening and baseline

2 LIST OF ABBREVIATIONS

AE	Adverse Event
AUC	Area Under the Curve
BP	Blood Pressure
CARIFS	Canadian Acute Respiratory Illness Flu Scales
CRO	Contract Research Organization
eCRF	Electronic Case Report Form
etc.	*"and so forth"*
e.g.	*"for example"*
GCP	Good Clinical Practice
GITI	Gastrointestinal Tract Infection
HR	Heart Rate
ICF	Informed Consent Form
ICH	International Conference of Harmonization
Ig	Immunoglobulin
i.e.	*"in other words"*
IEC	Independent Ethics Committee
IP	Investigational Product
IRB	Institutional Review Board
ITT	Intention to Treat
kg	Kilogram
mL	Milliliter
NNHPD	Natural and Non-Prescription Health Product Directorate
OTC	Over the Counter
PP	Per Protocol
QI	Qualified Investigator
SAE	Serious Adverse Event
URTI	Upper Respiratory Tract Infection

3 TABLE OF CONTENTS

4 INTRODUCTION

The flu season in North America typically occurs between late November and March (1,2). A typical year in Canada sees 12,200 hospitalizations and approximately 3,500 deaths due to flu (3). In the United States, over 200,000 hospitalizations and over 30,000 deaths (4) occur due to respiratory infections, which creates a large economic burden of $40 billion/year and $87 billion/year for the common cold (5) and influenza, respectively (6). Worldwide seasonal influenza can cause one billion infections, leading to three-to-five million severe cases, and from 250,000 to 500,000 deaths world-wide each year (1). The influenza vaccine is effective in providing protection against infection and illness from influenza (2), however a small percentage of school age children receive the flu vaccine. Overall, 31% of children 6 months to 17 years received the seasonal influenza vaccine during the 2023-2024 influenza season (7). Furthermore, rotavirus (RV) is a common cause of gastroenteritis in children; approximately 36% of children with RV gastroenteritis see a physician, 15% visit an emergency department, and 7% require hospitalization (8). Most unimmunized children are infected by 5 years of age whereas the first infection usually does not lead to immunity. In Canada RV is a common cause of gastroenteritis accounting for 10% to 40% of all childhood gastroenteritis (8). Similarly, norovirus is an increasingly important player in childhood gastroenteritis with the uptake in rotavirus vaccination (9). A meta-analysis on 6 years of research from 2014-2020 found the pooled prevalence of norovirus infection among 120,531 children with gastroenteritis from 45 countries was 17.7% (9).

The common cold is highly prevalent in the under 12 population (10), with the most common methods to reduce the risk of catching the cold being to wash your hands and avoid contact with your face. For adults with a severe cold, medications exist with codeine or hydrocodone, however, these are not recommended for children under 18 years of age (11). Further, decongestants are not advised in children 6-12 years of age (10). Almost one-third of common cold cases in children result in a visit to a physician, and over 75% of parents report giving their children over-the-counter (OTC) cold medication. However, there is little evidence these therapies are effective for children (12). Consequently, there is an increased need to determine efficacious alternatives that are safe. There have been several dietary supplements known to have immunomodulating effects examined in children, yet results are inconsistent. A meta-analysis of 31 randomized, placebo-controlled trials investigating the effects of regular vitamin C supplementation found a reduction in cold duration in adults and children (13). However, the effect of other supplements, such as echinacea, zinc, and garlic, are not as efficacious or clear. A meta-analysis examining the effects of echinacea involving 12 placebo-controlled randomized clinical trials (one involving children) showed no benefit for the common cold (14). In a meta-analysis investigating the effects of zinc supplementation on common cold symptoms results showed no benefit for children across three randomized, placebo-controlled trials (15). Another meta-analysis for preventing the common cold with garlic showed a reduction in incidence of common cold, however, more randomized, placebo-controlled trials are needed as only a single trial met these criteria (16). There is a noticeable lack of options for natural health supplements that provide strong evidence for reducing the burden of cold and flu-like symptoms for children, even though they are at the highest risk for catching common colds.

The current study will examine the efficacy of *Bacillus coagulans SNZ 1969* (*B. coagulans*) on immune health in children attending school. The primary outcome will assess the difference between the investigational product and placebo from baseline to day 84 in incidence, duration, and severity of (1) Upper Respiratory Tract Infection (URTI) and (2) Gastrointestinal Tract Infection (GITI) symptoms. This will be assessed by use of the Canadian Acute Respiratory Illness and Flu Scale (CARIFS) and a GITI symptoms questionnaire. A randomized, double-blind placebo controlled study of healthy school aged children (6-8

years) evaluating *B. coagulans* demonstrated a significant decrease in incidence of Upper Respiratory Tract Infections (URTIs) symptoms including nasal congestion, bloody nasal mucus, itchy nose, and hoarseness (17). There were corresponding decreases in duration of URTI symptoms of hoarseness, headache, red eyes and fatigue (17). In the same study *B. coagulans* significantly decreased the incidence of flatulence, a potentially symptom of GITI. These beneficial effects were associated with modulation of serum immune markers for TNFα, CD163, G-CSF, ICAM-1, IL-6, IL-8, MCP-2, RAGE, uPAR, and PF4 (17). Similarly, a randomized double blind placebo control study of malnourished children between 1 and 5 years of age demonstrated a significant reduction in the total days of illness with *B. coagulans SNZ 1969*® compared to placebo over a period of 3 months (18). This study also demonstrated a non-significant reduction gastrointestinal and respiratory tract infections along with a shorter duration of infection (18).

Enrolled participants will include children 6-12 years of age currently attending school during the 2025-2026 cold and flu season to allow for adequate exposure to URTI or GITI pathogens. To avoid confounding effects of pre-existing medical conditions children presenting with a history or presence of a clinically relevant respiratory, pulmonary, or gastrointestinal condition will be excluded at the discretion of the Qualified Investigator. Furthermore, participants consuming immune modulating medications, antibiotics, products containing *B. coagulans*, or any other probiotic supplement will be excluded unless they have undergone the specified washout. The strict eligibility criteria is designed to reduce confounders on immune health affecting both upper respiratory tract infections and gastrointestinal tract infection symptoms. Children presenting with any other medical condition or lifestyle factor which may affect the safety of their participation or study outcomes will also be excluded.

5 STUDY OBJECTIVES

The objective of this study is to investigate the safety and efficacy of *Bacillus coagulans* SNZ 1969 on the incidence rate, duration, and severity of acute upper respiratory tract infections (URTI) and gastrointestinal tract infection (GITI) symptoms in healthy school-aged children.

Primary outcomes:

1. The difference in incidence, duration, and severity (area-under-the-curve (AUC)) of URTI as assessed by the Canadian Acute Respiratory Illness and Flu Scale (CARIFS) from baseline to day 84 between *Bacillus coagulans* SNZ 1969 and placebo
2. The difference in incidence, duration, and severity (area-under-the-curve (AUC)) of Additional Respiratory Tract Symptoms as assessed by Additional Respiratory Tract Symptoms questionnare from baseline to day 84 between *Bacillus coagulans* SNZ 1969 and placebo
3. The difference in incidence, duration, and severity (AUC) of GITI symptoms as assessed by the GITI Symptoms Questionnaire from baseline to day 84 between *Bacillus coagulans* SNZ 1969 and placebo

Secondary outcomes:

1. The difference in incidence, duration, and severity (AUC) of URTI as assessed by the CARIFS from baseline to days 28 and 56 between *B coagulans* SNZ 1969 and placebo
2. The difference in incidence, duration, and severity (AUC) of Additional Respiratory tract symptoms as assessed by Additional Respiratory Tract Symptoms questionnare from baseline to days 28 and 56 between *B coagulans* SNZ 1969 and placebo
3. The difference in incidence, duration, and severity (AUC) of GITI symptoms as assessed by the GITI Symptoms Questionnaire from baseline to days 28 and 56 between *B. coagulans* SNZ 1969 and placebo
4. The difference between *B. coagulans* SNZ 1969 and placebo from baseline to days 28, 56, and 84 on:
    a. Severity of cold/flu and GITI symptoms as assessed by total and individual daily symptom scores
    b. Number of missed school days
    c. Number of well days, related to the absence of cold/flu and GITI symptoms
    d. Use of prescription and non-prescription cold/flu medications to treat cold or flu symptoms
    e. Total days of illness
5. The difference in change between *B. coagulans* SNZ 1969 and placebo from baseline to day 84 on:
    a. Saliva secretory immunoglobulin A (sIgA) concentrations
    b. Serum levels of Immunoglobulin A (IgA), G (IgG), E (IgE) and M (IgM)
    c. Immune response biomarkers: CD14, CD163, CD40 (TNFRSF5), CRP (C-Reactive Protein), E-Selectin, Fas (TNFRSF6/Apo-1), Fas Ligand (TNFSF6), GCSF, ICAM-1 (CD54), IL-1 alpha (IL-1 F1), IL-1 beta (IL-1 F2), IL-1 R4 (ST2), IL-10, IL-12 p70, IL-13, IL18, IL-2, IL-2 R alpha, IL-4, IL-6, IL-8 (CXCL8), Lipocalin-2 (NGAL), MCP-1 (CCL2), MCP-2 (CCL8), MIF, MIP-1 alpha (CCL3), MIP-1 beta (CCL4), Osteopontin (SPP1), PAI-1, Platelet Factor 4 (CXCL4), Procalcitonin, RAGE, Resistin, Thrombomodulin, TNF alpha, TREM-1, Troponin I, uPAR, VCAM-1 (CD106), VEGF-A.
    d. Microbiome as assessed by 16s rRNA fecal microbiome analysis

Safety outcomes:

1. Incidence of post-emergent adverse events (AE)
2. Clinically relevant changes in vital signs (blood pressure (BP) and heart rate (HR)) after supplementation

6 STUDY DESIGN

The planned sample size for this study is 100. In order to evaluate primary, secondary, and safety outcomes, study assessments will be conducted as per the Schedule of Assessments in Section 1.

Study Arm	Number of Participants
Bacillus coagulans SNZ 1969	n = 50
Placebo	n = 50
Total	n = 100

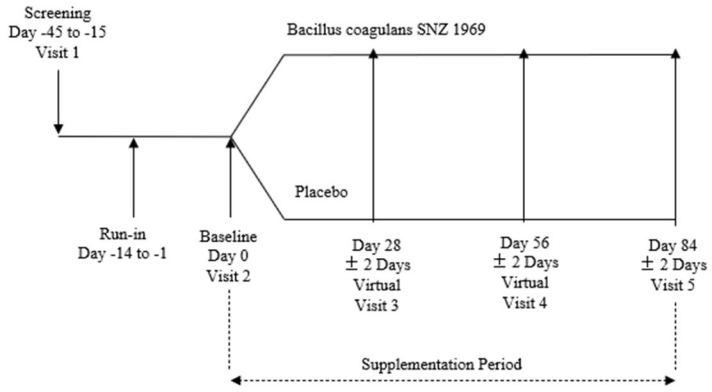

Figure 1. Study Design

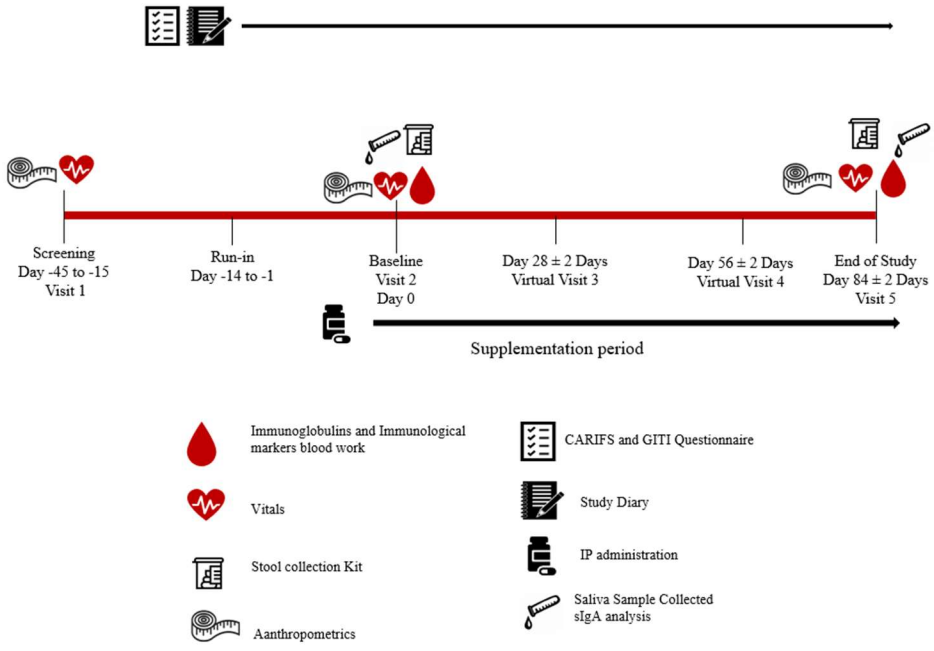

Figure 2. Study Schematic

7 SELECTION OF STUDY POPULATION

This study will enroll 100 healthy participants. Each participant must fulfill the inclusion criteria and not meet any of the exclusion criteria as described in Sections 7.1 and 7.2, respectively.

7.1 Inclusion Criteria

1. Males and females between 6 and 12 years of age at screening, inclusive
2. Children enrolled in and attending school in person at baseline
3. Willingness to complete questionnaires, records, and diaries associated with the study and to complete all clinic and remote visits
4. A care provider who can reliably bring the participant to study visits. The participant's primary caregiver must be willing and able to complete the questionnaires
5. The participant or the participant's parents/guardian are willing and able to provide written assent and/or informed consent as appropriate.
6. Agrees to maintain current lifestyle habits (diet, physical activity, medications, supplements, and sleep) as much as possible throughout the study
7. Healthy as determined by medical history as assessed by the Qualified Investigator (QI)

7.2 Exclusion Criteria

1. Individuals who are pregnant.
2. Allergy, sensitivity, intolerance, or dietary restriction preventing consumption of the investigational product or placebo ingredients
3. History or presence of a clinically relevant cardiac, renal, hepatic, endocrine (including diabetes mellitus), respiratory, pulmonary, biliary, metabolic, haematologic, gastrointestinal, or pancreatic disorders, that may affect participation or outcomes as assessed by the QI.
4. Confirmed history of COVID-19 infection in the 3 months prior to baseline.
5. Immune dysfunction, autoimmune disease, immune compromised and/or taking an immunosuppressive medication, as assessed by the QI
6. Severe environmental allergies requiring medical or need for allergy shots, as assessed by the QI.
7. Major surgery in the past 3 months or individuals who have planned surgery during the course of the study. Participants with minor surgery will be considered on a case-by-case basis by the QI
8. Cancer, except skin basal cell carcinoma completely excised with no chemotherapy or radiation with a follow up that is negative. Volunteers with cancer in full remission for more than five years after diagnosis are acceptable
9. Asthma, as assessed by the QI
10. Current use of prescribed and/or over-the-counter (OTC) medications, supplements, and/or consumption of food/drinks that may impact the efficacy and or safety of the investigational product (Sections 7.3.1 and 7.3.2)
11. Participation in other clinical research studies 30 days prior to baseline, as assessed by the QI
12. Participant or participant's caregiver who are cognitively or neurodevelopmentally impaired affecting their ability to give informed consent and/or assent
13. Any other condition or lifestyle factor, that, in the opinion of the QI, may adversely affect the participant's ability to complete the study or its measures or pose significant risk to the participant

7.3 Concomitant Medications

Participants who are taking any prescribed medications that are considered not to affect the study outcomes must agree to maintain their current dosing regimen during the study unless otherwise recommended by their general practitioner/nurse practitioner.

7.3.1 Prescribed Medications

Participants on the following concurrent prescribed medications and/or treatments will be excluded during enrollment unless they have been taken off these therapies by their general practitioner/nurse practitioner. In the latter event, the frequency of use and/or dosage may be considered by the QI on a case-by-case basis prior to recommending an appropriate washout or their enrollment in the study.

1. Immunomodulators (inc. corticosteroids) such as immunosuppressants or immunostimulants (4 weeks of baseline)
2. Cyclosporine, methotrexate, azathioprine, mycophenolate mofetil (1 week of baseline)
3. Antibiotics (4 weeks of baseline)

7.3.2 Over-the-counter Medications, Supplements/Natural Health Products, and Food/Drinks

Participants who are currently consuming the following over-the-counter (OTC) medications, supplement and food/drinks will not be allowed to participate unless willing to undergo the specified washout period prior to their baseline visit and agree not to take the supplements during the study. Other OTC supplements and food/drinks will require a case-by-case assessment by the QI based on dose and/or frequency of use to determine adequate washout.

1. Other supplements containing the IP (4 weeks of baseline)
2. Probiotic Supplements (4 weeks of baseline)
3. Natural health products used for cold, flu, or GI health (2 weeks washout)

7.3.3 Washout Periods

Please refer to Section 7.3.2 for washout periods for OTC medications, supplements, and drinks.

7.4 Early Withdrawal

Personal reasons

As stated in the Informed Consent Form (ICF), a participant may withdraw from the study for any reason at any time.

Removal by Qualified Investigator

Participant discontinuation should be considered at the discretion of the Qualified Investigator. The circumstances of any discontinuation must be documented in detail in the participant file and final report. If possible, the evaluations planned for the end of study will be carried out at the time when the participant is withdrawn from the study.

Criteria for removal of participants from the study includes:

Clinical reasons

A participant may be withdrawn from the study if, in the opinion of the Qualified Investigator, it is not in the participant's best interest to continue. Any participant who experiences a serious adverse event (SAE) may be withdrawn from the study at the discretion of the Qualified Investigator. A participant will also be withdrawn due to AEs causing clinically significant illness or the need for prohibited medication(s) during the study. Any participant who becomes pregnant during the course of the study will be withdrawn and followed up with until giving birth.

Protocol violation

Any participant found to have entered this study in violation of the protocol will be discontinued from the study at the discretion of the Qualified Investigator. This will include any participant found to have been inappropriately enrolled (did not meet eligibility criteria). Participant non-compliance includes failure to show up for study visits, failure to take the investigational product as directed, or refusal to undergo study visit procedures. Participants who are found to be taking prohibited medications or supplements without the knowledge of the Qualified Investigator will also be withdrawn. Any major protocol deviations (i.e. those that increase the risk to participants and/or compromise the integrity of the study or its results) will result in participant discontinuation.

Participant Replacement

For all early withdrawals, a participant leaving the study prematurely or in the event of participant removal will NOT be replaced by unless attrition rates are high. It is understood by all concerned that an excessive rate of withdrawals can render the study un-interpretable, thus unnecessary withdrawal of participants should be avoided.

If in the event attrition rates begin to approach 20% and the study sponsor is in agreeance, additional participants may be enrolled at the discretion of the qualified investigator to safeguard study outcomes.

8 INVESTIGATIONAL PRODUCT

8.1 Manufacturing and Storage

The investigational product will be provided to KGK Science Inc. by the Sponsor. The investigational product will be carefully stored at the study site in a lockable, limited access area, accessible only to study team personnel in compliance with pertinent regulations. Only authorized persons will have access to the investigational product. The products will be stored as per storage conditions on the label and will not be exposed to direct sunlight or heat. The investigational products will be kept in a locked investigational product storage room at KGK Science Inc. on receipt. An accountability log will be kept for the investigational products.

All unused investigational product will be returned to the study Sponsor by KGK Science Inc. (at the Sponsor's expense) or destroyed on receipt of written confirmation from the Sponsor at study closeout (within one month of the last participant's last visit).

Manufactured by:

Genomelabs Bio Pvt Ltd.

Plot No.: 6 & 6A, Synergy Square I, Genome Valley, Turkapally, Hyderabad, Shamirpet, Medchal-Malkajgiri, Telangana-500078

8.2 Labelling and Coding

The investigational product will be labeled according to the requirements of ICH-GCP guidelines and applicable local regulatory guidelines. Investigational product will be randomized and coded by an unblinded person at KGK who is not involved in data collection or analysis.

8.3 Investigational Product (*Bacillus coagulans* SNZ 1969)

Medicinal Ingredient	Quantity (per dose)
Bacillus coagulans SNZ 1969	1 Billion CFU/g

Non-medicinal ingredients: Glucidex (maltodextrin), Magnesium stearate, Banana dry mix flavour.

8.4 Placebo

Ingredients: Glucidex (Maltodextrin), Magnesium Stearate, Banana dry mix flavour.

8.5 Directions

Participants will be instructed to fully consume 1 sachet completely dissolved in approximately 50 ml of water before breakfast starting on Day 1 and throughout the duration of the study. If after consuming the dose residual powder remains in the cup add more water to dissolve remaining powder and consume remaining product. Clinic staff will instruct participants to save all unused and open packages and return them to KGK Science Inc. for a determination of compliance. If a dose is missed participants are instructed

to take it as soon as they remember on the same day or the next day. Participants will be advised not to exceed 2 sachets daily.

8.6 Rescue Medication

Rescue medication is not applicable for this study

8.7 Randomization

Each participant will be assigned a randomization code according to the order of the randomization list generated. Enrolled participants will be randomized to the different study arms at Day 0.

Block randomization will be implemented for this study. It is a method that helps to reduce bias and achieve balance in the allocation of participants to study arms. This technique helps to increase the probability that each arm will contain an equal number of individuals by sequencing participant assignments by blocks. Participants are randomized within blocks so that an equal number are assigned to each study arm. Allocation proceeds by randomly selecting one ordering and assigning the next block of participants to a study group based on a specified sequence. The block size needs to be divisible by the number of study groups (19).

8.8 Blinding and Allocation Concealment

Concealment of the allocation of study arms will be employed using a secure document located on Microsoft SharePoint. Information regarding the study arm associated with each randomization number will be readily available for the Qualified Investigator to access in the event that it becomes necessary to know which product a participant is taking for the sake of the participant's health care.

Unblinding should not occur except in the case of emergency situations. If a SAE occurs, for which the identity of the investigational product administered is necessary to manage the participant's condition, the study arm assigned to the participant will be unblinded and the investigational product identified. The Sponsor must be notified of any unblinding within 24 hours. Details of participants who are unblinded during the study will be included in the Final Report.

9 STUDY ASSESSMENTS

9.1 Screening (Day -45 to Day -15; Visit 1)*

** At the discretion of the Qualified Investigator, any participants falling outside of the screening window (Day -45 to Day -15) due to scheduling issues will be asked to repeat eligibility/screening procedures prior to randomization at baseline.*

At screening, an ICF will be given to the potential volunteer. They will be required to read the information and will be given the opportunity to seek more information if needed or provided with the option of taking the consent form home to review prior to making their decision. If agreeable, the caregiver and volunteer will sign the consent and assent forms as applicable and receive a duplicate of the signed copy. Once consent has been obtained, screening will proceed. Each volunteer will be assigned a screening number to be entered in the screening and enrollment log.

Screening assessments include:

1. Review medical history, concomitant therapies (inclusive of previous vaccinations), and current health status
2. Assess inclusion and exclusion criteria
3. Review any pre-emergent AEs
4. Urine pregnancy test for potential volunteers that are of child-bearing potential
5. Seated resting BP and HR measurements **(see Section 9.7.2)**
6. Weight and height measurements **(see Section 9.7.1)**
7. Dispense study diary inclusive of the CARIFS questionnaire, Additional respiratory tract symptoms questionnaire, and GITI Symptom Questionnaire and instruct participants on completion **(see Section 9.7.6)**
8. Dispense saliva collection kit and instruct participants on use **(See Section 9.7.5)**
9. Dispense stool collection kit and instruct participants on use **(see Section 9.7.4)**

The next appointment will be scheduled for potentially eligible volunteers for their baseline visit.

9.2 Run-in (Day -14 to Day -1)

Participants will complete the study diary inclusive of the CARIFS, Additional respiratory tract symptoms, and GITI symptoms questionnaires during the 14-day run-in period.

9.3 Day 0 (Baseline, Visit 2)

Eligible volunteers will return to the clinic for baseline assessments with collected stool and saliva samples.

Baseline (Day 0) assessments include:

1. Review concomitant therapies (inclusive of previous vaccinations) and current health status
2. Assess inclusion and exclusion criteria
3. Review any pre-emergent AEs
4. Urine pregnancy test for potential volunteers that are of child-bearing potential
5. Vital sign measurements (BP and HR)
6. Weight and height measurements

7. Randomization of eligible participants
8. Collect blood samples for analysis of: **(see Section 9.7.3)**
    a. Quantibody® Human Immune Response Array
    b. Immunoglobulins A (IgA), G (IgG), E (IgE) and M (IgM) serum levels
9. Collect saliva sample for the analysis of Salivary Ig A Levels
10. Collect stool samples for microbiome analysis
11. Review completed study diaries including CARIFS, Additional respiratory tract symptoms, and GITI symptoms questionnaires
12. Dispense investigational product and instruct participants on use
13. Dispense study diary inclusive of the CARIFS, Additional respiratory tract symptoms, and GITI symptoms questionnaires
14. Dispense stool collection kit for microbiome analysis for Visit 5 (End of Study visit)
15. Dispense saliva collection kit for Visit 5 (End of Study visit)

The next visit will be conducted remotely and scheduled for Day 28 (± 2 days)

9.4 Visit 3 (Day 28 ± 2 days)

Participants will attend remotely for Visit 3 assessments with completed study diaries inclusive of the CARIFS, Additional respiratory tract symptoms, and GITI symptoms questionnaires.

Visit 3 assessments include:

1. Review compliance by referencing study diaries and participant/caregiver count of study product
2. Review completed study diaries including CARIFS, Additional respiratory tract symptoms, and GITI symptoms questionnaires
3. Review concomitant therapies and AEs
4. Dispense new study diary

The next visit will be conducted remotely and scheduled for Day 56 (± 2 days).

9.5 Visit 4 (Day 56 ± 2 days)

Participants will attend remotely for Visit 4 assessments with completed study diaries inclusive of the CARIFS, Additional respiratory tract symptoms, and GITI symptoms questionnaires.

Visit 4 assessments include:

1. Review compliance by referencing study diaries and participant/caregiver count of study product.
2. Review completed study diaries including CARIFS, Additional respiratory tract symptoms, and GITI symptoms questionnaires
3. Review concomitant therapies and AEs
4. Dispense new study diary
5. Participants will be reminded to bring saliva and stool samples for the End of Study visit.

The next visit will be scheduled for Day 84 (± 2 days).

9.6 Visit 5 - End of Study (Day 84 ± 2 days)

Participants will return to the clinic for end of study assessments, with unused investigational product, completed study diaries (inclusive of the CARIFs, Additional respiratory tract symptoms, and GITI symptoms questionnaires), and stool and saliva samples.

Visit 5 assessments include:

1. Return and review study diary
2. Return unused investigational product in the original packaging and remnants and calculate compliance by counting the returned unused investigational product
3. Review concomitant therapies and AEs
4. Vital sign measurements (BP and HR)
5. Weight and height measurements
6. Urine pregnancy test for participants that are of childbearing potential
7. Collect blood samples for the analysis of:
    a. Quantibody® Human Immune Response Array
    b. Immunoglobulins A (IgA), G (IgG), E (IgE) and M (IgM) serum levels
8. Collect saliva samples for the analysis of Salivary Ig A Levels
9. Collect stool samples for microbiome analysis
10. Review completed study diaries including CARIFS, Additional respiratory tract symptoms, and GITI symptoms questionnaires

9.7 Clinical Assessments and Procedures

Calculations or measurements of specific parameters are required, as indicated in the Schedule of Assessments. Instructions for determining these parameters are provided in the following sections.

9.7.1 Height and Weight

Weight measurements will be performed with shoes removed and bladder empty on calibrated scales at all visits.

At least two separate measurements will be taken at each visit. If the two measurements are more than 0.5 kg (1.1 lbs) apart, a third measurement will be taken. The two closest values will be selected for computation.

Measurement of height will be performed with the participant's shoes removed. The participant's knees will be straightened, and head held upright.

9.7.2 Blood Pressure and Heart Rate

In-office, seated, resting BP assessment:

The participant should be seated comfortably with their back supported and the upper arm bared without restrictive clothing. Feet should be flat on the floor and legs will not be crossed. The participant will rest in this position for at least five minutes prior to the first reading.

At screening:

Seated BP will be checked in both arms to ensure that the arm with the higher BP reading will be used for BP measurements of the study. The arm chosen for use at the initial visit will be documented in the study file and used in all subsequent visits. Participants and/or Caregivers of participants with elevated BP should be queried about the participants usual BP. As per the QI's opinion, a high office BP should be rechecked after the participant is given a glass of water and is rested for 15 minutes.

At subsequent study visits:

Once enrolled in the study, BP will be measured.

HR (beats/min) will be measured simultaneously during BP recording.

9.7.3 Blood Sample Collection

An appropriately trained and qualified personnel will perform the venipuncture procedure to collect the necessary blood samples as per standard phlebotomy techniques.

Blood samples will be collected for the analysis of:

- Serum levels of Immunoglobulin A (IgA), G (IgG), E (IgE) and M (IgM)
- Immune response biomarkers: CD14, CD163, CD40 (TNFRSF5), CRP (C-Reactive Protein), E-Selectin, Fas (TNFRSF6/Apo-1), Fas Ligand (TNFSF6), GCSF, ICAM-1 (CD54), IL-1 alpha (IL-1 F1), IL-1 beta (IL-1 F2), IL-1 R4 (ST2), IL-10, IL-12 p70, IL-13, IL18, IL-2, IL-2 R alpha, IL-4, IL-6, IL-8 (CXCL8), Lipocalin-2 (NGAL), MCP-1 (CCL2), MCP-2 (CCL8), MIF, MIP-1 alpha (CCL3), MIP-1 beta (CCL4), Osteopontin (SPP1), PAI-1, Platelet Factor 4 (CXCL4), Procalcitonin, RAGE, Resistin, Thrombomodulin, TNF alpha, TREM-1, Troponin I, uPAR, VCAM-1 (CD106), VEGF-A.

9.7.4 Stool Collection

Stool samples will be collected for study visit 2-Baseline-Day 0, and Visit 5- End of Study, Day 84 ± 2 days using the dispensed collection kits. Participants will be instructed on stool sample collection, storage, and transportation. Participants will be given a stool collection kit containing materials required to collect their stool sample. Stool samples should be collected within 4 calendar days of their next visit. The sample will be stored at room temperature after collection and transported to the clinic within 4 days of their clinic visit.

Stool samples will be used for a microbiome analysis. Microbiome analysis will be completed in n=80 participants. At the discretion of the Study sponsor microbiome analysis may be completed in additional completers to bolster study outcomes.

Genomic DNA will be extracted from the stool samples and stored at -80ºC until PCR amplification. Upon completion of amplification amplicons will be stored at again at -80ºC until further processing and time of analysis. Analysis sequence reads will be completed to infer exact sequence variants and taxonomy assigned including kingdom 'Eukaryota', order 'Chloroplasts' or the family 'Mitochondria'. Microbiota α-diversity analyses will be performed on unfiltered sequence variant counts tables.

9.7.5 Saliva Sample Collection

Participants will be instructed on the collection of salivary samples for the analysis of IgA levels by study staff. Samples will be collected using saliva collection kits as per manufacturer's instructions and frozen immediately following sample collection at Visit 2- baseline (Day 0) and Visit 5-Day 84 ± 2 days. Saliva sample should be collected in the morning on the day of or day preceding their in-clinic visit.

9.7.6 Study Diary

Participant caregiver's will be instructed to complete the study diary daily. Questions pertaining to IP compliance, lifestyle habits, concomitant medications (inclusive of OTC medications and vaccinations), tracking cold/flu and GITI symptoms (see **Sections 9.7.7, 9.7.8, and 9.7.9** below), missed school days, and any adverse events and symptoms throughout the study will be included. Each day, caregivers will ask if their child has any of the symptoms listed in the CARIFS, Additional Respiratory Tract Symptoms or GITI Symptom Questionnaires (**Sections 16.1, 16.2, and 16.3**). Caregivers will report all symptoms in the study diary daily using the CARIFS, Additional Respiratory Tract Symptoms, and GITI Symptoms Questionnaires.

9.7.7 Canadian Acute Respiratory Illness Flu Scale (CARIFS)

The Canadian Acute Respiratory Illness Flu Scales (CARIFS) is an 18-item questionnaire for children that assesses respiratory illness severity across three domains: symptoms, function and parental impact (**Section 16.2**). Each item is answered on a 4-point scale (no problem = 0, minor problem = 1, moderate problem = 2, major problem = 3), with a higher score denoting a more severe illness (20,21). Participants will complete the CARIFS daily throughout run-in and the study period as part of their study diary. As referenced by *Lazou Ahren et al. (2020)* and *Singh et al. (2024)*, incidence, duration, and severity of cold and flu will be defined as the following (21,22):

- *Incidence* will be defined as two or more consecutive days with scores above zero (no problem) in at least two symptoms (headache, sore throat, muscle aches or pain, fever, cough, nasal congestion or runny nose, and vomiting; items 10–16) listed on the CARIFS (21).
- *Duration* will be defined as the length of time from an onset of an incidence (defined above) until the last day with symptoms followed by two days with a 0 score
- *Severity* will be calculated using two methods. 1) The mean total severity score per day of the individual items per incidence of cold or flu symptoms, and 2) area under the curve (AUC) of daily total symptom scores.

9.7.8 Gastrointestinal Tract Infection (GITI) Symptoms Questionnaire

Gastrointestinal tract infection (GITI) symptoms will be assessed by completion of the Gastrointestinal tract infection symptoms questionnaire. These questions will completed daily throughout run-in and the study period as part of their study diary. See **Section 16.1** for the questions in this questionnaire.

The GITI symptom questionnaire will comprise the record of the incidence, duration, and severity of individual GITI symptoms. Each item is answered on a 4-point scale (no problem = 0, minor problem = 1, moderate problem = 2, major problem = 3), with a higher score denoting a more severe illness. Participants

will complete the GITI symptom questionnaire daily throughout run-in and the study period as part of their study diary.

Incidence, duration, and severity of GITI symptom will be defined as the following:

- *Incidence of a symptom* will be defined as the first day with a score of greater than 0
- *Duration of a symptom* will be defined as the length of time from the onset of an incidence (defined above) until the last day the symptom is reported followed by a day with a score of 0
- *Severity of a symptom* will be calculated using two methods: 1) The mean of the specific symptom scores during the investigational period, and 2) the area under the curve (AUC) of individual symptom scores during the investigational period.

9.7.9 Additional Respiratory Tract Symptoms Questionnaire

Additional respiratory tract symptoms will be collected and administered in the same format as the CARIFS. This questionnaire will comprise the record of the incidence, duration, and severity of the individual symptoms. Each item is answered on a 4-point scale (no problem = 0, minor problem = 1, moderate problem = 2, major problem = 3), with a higher score denoting a more severe illness. Participants will complete the questionnaire daily throughout run-in and the study period as part of their study diary.

Additional Symptom data to be collected for the following respiratory symptoms: yellow mucus, bloody mucus, crystalline mucus, itchy nose, itchy throat, red eyes, hoarseness, and sneezing (**Section 16.3**).

Incidence, duration, and severity of symptoms will be defined as the following:

- *Incidence of a symptom* will be defined as the first day with a score of greater than 0
- *Duration of a symptom* will be defined as the length of time from the onset of an incidence (defined above) until the last day the symptom is reported followed by a day with a score of 0
- *Severity of a symptom* will be calculated using two methods: 1) The mean of the specific symptom scores during the investigational period, and 2) the area under the curve (AUC) of individual symptom scores during the investigational period.

9.8 Compliance

Compliance will be assessed at end of study by counting the returned unused study product at each visit. Compliance is calculated by determining the number of dosage units taken divided by the number of dosage units expected to have been taken multiplied by 100.

$$\frac{number\ of\ dosage\ units\ taken}{number\ of\ dosage\ units\ expected\ to\ have\ been\ taken} \times 100\%$$

In the event of discrepancy between the information in the study diary and the amount of study product returned, use will be based on the product returned unless an adequate explanation for the discrepancy has been provided. Participants found to have a compliance of <80% or >120% will be counseled.

During remote visits, compliance will be reviewed by asking participants and/or caregivers to count unused investigation product while also reviewing study diaries for possible discrepancies. Participants will be counseled during remote visits regarding study requirements as necessary.

9.9 Laboratory Analyses

Blood, saliva, and stool samples will be collected from the participants at baseline (Visit 2, Day 0) and at the end of study visit (Visit 5, Day 84± 2 days)or Early Termination Visit as indicated in the Schedule of Assessments.

Protection of participant confidentiality will extend to all data generated from the assaying of these samples. These samples will be alphanumerically coded, and the persons performing the analysis will not be aware of the participant's identity or the product they received.

The total blood volume collection for the laboratory assessments listed above will be approximately 60 mL, over the period from screening to end of study (approximately 131 days). At any study visit, blood loss per volunteer is not expected to exceed 30 mL. Additional blood samples may be collected during the course of the study in order to perform or repeat laboratory tests outlined in the Schedule of Assessments if needed.

External laboratories will be used in this study to measure blood parameters.

Urine pregnancy test will be performed at the clinic site during the screening visit (V1), baseline visit (V2), and the end of study visit (V5) or Early termination visit for participants of child bearing potential.

9.10 Termination of the Study

In the case of premature termination of the Study, participating investigators/participants, and the Institutional Review Board (IRB) must be promptly informed of the termination. In the event of early termination, as many assessments will be completed as agreed upon by the participant.

9.11 Protocol Amendments

If amendments to the study protocol are required after approval such changes will be captured in writing, the reasons for the change documented, and signed and dated by the Sponsor. Any such amendments may be subject to IRB and Health Canada review/approval prior to implementation. Exception: if it becomes necessary to alter the protocol to eliminate an immediate hazard to participants, an amendment may be implemented prior to IRB approval. In this circumstance, the Qualified Investigator must notify IRB and Health Canada in writing within five (5) working days of the implementation.

10 SAFETY INSTRUCTIONS AND GUIDANCE

10.1 Adverse Events

10.1.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a clinical investigation participant who has been administered an investigational product and does not necessarily present as having a causal relationship with this investigational product. The absence or presence of the causal relationship will be determined by the QI. An AE can be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a product, and whether or not it is considered related to that product. Pre-existing conditions that worsen during a study are to be reported as AEs and will be reviewed by the QI to provide classification.

During the study, participants should record any adverse effects in their diary. At each visit the participant will be asked, by the investigator: "Have you experienced any changes in your health since your last visit to the clinic?". Any AEs will be documented in the study record and will be classified according to the description, duration, intensity, frequency, and outcome by the medical team. The QI will assess all AEs and decide causality.

Intensity of AEs will be graded on a three-point scale (mild, moderate, severe) and reported in detail in the study record:

Mild: Awareness of event but easily tolerated

Moderate: Discomfort enough to cause some interference with usual activity

Severe: Inability to carry out usual activity

The causality relationship of investigational product to the AE will be assessed by the QI as either:

Most probable: There is a reasonable relationship between the investigational product and AEs. The event responds to withdrawal of investigational product (dechallenge) and recurs with rechallenge when clinically feasible.

Probable: There is a reasonable relationship between the investigational product and AEs. The event responds to dechallenge.

Possible: There is a reasonable relationship between the investigational product and AEs. Dechallenge information is lacking or unclear.

Unlikely: There is a temporal relationship to the investigational product administration but there is no reasonable causal relationship between the investigational product and the AEs.

Not related: There is no temporal relationship to investigational product administration or there is a reasonable causal relationship between non-investigational product, concurrent disease, or circumstance and the AEs.

10.1.2 Serious Adverse Event

A SAE is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that results in any of the following outcomes:

- Death
- A life-threatening AE
- Inpatient hospitalization for 24 hours or prolongation of existing hospitalization
- A persistent or significant disability of incapacity
- A congenital anomaly/birth defect in the offspring of a participant who received the study investigational product

Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require intervention to prevent one of the outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or the development of drug dependency or drug abuse.

10.1.3 Unexpected Adverse Reaction

An unexpected adverse reaction is an event that occurs the nature and severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product).

10.2 Treatment and Follow-up of AEs

All AEs, especially those for which the relationship to the investigational product is suspected, should be followed up, by the investigational team until they have returned to baseline status or stabilized in the participant. Such events and the follow-up need to be recorded to bring closure to the event, whenever possible

If after follow-up, return to baseline status or stabilization cannot be established, an explanation should be recorded in the study record.

10.3 Reporting of SAEs and Unexpected Adverse Reactions

The QI will be responsible for classification of an AE as an SAE within 24 hours of notification. Causality should be signed off by the QI prior to reporting to ethics and regulatory bodies. Notification of any SAEs must be made in writing to the study Sponsor. The IRB will be notified of all product related SAEs and unexpected adverse reactions. All blinded SAE's or unblinded-participant-on-active product SAE's will be reported to the Natural and Non-prescription Health Products Directorate (NNHPD) Clinical Trial Unit in an expedited manner.

KGK Science Inc. must notify the NNHPD of all blinded or unblinded-participant-on-active product SAEs and reactions as follows:

If it is neither fatal or life threatening, within 15 calendar days after the day on which the Sponsor becomes aware of the information; and

If it is fatal or life threatening, must be reported as soon as possible, but not later than seven (7) days after the day on which the Sponsor becomes aware of the information.

11 STATISTICAL EVALUATION

11.1 Determination of Sample Size

Assuming placebo participants would experience 1.43 episodes of URTIs and 7.42 duration days of URTI symptoms during 84 days of follow up (22), a sample size of 100 participants (50 participants per arm) has a power of 80% to detect a rate of ≤0.76 for the number of episodes of URTIs and ≤ 5.80 for the duration days of URTI symptoms in the Bacillus coagulans SNZ 1969 group using Poisson regression model.

In addition, a sample size of 100 participants could detect an effect size ≥ 0.63 in the AUC of GITI symptom scores and in the AUC of the Additional Respiratory Tract Symptoms questionnaire, and a difference of ≥27.8 score.day in the mean of AUC of CARIFS daily total symptom scores between Bacillus coagulans SNZ 1969 and placebo groups assuming standard deviations equal to 52.96 and 32.85 score.day for Bacillus coagulans SNZ 1969 and placebo groups (22), respectively, using two-sample t-test with 80% power, 2-sided 5% significance level, and 20% dropout rate.

11.2 Analysis Plan

The **Safety Population** will consist of all participants who received any amount of either product, and on whom any post-randomization safety information is available.

The **Intention-to-Treat (ITT) Population** consists of all participants who are randomized to a study group.

The **Per Protocol (PP) Population** consists of all participants who consumed at least 80% of investigational product or placebo doses, do not have any protocol deviations that affect primary outcomes and complete all study visits and procedures connected with measurement of the primary variable.

11.3 Statistical Analysis Plan

For both count and continuous primary and secondary outcomes, summary statistics including number of participants, mean, median, standard deviation, first quartile, third quartile, minimum, and maximum will be obtained for each available time interval and for each group. For categorical outcomes, frequencies and percentages will be presented.

The difference in the number of incidences of URTI, duration days of URTI, missed school days, well days, illness days between Bacillus coagulans SNZ 1969 and Placebo groups will be assessed by computing the rate ratio with 95% confidence interval using a generalized linear model with log link function (Poisson or negative binomial regression model).

The difference in the number of incidences of Additional Respiratory Symptoms and the duration days of Additional Respiratory Symptoms between Bacillus coagulans SNZ 1969 and Placebo groups will be assessed by computing the rate ratio with 95% confidence interval using a generalized linear model with log link function (Poisson or negative binomial regression model).

The difference in the number of incidences of GITI symptoms, duration days of GITI symptoms, missed school days, well days, illness days between Bacillus coagulans SNZ 1969 and Placebo groups will be assessed by computing the rate ratio with 95% confidence interval using a generalized linear model with log link function (Poisson or negative binomial regression model).

The difference in the AUC for daily total CARIFS scores, the mean total severity scores of URTI symptoms, the AUC for daily total Additional Respiratory Tract Symptoms questionnaire scores, the AUC of GITI symptom scores, and mean of GITI symptom scores between Bacillus coagulans SNZ 1969 and Placebo groups will be assessed by two-sample t-test if the outcome is normally distributed and Wilcoxon's rank sum test if the outcome is not normally distributed. Normality assumption will be assessed using quantile-quantile (Q-Q) plots.

Fisher's exact test will be used to assess the difference of proportion of participants who used prescription and non-prescription cold/flu medications to treat URTI/GITI symptoms between Bacillus coagulans SNZ 1969 and Placebo. 95% confidence interval of odds ratio will be calculated.

Differences between groups for sIgA, IgA, IgG, IgE, IgM, and immune response biomarkers at day 84 will be assessed by ANCOVA model adjusting for baseline values.

To assess the goodness of fit of the log link models, the Poisson regression model and the negative binomial regression model will be applied to the same dataset and then the Likelihood Ratio Test will be performed. If the p-value of the test is less than 0.05, then we will use the negative binomial model as it offers a better fit of the count data.

To evaluate the adequacy of ANCOVA model, diagnostics including Q-Q plots (for assessing the normality of error terms) and plots of residuals versus fitted values (to examine if the residuals are independent and have constant variance) will be generated. If these diagnostic plots indicate deviations from the model's assumptions, ranks or other transformation of the dependent variable, will be implemented.

Two-sided probabilities ≤ 0.05 will be considered statistically significant. All statistical analysis will be completed using the R Statistical Software Package Version 4.3.2 or newer for Microsoft Windows.

11.3.1 Premature Discontinuation Description

For each premature discontinuation, the following parameters will be listed: participant number, dates of start and end of study, and the reason of premature discontinuation.

11.3.2 Safety

For AEs, a descriptive analysis will be given. AEs will be presented in a frequency table by category and study arms. Furthermore, description, frequency, severity, and causality will be reported for each AE.

Continuous safety parameters (e.g. HR and BP) will be summarized using a table including mean, standard deviation, median, minimum value, and maximum value for each measurement point. The between group change will also be summarized similarly.

11.4 Protocol Deviation Description

Protocol deviations will be listed in the final study report.

11.5 Protocol Amendments

Once the protocol has been approved by the IRB and Health Canada, any changes to the protocol must be documented in the form of an amendment. All amendments will be documented in the final study report and amended documents provided as part of the final study report to the client.

12 DATA COLLECTION AND STORAGE

All data collection and record storage will be done in compliance with ICH-GCP Guidelines and applicable local regulatory guidelines.

13 ETHICAL ASPECTS OF THE STUDY

This study will be conducted with the highest respect for the individual participants (i.e., participants) according to the protocol, the ethical principles that have their origin in the Declaration of Helsinki, and the ICH Harmonised Tripartite Guideline for GCP.

13.1 IRB Approval

KGK Science Inc. will supply relevant documents for submission to an IRB for the protocol's review and approval. The following must be submitted to the IRB: this protocol, a copy of the ICF, and, if applicable, volunteer recruitment materials and/or advertisements and other documents required by all applicable laws and regulations. The IRB's written approval of the protocol and volunteer informed consent must be obtained before commencement of the study. The IRB approval must refer to the study by exact protocol title, number, and version date; identify versions of other documents (e.g., ICF) reviewed; and state the approval date.

KGK Science Inc. must adhere to all requirements stipulated by the IRB. This may include notification to the IRB regarding protocol amendments, updates to the ICF, recruitment materials intended for viewing by volunteers, local safety reporting requirements and submission of the Qualified Investigator's annual/final status report to the IRB.

13.2 Volunteer Information and Informed Consent

Written consent documents will embody the elements of informed consent as described in the declaration of Helsinki and the ICH Guidelines for GCP and will be in accordance with all applicable laws and regulations. The ICF describes the planned and permitted uses, transfers, and disclosures of the volunteer's personal and personal health information for purposes of conducting the study. The ICF further explains the nature of the study, its objectives, and potential risks and benefits, as well as the date informed consent is obtained. The ICF will detail the requirements of the volunteer and the fact that he or she is free to withdraw at any time without giving a reason and without prejudice to his or her further medical care.

13.3 Potential Risks and Procedures to Minimize Risk

All potential risks are disclosed to study participants prior to their participation. Potential side-effects of taking the study product may include:

- Abdominal discomfort (such as pain, nausea, changes in bowel habits)
- Dislike of taste
- Worsening of URTI or GITI symptoms

OTC medications for fever, cough etc. can be taken and use is to be documented as part of concomitant medications (see Section 9.7.6). If symptoms persist or worsen, participants will be instructed to contact their heathcare practitioner to ensure that any standard of care treatment can be provided.

Other potential risks associated with this study include venipuncture and the associated risks. Risks associated with venipuncture include pain, bruising, and infection at the site. Alcohol swabs and proper venipuncture procedure will be followed to minimize the risk of infection.

Potential Risks From E-Consent

Participants may receive a link via email/text message to download a PDF copy of the signed consent form. There may be risks of loss of privacy and confidentiality if the PDF copy of this consent form is viewed and/or stored on a personal electronic device (PED), especially if that PED is shared with other users or is lost, hacked, or subject to a search warrant or subpoena. Also, the PDF copy of the consent may not be able to be permanently removed from a PED.

13.4 Privacy and Confidentiality

By signing and dating the consent form, participants will give their consent to collect, use and disclose their health information as described, as stipulated in the ICF:

- All medical information will be kept confidential to the extent permitted by law.
- All research data (health information, past medical history, and test results from this study) will be kept in a secure location. Forms on which the participant's information will be entered will not contain their name (except for the study intake form and/or external requisitions if applicable)
- Any of personal information that is stored electronically will be password protected, accessible only to authorized personnel and coded wherever possible. Electronic data may be stored on secure servers which are physically located in Canada and/or the United States.
- Participants will not be identified in any publication that might result from the study. Unless required by law, only the following may have access to confidential study data (not personal identifying information) at the study site:
    - The study doctor and study staff
    - The Sponsor (including its monitors and auditors)
    - Members of the IRB/REB
    - Government regulatory authorities including Health Canada and other foreign regulatory agencies
- While the Sponsor will not have access to personal identifying information, the study doctor, study staff, monitors, auditors, IRB/REB, and regulatory authorities may review study records, which could include personal identifying information (such as the signed consent form) for compliance and verification purposes.
- Information from this study will be submitted to the Sponsor. Information sent from the study site will not contain the participant's name.
- Participants have the right to check their study records and request changes if the information is incorrect.

- While every effort will be made to protect the privacy of information, absolute confidentiality cannot be guaranteed. This does not limit the duty of the researchers and others to protect participant's privacy.

14 QUALITY ASSURANCE AND QUALITY CONTROL

14.1 Auditing

All material used in clinical studies are subjected to quality control. Quality assurance audits may be performed by the Sponsor or any health authority during the course of the study or after its completion.

The Qualified Investigator agrees to comply with the Sponsor and regulatory requirements in terms of auditing of the study. This includes access to the source documents for source data verification.

14.2 Monitoring

An initiation meeting will be conducted by the Sponsor or an approved representative (CRO). At this meeting, the protocol and logistical aspects of the study will be reviewed with the Qualified Investigator and all study staff.

Source documents will be reviewed to ensure that all items have been completed and that the data provided are accurate and obtained in the manner specified in the protocol. The participant files will be reviewed to confirm that:

1. Informed consent was obtained and documented
2. Enrolled participants fulfilled all inclusion criteria and did not meet any exclusion criteria;
3. AE/SAE reporting has been performed as applicable
4. Study visits have been conducted as per protocol and information has been recorded in the appropriate place in the source document
5. The study product is being stored correctly and an accurate record of its dispensation to the study participants is being maintained (accountability)

Incorrect, inappropriate, or illegible entries in the participant files will be returned to the Qualified Investigator or designee for correction. No data disclosing the identity of participants will leave the study center. The Qualified Investigator and any designees will maintain confidentiality of all participant records.

The Qualified Investigator will permit study-related monitoring, audits, IRB/IEC review, and regulatory inspections and will allow direct access to source data and documents for these purposes.

14.3 Data Management

Data required for the analysis will be acquired from source documentation (including laboratory reports) and captured through electronic data capture (EDC) case report forms specifically designed for this study. A password-protected user ID is created giving access to the delegated study personnel. The data management personnel designs the database, and once the database is finalized study-specific Data Management Plan is generated.

The standard data validation and edit checks are performed by designing study-specific rules and restrictions defined in the eCRF. The discrepancies will be queried and managed. Data sets will be created, queried, and exported during and at the end of the study.

For Statistical analysis, the data management personnel provides a validated locked blinded database to the Statistician to perform the analysis.

High safety standards for the transfer and storage of study data are guaranteed by the use of technologies such as password protection, firewalls, and periodic backup to protect stored data.

All study data is archived for a period not less than 15 years from the date of completion of the study in accordance with Health Canada regulatory requirements.

15 REFERENCE LIST

1. Sinha S, Dunning J, Wong I, Nicin M, Woodward S. The Underappreciated Burden of Influenza Amongst Canadas Older Population [Internet]. 2018 [cited 2025 May 11]. Available from: https://www.oacao.org/wp-content/uploads/2020/10/The-Underappreciated-Burden-of-Influenza-Amongst-Canadas-Older-Population.pdf

2. Canadian Immunization Guide [Internet]. 2021 [cited 2025 May 11]. Available from: https://www.canada.ca/en/public-health/services/canadian-immunization-guide.html

3. Schanzer DL, Sevenhuysen C, Winchester B, Mersereau T. Estimating influenza deaths in Canada, 1992-2009. PloS One 2013;8:e80481.

4. Lynch JP, Walsh EE. Influenza: evolving strategies in treatment and prevention. Semin Respir Crit Care Med 2007;28:144–58.

5. Fendrick AM, Monto AS, Nightengale B, Sarnes M. The economic burden of non-influenza-related viral respiratory tract infection in the United States. Arch Intern Med 2003;163:487–94.

6. Molinari N-AM, Ortega-Sanchez IR, Messonnier ML, Thompson WW, Wortley PM, Weintraub E, Bridges CB. The annual impact of seasonal influenza in the US: measuring disease burden and costs. Vaccine 2007;25:5086–96.

7. Canada PHA of. Childhood Seasonal Immunization Coverage Survey (CSICS): 2024 results [Internet]. 2025 [cited 2025 May 12]. Available from: https://www.canada.ca/en/public-health/services/immunization-vaccines/vaccination-coverage/childhood-seasonal-immunization-coverage-survey-2024-results.html

8. Canada PHA of. Rotavirus vaccines: Canadian Immunization Guide [Internet]. 2017 [cited 2025 May 12]. Available from: https://www.canada.ca/en/public-health/services/publications/healthy-living/canadian-immunization-guide-part-4-active-vaccines/page-19-rotavirus-vaccine.html

9. Farahmand M, Moghoofei M, Dorost A, Shoja Z, Ghorbani S, Kiani SJ, Khales P, Esteghamati A, Sayyahfar S, Jafarzadeh M, et al. Global prevalence and genotype distribution of norovirus infection in children with gastroenteritis: A meta-analysis on 6 years of research from 2015 to 2020. Rev Med Virol 2022;32:e2237.

10. van Driel ML, Scheire S, Deckx L, Gevaert P, De Sutter A. What treatments are effective for common cold in adults and children? BMJ 2018;363:k3786.

11. Commissioner O of the. Should You Give Kids Medicine for Coughs and Colds? FDA [Internet] FDA; 2025 [cited 2025 May 11]; Available from: https://www.fda.gov/consumers/consumer-updates/should-you-give-kids-medicine-coughs-and-colds

12. Ballengee CR, Turner RB. Supportive treatment for children with the common cold. Curr Opin Pediatr 2014;26:114–8.

13. Hemilä H, Chalker E. Vitamin C for preventing and treating the common cold. Cochrane Database Syst Rev 2013;2013:CD000980.

14. Karsch-Völk M, Barrett B, Kiefer D, Bauer R, Ardjomand-Woelkart K, Linde K. Echinacea for preventing and treating the common cold. Cochrane Database Syst Rev 2014;2014:CD000530.

15. Science M, Johnstone J, Roth DE, Guyatt G, Loeb M. Zinc for the treatment of the common cold: a systematic review and meta-analysis of randomized controlled trials. CMAJ Can Med Assoc J J Assoc Medicale Can 2012;184:E551-561.

16. Lissiman E, Bhasale AL, Cohen M. Garlic for the common cold. Cochrane Database Syst Rev 2014;2014:CD006206.

17. Anaya-Loyola MA, Enciso-Moreno JA, López-Ramos JE, García-Marín G, Orozco Álvarez MY, Vega-García AM, Mosqueda J, García-Gutiérrez DG, Keller D, Pérez-Ramírez IF. Bacillus coagulans GBI-30, 6068 decreases upper respiratory and gastrointestinal tract symptoms in healthy Mexican scholar-aged children by modulating immune-related proteins. Food Res Int Ott Ont 2019;125:108567.

18. Fernandes M, Daswani B, Kinikar A, Soman R. Safety and Efficacy of Bacillus coagulans SNZ 1969® Probiotic Supplementation in Reducing Infections in Malnourished Children: A Randomised, Double-blind, Placebo-controlled Study. J Clin Diagn Res [Internet] 2024 [cited 2025 May 11]; Available from: https://www.jcdr.net/article_fulltext.asp?issn=0973-709x&year=2024&month=September&volume=18&issue=9&page=FC01-FC06&id=19825

19. Efird J. Blocked Randomization with Randomly Selected Block Sizes. Int J Environ Res Public Health 2010;8:15–20.

20. Jacobs B, Young NL, Dick PT, Ipp MM, Dutkowski R, Davies HD, Langley JM, Greenberg S, Stephens D, Wang EEL. Canadian Acute Respiratory Illness and Flu Scale (CARIFS). J Clin Epidemiol 2000;53:793–9.

21. Lazou Ahrén I, Berggren A, Teixeira C, Martinsson Niskanen T, Larsson N. Evaluation of the efficacy of Lactobacillus plantarum HEAL9 and Lactobacillus paracasei 8700:2 on aspects of common cold infections in children attending day care: a randomised, double-blind, placebo-controlled clinical study. Eur J Nutr 2020;59:409–17.

22. Singh RG, Garcia-Campayo V, Green JB, Paton N, Saunders JD, Al-Wahsh H, Crowley DC, Lewis ED, Evans M, Moulin M. Efficacy of a yeast postbiotic on cold/flu symptoms in healthy children: A randomized-controlled trial. Pediatr Res 2024;96:1739–48.

16 APPENDICES

16.1 Gastrointestinal Tract Infection (GITI) Symptoms Questionnaire

	No problem	Minor problem	Moderate problem	Major problem	Don't know or Not Applicable
1. Constipation (Hard or Decreased passage of stools)					
2. Loose Stools (Diarrhea)					
3. Urgent need for defecation					
4. Flatulence (Passing Gas)					
5. Vomiting					
6. Abdominal Pains					
7. Burping/Belching					
8. Heartburn					
9. Bloating (Abdominal distention)					
10. Acid regurgitation (Sour taste)					
11. Nausea					
12. Halitosis (Bad breath)					
13. Feeling of incomplete evacuation					

16.2 Canadian Acute Respiratory Illness and Flu Scale (CARIFS)

English version of the Canadian Acute Respiratory Illness and Flu Scale (CARIFS)

	No problem	Minor problem	Moderate problem	Major problem	Don't know or Not Applicable
1.Poor appetite					
2. Not sleeping well					
3. Irritable, cranky, fussy					
4. Feels unwell					
5. Low energy, tired					
6. Not playing well					
7.Crying more than usual					
8. Needing extra care					
9. Clinginess					
10. Headache					
11.Sore throat					
12. Muscle aches or pains					
13.Fever					
14. Cough					
15.Nasal congestion, runny nose					
16.Vomiting					
17.Not interested in what's going on					
18.Unable to get out of bed					

16.3 Additional Respiratory Tract Symptoms Questionnaire

	No problem	Minor problem	Moderate problem	Major problem	Don't know or Not Applicable
1.Yellow mucus					
2. Bloody mucus					
3. Crystalline mucus					
4. Itchy nose					
5. Itchy throat					
6. Red eyes					
7.Hoarseness					
8. Sneezing					