2023

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CLINICAL TRIALS

A RANDOMIZED, DOUBLE-BLINDED, PLACEBO-CONTROLLED, PARALLEL STUDY, TO ASSESS THE EFFECT OF BIFIDOBACTERIAL SUPPLEMENTATION ON BOWEL MOVEMENTS

PROTOCOL NUMBER AFCRO-171

Version Number: 1.0
Date: 03 October 2023

ATLANTIA CLINICAL TRIALS LTD.
Heron House Offices, First Floor,
Blackpool, Cork, Ireland T23 R50R

Principal Investigator's Signature

I have carefully read this protocol entitled '*A randomized, double-blinded, placebo-controlled, parallel study, to assess the effect of Bifidobacterial Supplementation on bowel movements*' and agree that the conduct of this study is to be informed by the principles of Good Clinical Practice (GCP), the ethical principles that have their origin in the Declaration of Helsinki and further described in International Conference on Harmonization (ICH-GCP) E6 (R2), Title 21 of the Code of Federal Regulations 50, 56, and 312, General Data Protection Regulation (EU) 2016/679, and Clinical Trials Regulation EU No 536/2014 and 2005/28/EC for Good Clinical Practice.

In that it contains all the necessary information required to conduct the study. I agree to conduct this study as outlined in the protocol.

I understand that this study will not be initiated without approval of the appropriate Institutional Review Board/Independent Ethics Committee, and that all administrative requirements of the governing body of the institution will be complied with fully.

Informed written consent will be obtained from all Participants in accordance with the institutional requirements as set forth in the terms of the declaration of Helsinki as specified in the good clinical practice consolidation guidance issued by the International Conference on Harmonization.

I understand that my signature on the completed case report form(s) indicates that I have carefully reviewed each page and accept full responsibility for the contents thereof.

I understand that the information presented in this study protocol is confidential, and I hereby assure that no information based on the conduct of the study will be released without prior consent from Morinaga Milk Industry, unless the requirement is superseded by the appropriate regulatory authority.

Synopsis

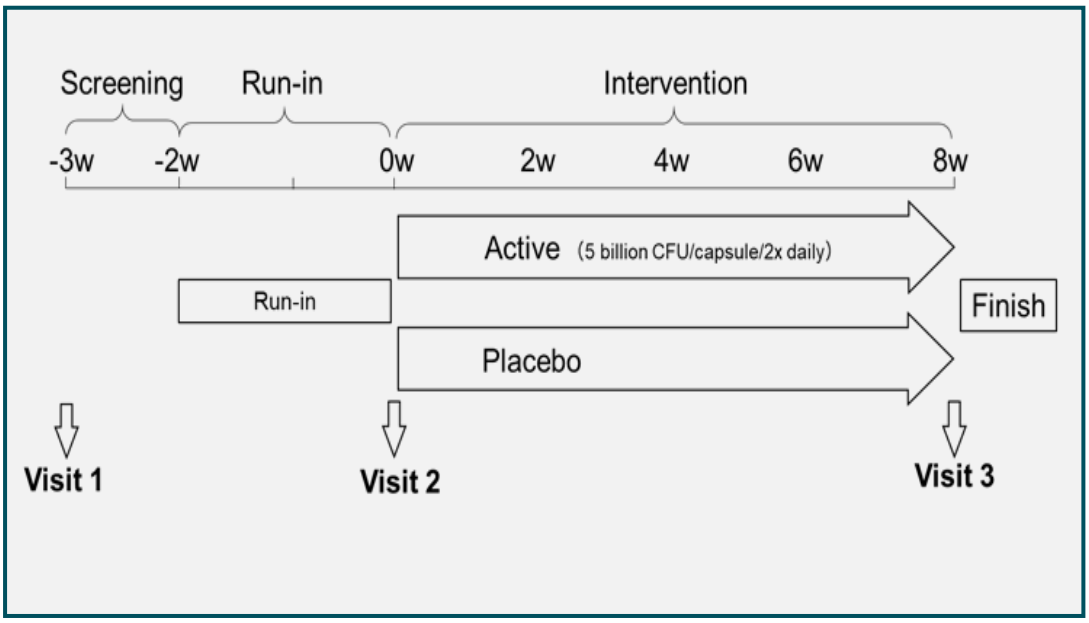
TITLE	A randomized, double-blinded, placebo-controlled, parallel study, to assess the effect of Bifidobacterial Supplementation on bowel movements.			
STUDY ACRONYM	N/A			
PROTOCOL NUMBER	AFCRO-171	**Eudract and/or IND No.**	N/A	
VERSION	1.0	**Date0/**	03 October 2023	
SPONSOR	Morinaga Milk Industry			
PLANNED START OF STUDY		**Estimated study duration**		
PRINCIPAL INVESTIGATOR	Stacey Boetto, FNP-C, NRCME, CCM			
STUDY SITE	Atlantia Clinical Trials, 142 E. Ontario, Suite 1200, Chicago, Illinois, 60611, USA			
RESEARCH AREA/APPLICATION FIELD	Constipation			
PRIMARY OBJECTIVE	To evaluate the effect of eight weeks daily consumption of *B. longum* compared to Placebo on Spontaneous Bowel Movement (SBM) frequency, in a population who experience constipation.			
SECONDARY OBJECTIVES	To evaluate the effect of eight weeks daily consumption of *B. longum* compared to Placebo, in a population who experience constipation on: - SBM frequency measured by the daily bowel diary from baseline to week 6. - Stool consistency as assessed by the Bristol Stool Scale (BSS). - Constipation related symptoms as assessed by Patient Assessment of Constipation Symptoms (PAC-SYM) Total Score. - Impact of constipation on Quality of life as assessed by Patient Assessment of Constipation Quality of Life Questionnaire (PAC-QoL) Total Score.			
SAFETY AND TOLERABILITY OBJECTIVES	To evaluate in otherwise healthy adults with constipation, the safety and tolerability of consuming two capsules of *B. longum* consumed daily compared to Placebo as assessed by: - Occurrence of any Adverse Events (AEs/SAEs) - Vital signs (Blood Pressure, Heart Rate, and Temperature) - Safety blood parameters			
STUDY DESIGN	Randomized, double-blinded, placebo-controlled, parallel study in healthy adults.			
STUDY POPULATION	Otherwise, generally healthy adults aged 18 to 65 who regularly experience constipation.			
SAMPLE SIZE	Screen *n* = 160 Randomize *n* = 80 (Two arms with 40 subjects per group) Evaluable *n* = 72 Expected dropout rate is 10% - dropouts will not be replaced.			

NUMBER OF SITES	Single centre; Atlantia Clinical Trials, 142 E. Ontario, Suite 1200, Chicago, Illinois, 60611, USA
STUDY PRODUCT	I. *B. longum* II. Placebo
NUMBER OF ARMS	2
DOSAGE AND REGIMEN	Two capsules of the Study Product per day, before breakfast, with a glass of water for the duration of the study (56 days).
PARTICIPANT SELECTION CRITERIA	**Inclusion criteria:** To be eligible for inclusion, the Participant must fulfil all the following criteria: 1. Is willing to participate in the study and comply with its procedures. 2. Be able to give written informed consent. 3. Adults aged ≥18 years and ≤65 years. 4. Experience less than 3 bowel movements per week within the past three months. 5. ≥25% of bowel movements with stool firmness, less than Type-3 based on Bristol Stool Scale (BSS), within the past three months. 6. Record less than 6 bowel movements in eDiary App during the two-week run-in period. 7. Is willing to maintain the current diet and level of physical activity throughout the study period. **Exclusion criteria:** The presence of any of the following criteria will exclude the Participant from participating in the study: 1. Participants who are pregnant or wish to become pregnant during the study, or who are currently breastfeeding. 2. Participants currently of childbearing potential, but not using continuous effective method of contraception, as outlined below: a. Complete abstinence from intercourse two weeks prior to administration of the Study Product, throughout the clinical study, until the completion of follow-up procedures or for two weeks following discontinuation of the Study Product in cases where Participant discontinues the study prematurely. (Participants utilizing this method must agree to use an alternate method of contraception if they should become sexually active and will be queried on whether they have been abstinent in the preceding two weeks when they present to the clinic for the final Visit). b. Has a male sexual partner who is surgically sterilized prior to the Screening Visit and is the only male sexual partner for that Participant. c. Sexual partner(s) is/are exclusively female. d. Use of acceptable method of contraception, such as a spermicide, mechanical barrier (e.g., male condom, female diaphragm), tubal ligation, or contraceptive pill. The

	Participant must be using this method for at least one week prior to and one week following the end of the study. e. Use of any intrauterine device (IUD) or contraceptive implant. The Participant must have the device inserted at least two weeks prior to the first Screening Visit, throughout the study, and two weeks following the end of the study. 3. Participants reporting current, or historical alcohol or drug abuse in past year. 4. Participants who have quit smoking in the 6-months prior to screening. 5. Participants with a known allergy to the Study Product's active or inactive ingredients. 6. Participants with a chronic disease who require long-term medication throughout the whole study period. 7. Participants with a significant acute, chronic or un-controlled coexisting illness or is taking medication/supplements that would put the Participant at risk or confound the interpretation of the study results, as judged by the investigator. 8. Participants who regularly take medications, that are thought to affect defecation, including regular laxative use. (Occasional laxative use (PRN) < 3 times per month is acceptable). 9. Participants who regularly consume supplements that are thought affect defecation, including but not limited to fiber, iron, and magnesium. 10. Participants who are consuming oral antibiotics at screening, during run-in and for the duration of the study. 11. Participants who have consumed probiotic supplements and prebiotic supplements eight weeks prior to Visit 2. 12. Participants who have consumed fermented foods, that aid in bowel movement improvement such as yogurt, kefir, sauerkraut, kimchi, soy-based fermented food, miso, natto and fermented cheese, etc. within the four weeks prior to the Visit 2. 13. Participants who are diagnosed with or undergoing treatment for any gastrointestinal conditions such as coeliac disease, diarrhea, Crohn's disease, ulcerative colitis, irritable bowel syndrome, diverticulosis, stomach or duodenal ulcers, gastroesophageal reflux disease (GERD) or have undergone significant gastrointestinal surgery (appendectomy allowed). 14. Participants diagnosed with clinical depression, Parkinson's disease, anxiety or an anxiety related disorder, who are undergoing treatment with medication. 15. Participants with clinically significant abnormal laboratory results at screening. 16. Individuals who are cognitively impaired and/or who are unable to give informed consent. 17. Participants may not be participating in other clinical studies. If the participant has previously taken part in an experimental study, the

	Investigator must ensure sufficient time has elapsed before entry to this study to ensure the integrity of the results. 18. Any Participant who is an employee of the investigational site or an Atlantia Clinical Trials employee or their close family member or a member of their household. 19. Individuals who, in the opinion of the investigator, are considered to be poor attendees or unlikely for any reason to be able to comply with the study.
STATISTICAL CONSIDERATIONS	All statistical elements of the study will be informed by the principles of the International Conference on Harmonization -Good Clinical Practice (ICH-GCP) documents and executed by the study statistician under the standard operating procedures for Atlantia Clinical Trials Ltd. Details about these elements will be available in a Statistical Analysis Plan (SAP) which will be written prior to first Participant randomized and finalized prior to database lock. Any deviations from the SAP and/or additional analysis after database lock should be fully described and justified in the clinical study report. The SAP will be developed by the study statistician, in close collaboration with the study PI and study Sponsor. All analyses will be conducted using IBM SPSS Statistics Software with figures generated using GGPLOT script in e R Project for Statistical Computing, as appropriate. Reporting of the statistical results will be informed by the principles of ICH-GCP and CONSORT.
ETHICAL, LEGAL, AND REGULATORY REQUIREMENTS	An application will be submitted to the Institutional Review Board (IRB) for approval of the study, prior to the study starting. This study will be conducted according to the principles of ICH-GCP and the Declaration of Helsinki. The study will be registered by Morinaga Milk Industry at clinicaltrials.gov.

Study Overview



Abbreviations

AE	Adverse Event
AR	Adverse Reaction
BDR	Blinded Data Review
BMI	Body Mass Index
BSS	Bristol Stool Scale
CFU	Colony Forming Units
CONSORT	**Con**solidated **S**tandards **o**f **R**eporting **T**rials
CRF	Case Report Form
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EP	Enrolled Population
GERD	Gastroesophageal Reflux Disease
GI	Gastrointestinal
GRAS	Generally Recognized As Safe
ICH-GCP	International Council for Harmonization- Good Clinical Practice
IDMC	Independent Data Monitoring Committee
IEC/IRB	Independent Ethics Committee/Institutional Review Board
ISF	Investigator Site File
ITT	Intention-to-Treat
IWRS	Interactive Web Response System
LPLV	Last Participant Last Visit
MedDRA	**Med**ical **D**ictionary for **R**egulatory **A**ctivities
PAC-QoL	Patient Assessment of Constipation Quality of Life
PAC-SYM	Patient Assessment of Constipation Symptoms
PI	Principal Investigator
PP	Per Protocol population
PT	Preferred Term
S	Safety Population
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SBM	Spontaneous Bowel Movement, defined as no manual maneuver and no laxatives, enemas, or suppositories in the preceding 24 hours
SOC	System Organ Classification
SOP	Standard Operating Procedure
SP	Study Product
SUSAR	Suspected Unexpected Serious Adverse Reaction
TFL	Tables, Figures, Listings
TMF	Trial Master File

Table of Contents

1 Introduction

1.1 Background information

Probiotics have been reported to have a beneficial effect on the intestines, and Bifidobacteria, in particular, have shown functionality in relieving constipation[(1)]. For this study, bowel movements are defined as any bowel movement, irrespective of its characteristics. In contrast, Spontaneous Bowel Movements (SBM) are defined as no manual maneuver and no laxatives, enemas, or suppositories in the preceding 24 hours. To obtain a holistic understanding of the effect of *B. longum* on gastrointestinal function, it is essential to select an appropriate type of bowel movement as an evaluating indicator. SBMs represent a natural and unstimulated event that reflects the body's ability to regulate bowel movements without external factors. Including SBMs in the study allows for a more comprehensive evaluation of bowel function.

1.2 Study Product

B. longum, which is isolated from infants, has been proven to be safe and has been widely used in fermented foods and supplements for a long time.

In this study, the active Study Product, *B. longum* capsule, contains lyophilized powder of live *B. longum* at 5×10^9 CFU/capsule blended with corn starch, and placebo capsule contains corn starch only.

High survivability of *B. longum* in host feces has been identified after oral intake. As a bifidobacteria, *B. longum* produces not only lactic acid but also a significant amount of acetic acid in the colon, which can reduce the luminal pH and improve the bowel movement. It is considered that B. LONGUM can promote defecation and improve constipation.

1.3 Rationale for conducting the clinical study

Due to limitations in recruiting clinical subjects, previous clinical trials investigating the effects of B. longum on constipation improvement only focused on East Asian individuals as participants. Consequently, there is currently a lack of clinical evidence regarding the efficacy of B. longum in regulation of bowel movement frequency and determining the appropriate dosage for European and North American populations, which have significantly different ethnicities, diets, and lifestyle habits.

2 Objectives

2.1 Primary objective

To evaluate the effect of eight weeks daily consumption of *B. longum* compared to Placebo on Spontaneous Bowel Movement (SBM) frequency, in a population who experience constipation.

2.1.1 Primary endpoint

Change in mean weekly frequency of SBM, as measured by the daily bowel diary from baseline to the end of intervention. Frequency will be defined as the mean weekly number of SBM during the 2-week run-in period prior to Visit 2, for baseline, and during the final 2-week period of the intervention prior to Visit 3, for end of intervention.

2.2 Secondary objectives

To evaluate the effect of eight weeks daily consumption of *B. longum* compared to Placebo, in a population who experience constipation on:

- SBM frequency measured by the daily bowel diary from baseline to week 6.
- Stool consistency as assessed by the Bristol Stool Scale (BSS).
- Constipation related symptoms as assessed by Patient Assessment of Constipation Symptoms (PAC-SYM) Total Score.
- Impact of constipation on Quality of life as assessed by Patient Assessment of Constipation Quality of Life Questionnaire (PAC-QoL) Total Score.

2.2.1 Secondary endpoints

- Change in mean weekly frequency of SBM, as measured by the daily bowel diary from baseline to Week 6. Frequency will be defined as the mean weekly number of SBM from baseline to Week 1-2, baseline to Week 3 -4, baseline to Week 5-6.
- Change in mean daily stool consistency as measured from the Bristol Stool Scale (BSS) in the daily bowel dairy from baseline to the end of intervention. Stool Consistency will be defined as the mean daily stool consistency during the 2-week run-in period prior to Visit 2, for baseline, and during the final 2-week period of the intervention, for end of intervention.
- Change in self-reported constipation related symptoms as assessed by the Total Score of the Patient Assessment of Constipation Symptoms (PAC-SYM) Questionnaire from baseline to:
  - Week 4
  - Week 8
- Change in self-reported impact of constipation on Quality of Life as assessed by the Total Score of the Patient Assessment of Constipation Quality of Life (PAC-QoL) Questionnaire from baseline to:
  - Week 4
  - Week 8

2.3 Exploratory objective(s)

To evaluate the effect of eight weeks daily consumption of *B. longum* compared to Placebo, in a population who experience constipation on:

- PAC-SYM sub-scores (Abdominal, Rectal, and Stool related symptoms)
- PAC-QoL sub-scores (Physical Discomfort, Psychosocial Discomfort, Treatment Satisfaction, and Worries/ Concerns)
- Microbiome and fecal metabolite analysis (stool sampling only, analysis by Morinaga Milk Industry)

2.3.1 Exploratory endpoints

- Change in PAC-SYM sub-scores (Abdominal, Rectal, and Stool related symptoms) from baseline to:
  - Week 4
  - Week 8
- Change in PAC-QoL sub-scores (Physical Discomfort, Psychosocial Discomfort, Treatment Satisfaction, and Worries/ Concerns) from baseline to:
  - Week 4
  - Week 8

2.4 Safety objectives and tolerability objective(s) and endpoint variables

2.4.1 Safety and tolerability objective(s)

To evaluate in otherwise healthy adults with constipation, the safety and tolerability of *B. longum* consumed twice daily compared to Placebo as assessed by:

- Occurrence of any Adverse Events (AEs/SAEs)
- Vital signs (Blood Pressure, Heart Rate, and Temperature)
- Safety blood parameters

2.4.2 Safety and tolerability endpoint variable(s)

- Incidence of AEs per group by product causality.
- Incidence of mild, moderate, and severe AEs per treatment group by product causality.
- Incidence of AEs by MedDRA System Organ Classification (SOC) and preferred term (PT) by product allocation.
- Incidence of Safety Blood Parameter AEs by product allocation.

- Incidence of Vital Parameter (blood pressure, heart rate, and body temperature) AEs by product allocation.
- Incidence of SAEs per group by product causality.

3 Study Design

Randomized, double-blinded, placebo-controlled, parallel study in healthy adults.

3.1 Duration of clinical study

The study will involve three on-site visits over a period of up to eleven weeks.

3.2 Duration of clinical study per Participant

- Screening: 1 - 7 days
- Run-in phase: 14 days
- Intervention phase: 56 days
- Total time: 77 days

4 Participants and site

4.1 Number of Participants

Screen *n* = 160
Randomize *n* = 80 (Two arms with 40 subjects per group)
Evaluable *n* = 72
Expected dropout rate is 10% - dropouts will not be replaced.

4.2 Clinical study population

Otherwise, generally healthy adults aged 18 to 65 who regularly experience constipation.

4.3 Inclusion criteria

To be eligible for inclusion, the Participant must fulfil all the following criteria:

1. Is willing to participate in the study and comply with its procedures.
2. Be able to give written informed consent.
3. Adults aged ≥18 years and ≤65 years.
4. Experience less than 3 bowel movements per week within the past three months.
5. ≥25% of bowel movements with stool firmness, less than Type-3 based on Bristol Stool Scale (BSS), within the past three months.
6. Record less than 6 bowel movements in eDiary App during the two-week run-in period.
7. Is willing to maintain the current diet and level of physical activity throughout the study period.

4.4 Exclusion criteria

Participants will be excluded from the study if they meet any of the following criteria:

1. Participants who are pregnant or wish to become pregnant during the study, or who are currently breastfeeding.

2. Participants currently of childbearing potential, but not using continuous effective method of contraception, as outlined below:
    a. Complete abstinence from intercourse two weeks prior to administration of the Study Product, throughout the clinical study, until the completion of follow-up procedures or for two weeks following discontinuation of the Study Product in cases where Participant discontinues the study prematurely. (Participants utilizing this method must agree to use an alternate method of contraception if they should become sexually active and will be queried on whether they have been abstinent in the preceding two weeks when they present to the clinic for the final Visit).
    b. Has a male sexual partner who is surgically sterilized prior to the Screening Visit and is the only male sexual partner for that Participant.
    c. Sexual partner(s) is/are exclusively female.
    d. Use of acceptable method of contraception, such as a spermicide, mechanical barrier (e.g., male condom, female diaphragm), tubal ligation, or contraceptive pill. The Participant must be using this method for at least one week prior to and one week following the end of the study.
    e. Use of any intrauterine device (IUD) or contraceptive implant. The Participant must have the device inserted at least two weeks prior to the first Screening Visit, throughout the study, and two weeks following the end of the study.
3. Participants reporting current, or historical alcohol or drug abuse in past year.
4. Participants who have quit smoking in the 6-months prior to screening.
5. Participants with a known allergy to the Study Product's active or inactive ingredients.
6. Participants with a chronic disease who require long-term medication throughout the whole study period.
7. Participants with a significant acute, chronic or un-controlled coexisting illness or is taking medication/supplements that would put the Participant at risk or confound the interpretation of the study results, as judged by the investigator.
8. Participants who regularly take medications, that are thought to affect defecation, including regular laxative use. (Occasional laxative use (PRN) < 3 times per month is acceptable).
9. Participants who regularly consume supplements that are thought affect defecation, including but not limited to fiber, iron, and magnesium.
10. Participants who are consuming oral antibiotics at screening, during run-in and for the duration of the study.
11. Participants who have consumed probiotic supplements and prebiotic supplements eight weeks prior to Visit 2.
12. Participants who have consumed fermented foods, that aid in bowel movement improvement such as yogurt, kefir, sauerkraut, kimchi, soy-based fermented food, miso, natto and fermented cheese, etc. within the four weeks prior to the Visit 2.
13. Participants who are diagnosed with or undergoing treatment for any gastrointestinal conditions such as coeliac disease, diarrhea, Crohn's disease, ulcerative colitis, irritable bowel syndrome, diverticulosis, stomach or duodenal ulcers, gastroesophageal reflux disease (GERD) or have undergone significant gastrointestinal surgery (appendectomy allowed).

14. Participants diagnosed with clinical depression, Parkinson's disease, anxiety or an anxiety related disorder, who are undergoing treatment with medication.
15. Participants with clinically significant abnormal laboratory results at screening.
16. Individuals who are cognitively impaired and/or who are unable to give informed consent.
17. Participants may not be participating in other clinical studies. If the participant has previously taken part in an experimental study, the Investigator must ensure sufficient time has elapsed before entry to this study to ensure the integrity of the results.
18. Any Participant who is an employee of the investigational site or an Atlantia Clinical Trials employee or their close family member or a member of their household.
19. Individuals who, in the opinion of the investigator, are considered to be poor attendees or unlikely for any reason to be able to comply with the study.

4.5 Randomization, blinding, and treatment allocation

Participants who meet the eligibility criteria/have successfully completed the Screening Visit will be randomized on a 1:1 basis to the two arms of this study to determine whether they receive the Study Product (SP) or the Placebo. The randomization list and procedure will be prepared by a statistician not involved in the conduct phase of the study (i.e., blinded data reviews) and securely conveyed to the science and quality team.

Randomization will be completed using a minimization procedure via an Interactive Web Response System **(**IWRS) with the aim to minimize imbalance on sex and bowel movement frequency. Each eligible participant will be assigned a randomization number, which corresponds to one of the two products. The relationship between the randomization number and the product assignment will be unknown to the clinical research team, the sponsor, study site staff, or the Participants, i.e., the study will be double blinded.

Blinding of both study team and Participants will be ensured by only providing access to the randomization list to the science and quality team which is stored in the quality drive.

4.6 Emergency Unblinding procedure

The investigator will receive access to an IWRS to unblind if required.

Unblinding access is for emergency use only, i.e., when it becomes absolutely necessary to know what treatment the participant receives in case of a serious adverse event (SAE) in order to manage the participant's condition. A broken code requires the participant to be withdrawn from the clinical trial.

The investigator should promptly document and notify the Sponsor any premature unblinding (e.g., accidental unblinding, unblinding due to a SAE) of the IP. The date, time, and reason for unblinding must be written onto the envelope and signed by the investigator.

If appropriate, the monitor will check the IWRS during monitoring visits and obtain adequate documentation for any unblinding.

4.7 Number of sites

This is a single-site study, with all Participants to be recruited by Atlantia Clinical Trials.

5 Study Product and Supplementation Regimen

5.1 Study Product

5.1.1 Study Product name and formulation

- Product name: Bifidobacterial capsule, Morinaga Milk Industry, 5-33-1 Shiba, Minato-ku, Tokyo, 108-8403, Japan.
- Active compound: *Bifidobacterium longum*, 1×10^{10} CFU
- Capsules: 5×10^{9} CFU active compound per capsule

5.2 Other product name and formulation

5.2.1 Placebo Product:

- Ingredients: 0 CFU compound per capsule

5.3 Packaging and labelling

The label will contain the following information:

Atlantia Study Code - AFCRO-171
Sponsor: Morinaga Milk Industry
Participant Randomization Code
Investigator name
For clinical study use only
Batch/Lot number
Storage conditions / keep away from children (if applicable)
Directions for use
Emergency Contact Number
Expiry date

Packaging will be performed by Morinaga Milk Industry and labelling will be carried out by Atlantia Clinical Trials, 142 E. Ontario, Suite 1200, Chicago, Illinois, 60611, USA.

Each aluminum bag will contain 28 capsules of either the SP or the Placebo. Each Participant will be provided with five aluminum bags (total 140 capsules) allowing for two capsules per day, for 56 days and an extra 14-day supply, in case of delay of visit or loss of Study Product.

5.4 Handling and storage conditions

Study Product will be stored in a secure area with restricted access. The Principal Investigator/Sub-Investigator agrees that the Study Product will be dispensed only to study Participants who have provided written consent. Compliance will be monitored by return of all product containers (used and unused). Any deviations will be noted in the source documents and the Participant's eCRF. The Study Product should be stored at refrigerated temperature, 2°- 8°C (36°-46°F) before being dispensed to the Participants.

Study Participants will be instructed to store the Study Product at room temperature.

5.5 Supplementation regimen

5.5.1 Dosage regimen and dose adjustment

- Participants will be provided an eight-week supply of Study Product (5 bags) at Visit 2 in a transparent UniPak, wherein 4 bags will be used for consumption during the eight-week intervention period and 1 bag will be provided as an additional supply in case of delay in study visit or loss of Study Product.
- Participants will be instructed to consume two capsules of the Study Product per day, before breakfast, with a glass of water for the duration of the study (56 days).
- If the dose is missed, the Participant should consume the same dosage after dinner on the same day.
- Missed doses should not be moved to the following day.
- Participants will be instructed to return any unused Study Product at Visit 3.
- Participants will consume their first dose of Study Product on the day following their Visit 2 (Day 1) and will consume their final dose of Study Product on the morning of their Visit 3 (Day 56).

In the event of significant worsening, severe or intolerable new GI symptoms, or other AEs after product consumption/use or overdose, the dose may be interrupted or titrated after consultation with the sponsor representative and PI/Sub-Investigator. The dose may be temporarily interrupted for the shortest time possible without increasing the risk to the Participants continued safety on the study. If the Participant requires dose interruption for more than three days with no improvement in GI symptoms and/or other AEs, then consultation with the Sponsor representative and PI/Sub-Investigator must occur, and the Participant may be discontinued from SP.

5.6 Compliance

Individual Product Dispensing and Accountability Logs will be completed for each Participant and kept up to date. The log lists the Participant Number, the date of dispensing, number of capsules dispensed, the number of capsules returned, and the date returned. Participants will be instructed to return all unused product. If applicable, the monitor will inspect the amount of SP dispensed and returned.

Compliance will be assessed at Visit 3, in the statistical analysis, overall compliance will be considered. If a Participant states that they have not returned all unused capsules (e.g., forgot to return, loss, contaminated), details will be recorded in the eCRF. Participants must have consumed a minimum of 80% of the Study Product, in order to be deemed product compliant. Non-compliant Participants will be included in the Intention-to-Treat (ITT) population set (Analyzed on as-randomized basis) and will be excluded withdrawn from the Per-Protocol (PP) analysis.

5.7 Warnings and precautions

- For clinical study use only.
- Keep out of reach of children.

5.8 Concomitant treatments/supplements and restrictions

Participants will be questioned about their general medical history within the previous year, and medications within the previous three months. The details of any medication taken will be recorded in the Participant case notes and case report form.

Participants will be instructed to follow their habitual diet and exercise routine and to not consume any disallowed medications, dietary supplements, probiotic, prebiotic supplements, and fermented products that could interfere with the assessment of the Study Product for the duration of the study.

5.9 Rescue Medication

In case of no bowel movement for more than four days: bisacodyl suppositories or tablets (5- 10 mg) or other rescue medication in line with local guidelines at the dose specified in the local guidelines (no more than three consecutive days over a period of 1 week) will be allowed as rescue medication. Maximum dosage: 15 mg/ day.

Its use needs to be documented at relevant study visits (including concomitant medication, diet, supplements, and any related adverse event). The date and time of rescue medication administration as well as the name and dosage regimen of the rescue medication must be recorded.

The study site will not supply any rescue medication.

6 Study Procedures

Table 2. Schedule of Assessments

Assessment periods					
Visit Number	Visit 1	Run-in	Visit 2	Visit 3	Early Termination Visit
Visit Window	Day -21 to -15	Day -14 to -1	Day 0	Day 56 + 3 Days	
Study Phase	Screening	Run-in	Intervention		
Screening					
Informed consent	x				
Eligibility Screen	x		x		
Demographic/ Lifestyle data	x				
Medical history	x		x		x
Anthropometric measurements (Height, weight, BMI)	x				
Prior and concomitant medications	x		x	x	x
Safety and Tolerability					
Vitals (Blood pressure, heart rate, temperature)	x		x	x	x
Safety blood parameters	x				

Assessment periods					
Visit Number	Visit 1	Run-in	Visit 2	Visit 3	Early Termination Visit
Visit Window	Day -21 to -15	Day -14 to -1	Day 0	Day 56 + 3 Days	
Study Phase	Screening	Run-in	Intervention		
(Chemistry profile and Hematology profile)					
AE/SAE			<----------	---------->	x
Assessments/Procedures					
Stool sample collection					
Dispense stool collection kit	x		x		
Return Stool Sample for microbiome analysis			x	x	x
Urine collection					
Urine pregnancy test	x		x	x	x
Questionnaires					
Download eDiary App	x				
Bowel Movement & Bristol Stool Scale		x	<----------	---------->	
PAC-SYM (Week 0, 4 & 8)			<----------	---------->	
PAQ-QoL (Week 0, 4 & 8)			<----------	---------->	
Delete eDiary App				x	x
Dietary analysis					
Dispense 3-Day food Diary	x		x		
Return 3-Day food Diary			x	x	x
Supplementation					
Randomization			x		
Administer SP			x		
Return SP				x	x
Assess SP Compliance				x	x
Participant Compensation					
Dispense Participant Compensation				x	x

All visits will have the option of being completed remotely via telephone where feasible based on the assessments required for the visit.

6.1 Recruitment procedures

Participants may be recruited through Atlantia's database, social media, radio advertisements and adverts in local newspapers. Participants will undergo an initial pre-screening questionnaire and will be asked questions regarding their age, general health, and medications. Eligible Participants will be scheduled for a Screening Visit.

6.2 Obtaining informed consent

Participants will receive a copy of the Informed Consent Form, via email prior to the Screening Visit. Participants who are foreseen to fulfil the inclusion/exclusion criteria for enrolment into the clinical study will be asked to give informed consent in writing prior to any clinical study specific procedures. Both the Participant (or legal representative) and PI or a person designated by the PI (if acceptable by local regulations) will sign and date the informed consent form. All Participants who have signed the informed consent form will be listed on the *Participant Screening and Enrolment Log* and *Participant Identification Log*.

7 E-Diary/Questionnaire Data Collection

7.1 Daily eDiary Questions

Participants will download an eDiary App at Visit 1 which they will be directed to complete throughout the study. This eDiary will record the following information:

- Bowel movement diary will collect daily data relating to stool frequency and stool consistency.
- AEs and medication use (Weekly)
- Study Product compliance (Weekly)

7.2 eDiary Questionnaires

7.2.1 Patient Assessment of Constipation Symptoms (PAC-SYM) Questionnaire

- Participants will complete the questionnaire at Week 0, Week 4, and Week 8.
- PAC-SYM collects self-reported constipation related symptoms – has a Total Score, and three sub-scores for key symptom areas (Abdominal, Rectal, and Stool related symptoms).

7.2.2 Patient Assessment of Constipation – Quality of Life (PAC-QoL) Questionnaire

- Participants will complete the questionnaire at Week 0, Week 4, and Week 8.
- PAC-QoL assessment collects self-reported impact of constipation on Quality of Life -has a Total Score, and four sub-scores for key areas of wellbeing (Physical Discomfort, Psychosocial Discomfort, Treatment Satisfaction, and Worries & Concerns).

7.3 3-Day Food diary

Participants will complete a 3-Day Food Diary, logging any food eaten for three days prior to Visit 2 to establish their dietary habits at Baseline, and again at Visit 3 to reassess it at end of intervention. This data will be used to confirm that Participants maintained their habitual diet throughout the study via evaluation of key dietary components at baseline compared to end-of-intervention (i.e., energy, total carbs, total sugar, fiber, and fat).

8 Biological measurements and assessments

8.1 Blood sample collection

A total of approximately 7.5mL/0.25 fl.oz of blood should be collected from all Participants.

8.1.1 Failed or partial blood draws

In the case of a failed attempt at a blood draw, or a partial blood draw which did not successfully collect the required amount of blood for analysis, the clinical phlebotomist may either make additional attempts on the Participants other arm, or, if willing, the Participant may be asked to return within the visit window for a second attempt. This will only be carried out if required and will be entirely at the Participant's discretion.

8.1.2 Rejected blood samples or failed blood sample analysis

In the case of a rejected blood sample or failed blood sample analysis, refer to the sub-investigator and the Safety Monitoring Plan.

8.2 Storage of Samples

All blood and fecal samples will be listed on study specific sample logs and stored at the clinical site during the study where applicable.

8.3 Shipment of Samples

Shipment of samples will be performed in accordance with the Laboratory manual.

8.4 Analysis of Samples

All samples will be analyzed using validated assays and methods.

9 Early Participant Withdrawal

Participants have the right to withdraw from the study at any time for any reason, without giving a reason and without penalty or loss of benefits they are entitled to. The PI/Sub-Investigator also has the right to discontinue Participants from SP consumption and/or withdrawal from the study if this is in the best interest of the Participant or if Participant is persistently not compliant with study procedures and SP consumption.

Any of the following conditions may lead to premature withdrawal from SP/study:

- Request by Participant to discontinue for any reason during the study.
- erroneously included / randomized Participant.
- adverse event(s) or concurrent illness that, in the opinion of the PI/Sub-Investigator, warrants the Participant's withdrawal from intervention.
- intake of concomitant medication/dietary supplements prohibited by the protocol.
- Participants who do not follow the requirements of the Protocol, especially those concerning safety and/or if the Participant after their enrolment is uncooperative or not willing to comply with the protocol (non-compliant).

- failure to return for follow-up visits despite numerous attempts to reschedule (lost to follow-up).
- request by regulatory agencies for termination of supplementation of an individual Participant or all Participants under this protocol.
- pregnancy, if a protocol exclusion criterion.

The circumstances of any discontinuation will be in consultation with the Sponsor and must be documented in detail in the corresponding study termination form in the case report form. Whenever a Participant will be withdrawn from the study for whatever reason, adequate efforts will be made to perform a final assessment to include standard medical and laboratory tests which would have been done on normal completion of the study. The data of the withdrawn Participants will be documented in the clinical study report.

Note: Participants who withdraw prematurely from the study will not be replaced.

10 Data processing

10.1 Case Report Form

10.1.1 Electronic Data Capture

It is the responsibility of the PI (or designee) to ensure the completeness and accuracy of the electronic CRFs (eCRF). One eCRF must be compiled for each Participant participating in the study.

Data required for the analysis will be acquired and transferred electronically to a central database by means of an Electronic Data Capture system (the "EDC-tool"). The system operates over the Internet according to the principle of online data capture. Data entered by investigators or data managers into local computers are directly transferred over the Internet to a central database, without any permanent local storage.

High security standards for the transfer and storage study data are guaranteed using technologies such as encrypted data transfer, firewalls, and periodic backup to protect centrally stored data. Writing access to the system will be limited to authorized personnel and the system will automatically keep an audit trail of all entries and corrections to the CRF. Training in the use of the EDC-tool will be provided by data management, study coordinator, monitor, or delegates.

The EDC-tool will comprise of an eCRF, designed specifically for this study.

The EDC-tool is operated under an electronic signature, consisting of the combination of an identification code and a password. In relation to the present study, passwords and electronic signatures will be distributed to users of the EDC tool, and used for entering, modifying, or viewing study data.

It is the responsibility of individuals who received an electronic signature to keep their password secret, i.e., not to reveal it to third parties, and to access the EDC-tool, to enter or modify study data using the EDC-tool only under their personal identification code and password.

10.1.2 Data Management

The eCRF is based on the electronic data capture system developed by Clindox, which is fully compliant with and a Gold Member of the Clinical Data Interchange Standards Consortium.

The eCRF will be hosted on a dedicated validated stand-alone server placed in a double locked server room. All access to the server and other server maintenances will be logged. Study setup and hosting will be managed by Clindox and Atlantia according to ICH-GCP and Standard Operating Procedures.

Only authorized access to the eCRF will be possible using encrypted username and password. Roles in the system are given according to functions (researcher, monitor, project manager, data manager, data analyst etc.). All tasks performed in the eCRF are logged in an audit trail.

The eCRF will contain data ranges and validation checks to maintain an ongoing quality check of data entered. All data validation will be performed as part of the system using Data Clarification Report tools. Ongoing and prior to database lock a blinded database review will be performed, and any discrepancies or missing data will be queried and resolved. A log will be kept on site to document location of source data.

Medical history data, adverse events, and prior and concomitant medication data entry will be standardized.

The PI/Sub-Investigator and authorized staff at the clinical site can add data to the eCRF and must keep the eCRF current to reflect Participant status during the study. A screening and randomization number and the date of birth identify the Participants on the eCRF. The study personnel must make a separate confidential record of personalized details (name and date of birth) on the Participant identification and enrolment log which is kept separately from the eCRF. Once the eCRF for a Participant is completed, the PI/Sub-Investigator will approve the data using an electronic signature and thereby confirm the accuracy of the data recorded.

Laboratory data will be collected electronically from the laboratory and combined with the other clinical data. The data entry of the eCRFs and laboratory data may generate additional requests (Data query) to which the researcher is obliged to respond by confirming or modifying the data questioned.

The eCRFs for any Participant leaving the study should be completed at the time of the final Visit or shortly thereafter. Any ongoing AEs at the final Visit will be characterized as ‘Ongoing at End of Study’ and there will be no further follow up.

11 Safety and Tolerability

11.1 Definitions and Standards

Adverse Event (AE):

Any untoward medical occurrence in a Participant involved in a clinical study administered a Study Product whether or not related to this product.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding for example), subjective and objective symptom, or disease temporally associated with the use of a product, accidents, whether or not considered related to the product or study-related procedure and reported by the Participant or observed by the PI/Sub-Investigator.

Serious Adverse Event (SAE):

Any AE that at any dose fulfils at least one of the following criteria:

1. Is fatal (results in death)
   (*note*: death is an outcome, not an event)
2. is life-threatening
   (*note*: the term "life-threatening" refers to an event in which the Participant was at risk of death at the time of the event; it does not refer to an event which could hypothetically have caused death had it been more severe)
3. requires inpatient hospitalization or prolongation of existing hospitalization
   (*note*: "inpatient hospitalization" refers to an unplanned, overnight hospitalization)
4. results in persistent or significant disability/incapacity
   (*note:* the term means substantial disruption of one's ability to conduct normal life function)
5. is a congenital anomaly/birth defect
   (*note:* congenital anomaly/birth defect in offspring of Participant taking the product regardless of time to diagnosis)
6. is medically significant
   (*note*: Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in other situations, such as 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 other outcomes listed in the definitions above)

Suspected Unexpected Serious Adverse Reaction (SUSAR)

A suspected Unexpected Serious Adverse Reaction is any adverse reaction for which the nature or severity is non consistent with the applicable product information.

11.2 Adverse event assessment

Expectedness:

An unexpected AE is an event of which the nature or severity is not consistent with the Participant's medical history, concomitant medications, or the applicable product information.

For purposes of this study, bloating and mild diarrhea may be expected according to previous reports. All other AEs reported will be unexpected.

11.2.1 Causality Assessment

The causality assessment of an AE to the investigational and/or study procedure(s) product will be rated as follows:

Unrelated: The event is clearly related to other factors such as the Participant's clinical state, therapeutic interventions, or a concomitant medication administered to the Participant and does not follow a known response pattern to the Study Product.

Unlikely: The event follows a reasonable temporal sequence from the time of Study Product administration, although there is another plausible explanation for the occurrence of the event.

Possible: The event follows a reasonable temporal sequence from the time of Study Product administration and/or follows a known response pattern to the study treatment but could have been produced by other factors such as the Participant's clinical state, therapeutic interventions, or concomitant medications administered to the Participant.

Probable: The event follows a reasonable temporal sequence from the time of Study Product administration and follows a known response pattern to the Study Product, where the event is more likely explained by the product than any other cause.

Definite: The event follows a reasonable temporal sequence from the time of Study Product administration and follows a known response pattern to the Study Product and cannot be reasonably explained by other factors such as the Participants clinical state, therapeutic interventions administered to the Participant and either occurs immediately following Study Product, or improves on stopping the Study Product, or reappears on repeat exposure, or there is a positive reaction at the application site.

11.2.2 Severity/Intensity

The severity of AEs must be recorded, including the start and stop dates for each change in severity. An event that worsens / increases in severity should be captured as a new event. After initiation of IP consumption, worsening of events that started prior to initiating intake of IP must be recorded as new AEs (for example, if a patient experiences mild intermittent dyspepsia prior to dosing of IP, but the dyspepsia becomes severe and more frequent after first dose of IP has been administered, a new AE of severe dyspepsia [with the appropriate date of onset] is recorded in the eCRF).

The severity/intensity of AEs where applicable will be graded on a five-point-scale in accordance with the Common Terminology Criteria for Adverse Events:

Grade 1:	Mild AE - Discomfort noted, but no disruption to normal daily activities
Grade 2:	Moderate AE - Discomfort sufficient to reduce or affect normal daily activities
Grade 3:	Severe AE - Inability to work or perform normal daily activities
Grade 4:	Life threatening or disabling AE
Grade 5:	Death related to AE

11.2.3 Outcome of event

The outcome of AEs must be followed up and recorded during the course of the study in the eCRF. Outcomes are as follows:

- Recovered
- Recovered with sequelae
- Ongoing
- Fatal
- Unknown/Lost to follow-up

11.2.4 Clinical laboratory and other safety evaluations

A change in the value of a clinical laboratory, vital sign, or other study assessment can represent an AE if the change is a clinically significant worsening from baseline. This includes abnormal assessments where there is a shift of a parameter from a normal value to an abnormal value, or a significant worsening of an already abnormal value. Clinical significance is defined as an abnormal study assessment that leads to a diagnosis (in which case the diagnosis is recorded rather than the abnormal assessment) or results in Participant intervention such as further monitoring (excluding confirmatory repeat testing) or medical treatment. If, at the end of the treatment phase, there are abnormal clinical laboratory, vital sign values or other study assessments which were not present pre-treatment, the value observed closest to the start of study treatment should be used as baseline. The PI or Sub-Investigator should decide, based on the above criteria and the clinical condition of a Participant, whether a worsening of an abnormal study assessment is clinically significant and therefore represents an AE.

11.3 Pregnancy

All pregnancies are to be reported from the time of enrolment to the end of follow-up in the Participants eCRF and reported using a Pregnancy Report Form. Any report of pregnancy for any study Participant must be reported to the Sponsor. Pregnancy itself is not an AE, however it should be reported for tracking purposes.

Pregnant Participants must be withdrawn from using the Study Product, and in consultation with the Sponsor and/or PI/Sub-Investigator may be withdrawn from the study or asked to remain and continue safety monitoring for the remainder of the study. Every effort should be made to gather information regarding the pregnancy outcome.

The Participant will be asked to notify the PI/Sub-Investigator of the outcome of the pregnancy (i.e., birth, loss, or termination). To help ensure this, the PI/Sub-Investigator will follow-up with the Participant until the end of pregnancy or last study Visit whichever occurs first, with the Participant's consent. This request and the Participant's response will be documented in the Participant's source document. Pregnancy outcome will be determined as live birth, still birth or pregnancy termination.

If the pregnancy is due to a Participant's failure to comply with the contraceptive requirements of the protocol, this should also be documented as a protocol deviation.

Pregnancy complications such as spontaneous abortion/miscarriage or congenital abnormality are considered SAEs and must be reported as such. Note: an elective abortion is not considered an SAE. In addition to the above, if the PI/Sub-Investigator determines that the pregnancy meets SAE criteria, it must be reported as an SAE. The test date of the first positive urine human chorionic gonadotropin test or ultrasound result will determine the pregnancy onset date.

11.4 Safety Management

11.4.1 Procedures

All Adverse Events (AEs) occurring during clinical studies are recorded in the electronic Case Report Form (eCRF).

Serious adverse events (SAEs) are reported and processed according to the applicable laws and regulatory requirements governing the conduct of clinical studies. SAEs must be recorded on the SAE Form.

11.5 Responsibilities

PI or Sub-Investigator will ensure that SAE reporting procedures outlined in the study protocol are adhered to and that all required documentation is up-to-date, and that regulatory and IRB/IEC SAE reporting procedures are followed.

All clinical research personnel at the study site (PI, Sub-Investigator, Project Manager, Clinical Research Coordinator, Clinical Trial Assistant, Research Nurse, Phlebotomist) who are in contact with Participants are responsible for collecting AE information from the Participant at each scheduled site visit or during telephone calls with the Participant. Therefore, Sponsor clinical research personnel or a delegate (e.g. CRO) who initiate and monitor the study are responsible for explaining the procedures for reporting and evaluating AEs to the PI or Sub-Investigator and all study personnel who come in contact with the Participants

During the course of the study, complete reports of all AEs will be entered in the Participants site source documents, and if applicable, on the appropriate study case report forms (CRFs).

A licensed clinician will be responsible for:

- identifying and evaluating the severity (mild, moderate, or severe) and clinical importance of the AE,
- taking appropriate medical action(s), and for
- notifying the Sponsor immediately of an SAE as specified in the protocol and for notifying the Science Department for reporting to the IRB/IEC.

Likewise, a licensed clinician will indicate the causality (relationship) of the AE to the Study Product and modify IP consumption in the event of a safety concern.

For any laboratory abnormality, the PI or Sub-Investigator will make a judgement as to its clinical significance. If the laboratory value is thought to be clinically significant, the Sponsor may be consulted, and an assessment will be made by the PI or Sub-Investigator as to its relationship to Study Product administration and it will be documented on the AE page of the CRF.

The PI or Sub-Investigator will comply with applicable regulatory requirement(s) related to the reporting of SAEs to the IRB/IEC.

The Monitor(s) will review completed CRF data and will compare CRF entries with information recorded in the source documents. Any discrepancies or omissions in either data source will be discussed with the site personnel who should make the appropriate corrections to the documents.

The CRO will report all SAEs to the Sponsor within 24 hours of becoming aware of the event, whether or not the SAE is considered to be related to the Study Product. All clinically relevant SAEs will be reported to the Clinical Research Ethics Committee of the Cork Teaching Hospitals.

12 Ethical Considerations

12.1 Local Regulations and Declaration of Helsinki

The PI/Sub-Investigator will ensure that this clinical study is conducted in line with the principles of the "Declaration of Helsinki" and/or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual.

The site, investigator, protocol, informed consent form and any other pertinent documents for this study will be approved by the IRB.

The study will not begin until the IRB has approved of the protocol and the Participant consent form along with any advertisements, diaries, and instructions to the Participants, if applicable. The IRB approval will be documented in writing to the PI/Sub-Investigator.

12.2 GCP Management Directive

The PI/Sub-Investigator will ensure that this clinical study is conducted in line with the principles of GCP, as outlined below:

- Clinical studies should be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with the principles of GCP (where applicable) and the applicable laws and regulatory requirements governing the conduct of human studies.
- Foreseeable risks and inconveniences should be weighed against the anticipated benefit for the individual trial Participant and the society.
- The rights, safety, and well-being of the Participants involved in clinical studies are the most important considerations and should prevail over interests of science and society.
- The available nonclinical and clinical information on any Study Product should be adequate to support the proposed clinical trial.
- Clinical studies should be scientifically sound, and described in a clear, detailed protocol.
- Clinical studies should be conducted in compliance with the protocol that has received prior institutional review board (IRB)/independent ethics committee (IEC) approval/favorable opinion.

- The medical care given to, and medical decisions made on behalf of, Participants involved in clinical studies should always be the responsibility of a qualified licensed clinician.
- Each individual involved in conducting clinical studies should be qualified by education, training, and experience to perform his or her respective task(s).
- Freely given written informed consent should be obtained from every Participant prior to participation in a clinical trial.
- All information from clinical studies should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation, and verification.
- The confidentiality of records that could identify Participants should be protected, respecting the privacy and confidentiality rules in accordance with the applicable laws and regulatory requirements.
- Study Product should be manufactured, handled, and stored in accordance with Morinaga Milk Industry quality standards, or if applicable, good manufacturing practice (GMP); IP(s) should be used in accordance with the approved protocol.
- Systems with processes and procedures (such as SOPs) that assure the quality of every aspect of clinical studies should be implemented.

12.3 Informed Consent

It is the responsibility of the PI, or a person designated by the PI (if acceptable by local regulations), to obtain written informed consent from each Participant involved in this clinical study, or from the Participant's legally acceptable representative, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. No study procedures may be performed prior to obtaining the informed consent. The PI or Designee must also explain that the Participants are completely free to refuse to enter the study or to withdraw from it at any time, for any reason.

12.4 IRB/IEC approval

This protocol and any accompanying material provided to the Participant (such as Participant information leaflets or descriptions of the study used to obtain informed consent) as well as any advertising or compensation given to the Participants, will be submitted by the PI/Sub-Investigator to an IRB/IEC. Approval from the IRB/IEC will be obtained before starting the study and should be documented in a letter to the PI/Sub-Investigator specifying the date on which the IRB/IEC met and granted the approval.

Any modifications (amendments) made to the protocol after receipt of the IRB/IEC approval must also be submitted by the PI/Sub-Investigator to the IRB/IEC in accordance with local procedures and regulatory requirements.

12.5 Changes to Protocol

Any changes to the protocol will be in the form of a written protocol amendment or note to file that will be issued by the Sponsor or designee.

All amendments to the approved protocol require approval by the IRB/IEC.

The protocol requirements should in no way prevent any immediate action from being taken by the PI/Sub-Investigator, or by the Sponsor (or designee), in the interest of preserving the safety of the Participants included in the study.

12.6 Protocol Adherence

It is the responsibility of the PIs to apply due diligence to avoid protocol deviations. The Sponsor may be consulted if protocol deviations are identified, to determine the best course of action to protect Participant safety and maintain the integrity of the study data. All protocol deviations will be recorded in the Participants eCRF and reported in the clinical study report.

13 Confidentiality

13.1 Data

All information regarding the nature of the proposed investigation provided by the Sponsor or its designee to the PI/Sub-Investigator (with the exception of information required by law or regulations to be disclosed to the IRB/IEC, the Participant, or the appropriate regulatory authority) must be kept in confidence by the PI/Sub-Investigator in accordance with all applicable laws and regulations as specified in the Clinical Trial Agreement.

13.2 Participant Pseudonymity

The pseudonymity of Participants must be maintained. Participants will be identified by an assigned Participant number on CRFs, and other documents submitted to the Sponsor or its designee. Documents that will not be submitted to the Sponsor or its designee and that identify the Participant (e.g., the signed informed consent document) must be maintained in strict confidence by the PI or designee, except to the extent necessary to allow auditing by the appropriate regulatory authority, the Sponsor, or its designee and in accordance with applicable regulatory requirements.

13.3 Participants' compensation / remuneration

Participants will receive compensation for completing the study to cover costs and expenses – details are specified in Section 6 Study Procedures.

14 Study Documentation and Record Keeping

14.1 Protocol amendments

Any modification to the agreed protocol must be approved in writing by both the Sponsor and the Investigator(s). Amendments will be submitted to the IRB/IEC, as required. These procedures must be fulfilled before any modification is put into effect.

14.2 Investigator Site File

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two different separate categories (1) Investigator's Site File (ISF), and (2) source documents.

The ISF will contain the protocol/amendments, CRFs, and Query Forms, IRB/IEC and governmental approval with correspondence, sample informed consent, staff curriculum vitae and authorization forms and other appropriate documents/correspondence etc.

Source documents (usually defined by the project in advance to record key efficacy/safety parameters independent of the CRFs) would include Participants' records, progress notes, appointment book, original laboratory reports, ECG, EEG, X-ray, pathology, and special assessment reports, signed informed consent forms, consultant letters, and Participant screening and enrolment logs.

The investigator should retain the study documents of the ISF for the retention time specified in the contract with the Sponsor.

Should the investigator wish to assign the study records to another party or move them to another location, the Sponsor must be notified in advance.

If the investigator cannot guarantee this archiving requirement at the study site for any or all of the documents, special arrangements must be made between the investigator and the Sponsor to store these in a sealed container(s) outside of the site so that they can be returned sealed to the investigator in case of a regulatory audit. Where source documents are required for the continued care of the Participant, appropriate copies should be made for storing outside the site.

14.2.1 Source document and source data verification

According to the standards of the data protection law, all data obtained in the course of a clinical study must be treated with discretion in order to guarantee the rights of the Participant's privacy. The investigator should agree to allow the monitor/auditor/inspector to have access to any or all the clinical study materials needed for source data verification and proper review of the clinical study progress.

15 Monitoring

It is understood that the responsible monitor will contact and visit the clinical site regularly and will be allowed, on request, to review the various records of the study (CRFs/eCRFs and other pertinent data) provided that Participant confidentiality is maintained in accordance with local requirements and as specified in the contract.

The Principal Investigator or designee at the site of the investigation will be instructed by the monitor to ensure that the following responsibilities and tasks are carried out:

- Monitoring the study supplies and, if requested, returning all undispensed supplies to the Sponsor at completion of the study.
- Maintaining all records of the study.
- Checking source documents for legibility and completion at the time they are received from the clinical research team. After review of study data with Data Management, the PM, and clinical study team will ensure completeness of the CRF/eCRF.
- Contacting Participants to remind them of their scheduled visits and obtaining a final disposition for every Participant who is entered in the study.

- Checking for reasonableness and completeness of source documents and of recorded CRF data before CRFs are reviewed by a Sponsor monitor at each routine monitoring visit.

It will be the monitor's responsibility to review the study documents (e.g., CRFs) at regular intervals throughout the study, to verify the adherence to the protocol and the legibility, completeness, consistency, and accuracy of the data being entered on them. The monitor must have access to laboratory test reports and other Participant records needed to verify the entries on the CRF/eCRF. This source data verification may be carried out remotely.

The investigator (or his/her deputy) facilitates the monitoring tasks including the source document verification and agrees to cooperate with the monitor to ensure that any issues detected in the course of these monitoring visits are resolved.

15.1 Quality assurance and quality control

All SP 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 clinical study or after its completion (see Archiving).

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

16 Final Study Report

When all completed CRFs have been collected and data have been analyzed, the results of this clinical study are to be documented in a Final Study Report as specified in the contract.

17 Archiving

The study documents will be retained for seven years after the completion of a clinical study. A clinical study is completed with the acceptance of the Final Study Report.

The Trial Master File (TMF) shall be retained for a longer period, where so required by other applicable legal or regulatory requirements and/or as specified in the contract between the Sponsor and the PI/Sub-Investigator.

18 References

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4.	Kondo J, Xiao JZ, Shirahata A, Baba M, Abe A, Ogawa K, Shimoda T. Modulatory effects of *Bifidobacterium longum* B. LONGUM on defecation in elderly patients receiving enteral feeding. World J Gastroenterol. 2013;19(14):2162-70. doi: 10.3748/wjg.v19.i14.2162. PMID: 23599642; PMCID: PMC3627880.