Title:

Survival of the Probiotic *Lacticaseibacillus paracasei* strain Shirota (LcS) in the Gastrointestinal Tract of Generally Healthy Adults

Protocol BIO-2208

Sponsor:

Yakult

Trial Managed by:

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CONFIDENTIAL

CLINICAL INVESTIGATOR
PROTOCOL SIGNATURE SHEET

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By my signature below, I attest that I have read, understood, and agree to abide by all conditions, instructions, and restrictions contained in this protocol (including appendices). I will not initiate this study without approval from the appropriate Institutional Review Board (IRB) and I understand that any changes to the protocol must be approved in writing by the Sponsor and the IRB before they can be implemented, except where necessary to eliminate immediate hazards to the subject.

Approval Signatures:

D. Beckman MD. 7/18/22

Clinical Investigator Signature **Date**

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By my signature below, I approve of this protocol.

Sponsor Company: _____ 7/16/2022

Signature Date

Yutaka Misumi
President & CEO

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1. Table of Contents

2. Abbreviations and Definitions

2.1 Abbreviations

AE	adverse event
ANOVA	analysis of variance
BMI	body mass index
C	Celsius
CFR	Code of Federal Regulations
CFU	colony forming unit
d	day(s)
DDC	direct data capture
DNA	deoxyribonucleic acid
eCRF	electronic case report file
FDA	Food and Drug Administration
g	gram(s)
GCP	Good Clinical Practice
GI	gastrointestinal
GRAS	generally recognized as safe
h	hour(s)
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Conference on Harmonization
IRB	Institutional Review Board
ITT	intent-to-treat
kg	kilogram
LcS	*Lacticaseibacillus paracasei* strain Shirota
LOD	limit of detection
mg	milligram
mL	milliliter
mm Hg	millimeter mercury
mo	month(s)
NSAID	non-steroidal anti-inflammatory drugs
OTC	over the counter
oz	ounce
PCR	polymerase chain reaction
PP	per protocol
RNA	ribonucleic acid
SAE	Serious Adverse Event
SD	standard deviation
SEM	standard error to the mean
SMP	specimen management plan
SOP	Standard Operating Procedures
wk	week(s)
y	year(s)

2.2 Definitions

Clinical Investigator	The person who is responsible for the conduct of the study at a clinic study site. If a study is conducted by a team of individuals at a clinic site, the Clinical Investigator is the responsible leader of the clinical study team and may be called a Principal or Co-Principal Investigator.
ITT	The principle that asserts that the effect of a treatment or intervention can be best assessed by evaluating the data on the basis of the intention to treat a subject rather than the actual treatment group. All subjects that are allocated to a treatment group should be followed-up, assessed and analyzed as members of that group irrespective of their compliance to the planned course of treatment.
PP	The set of data generated by the subset of subjects who complied with the protocol sufficiently to ensure that these data would be likely to exhibit the effects of treatment, according to the underlying scientific model.
Study Clinical Staff	The persons who are appointed by the Clinical Investigator to give support in conducting the study (e.g., cadre).
Study Team	All persons who are involved in the study, which may consist of the Study Clinical team, Principal Scientific Investigators, Project Manager, Statisticians, Data Managers, Monitors, and other support personnel.

3. Synopsis

STUDY TITLE	Survival of the Probiotic *Lacticaseibacillus paracasei* strain Shirota (LcS) in the Gastrointestinal Tract of Generally Healthy Adults
STUDY NUMBER	BIO-2208
SPONSOR	Yakult U.S.A. Inc. 17235 Newhope St. Fountain Valley, CA 92708
SPONSOR LEAD	Hideyuki Shibata Vice President for Science
STUDY SITE	Biofortis Innovation Services 800 S. Rohlwing Road, Suite A Addison, IL 60101
RESEARCH OBJECTIVE	The objective of this study is to investigate the survival of LcS in the human gastrointestinal (GI) tract after consumption of fermented milk.
STUDY PRODUCT	Fermented milk containing 8 billion LcS per 80 mL (10^8 CFU/mL) produced by Yakult. Participants will consume one bottle of study product per day within 30 minutes after breakfast for 14 days.
STUDY DESIGN AND DURATION	This study is a single-arm, open-label study with a 14-d run-in (baseline), 14-d consumption period, and 14-d follow-up. Participants will maintain habitual dietary and lifestyle practices with the exception of avoiding fermented foods and beverages throughout the 42-d trial. The number of live bacteria (by count of LcS) in fecal samples will be assessed after 14 d consumption of a fermented milk product containing 8 billion live bacteria per 80 mL milk product (10^8 CFU/mL). Participants will complete a Daily Diary on compliance with study instructions and changes in medications/supplements during the 14-d run-in. Collection of the baseline fecal sample will occur between 7 pm the night before and morning of Visit 2 (Day 14). Participants will then begin the 14-d study product ingestion period. One serving of study product will be consumed within 30 min after breakfast every day. Fecal samples will be collected at

	Day 21 (Visit 3) and Day 28 (Visit 4). Participants will continue with the Daily Diary, with inclusion of a daily product consumption log for compliance with study product during the ingestion period. Non-compliance will be defined as consuming less than 12 servings (missing 2 or more days) of study product consumption between Visits 2 and 4 (Days 14 and 28), or not consuming the product the day before the stool collection. At Visit 4 (Day 28), participants will enter a 14-d follow-up period, continuing with the Daily Diary and maintaining habitual dietary and lifestyle patterns, with continued exclusion of fermented products. Participants will provide a final fecal sample at Visit 5 (Day 42), collected between 7 pm the night before and morning of the visit, and then be discharged from the study. Adverse events (AEs) will be collected throughout the study and participants will be instructed to contact study clinic staff with any illness or AE occurring between visits.
STUDY POPULATION	Participants will be generally healthy adults, 18 to 40 years of age (inclusive), with a body mass index (BMI) $\geq$18.5 and $\leq$29.9 kg/m^2 and regular bowel habits including consistent bowel movement per day, preferably in the morning. A sample size of N=25 enrolled participants was determined based on previous similar studies. No participants will be replaced in the event of early terminations.
MAIN OUTCOMES	**Primary Outcome Variable** The primary outcome variable will be the change in live LcS numbers (CFU/g feces) from baseline (Visit 2; Day 14) to the end of the ingestion period (Visit 4; Day 28). **Secondary Outcome Variables** • Change in LcS number from baseline (Visit 2; Day 14) to during the ingestion period (Visit 3; Day 21). • Change in LcS from the end of the ingestion period (Visit 4; Day 28) and to the end of follow-up (Visit 5; Day 42) **Safety Outcomes** Safety will be assessed by: • Overall incidence of product-emergent AEs • Number, types and duration of Serious Adverse Events (SAEs)

STATISTICAL ANALYSIS	The safety analysis will be conducted based on the modified Intention-to-Treat (ITT) population defined as all participants who receive at least one serving of the study product. The efficacy analysis will be conducted on the Per Protocol (PP) population, defined as all participants who completed the intervention in compliance. The LcS number (CFU/g feces) will be evaluated with a repeated measures model. The LcS number measured at each time point will be log-transformed and included in the response vector. The absolute change from baseline to each measured time point will be estimated along with the corresponding 95% confidence interval (CI). Non-detectable levels will be replaced by zero. In the event model assumptions are violated (i.e., constant variance and/or normality of residuals), a rank-based transformation will be considered.
STUDY PERIOD	Baseline/ run-in period (between Visit 1 and Visit 2): 14 days Study product consumption period (Visit 2-Visit 4): 14 days Follow-up period (between Visit 4 and Visit 5): 14 days

4. Background/Rationale

The human gut microbiota contains an extraordinary complex variety of metabolically active bacteria. Microbial composition of fecal and mucosal samples using 16S ribosomal DNA and RNA have estimated as high as 1,800 genera and between 15,000-36,000 individual species. The total microbial load of the intestine (10^{13}-10^{14} microorganisms) collectively contains at least 100 times as many genes as the human genome. The concentration and complexity of these bacteria increase from the proximal gastric and duodenal population of 10^{2}-10^{3} bacteria/g luminal contents to 10^{11}-10^{12} bacteria /g of the cecum and colon contents [1-3] More than 99% of the microbiota in the human gastrointestinal (GI) tract are from four bacteria divisions: Firmicutes (64%), Bacteroidetes (23%), Proteobacteria (8%) and Actinobacter (~5%). These bacteria intimately interact with the host epithelial cells and normally do not have any acute adverse effects. On the other hand, some of them have been shown to be necessary for maintaining the well-being of their host while the others are pathogenic. The appropriate composition of gut microbiota is generally maintained and plays an important role for its beneficial effects.[3-5] The development of the gut microbiota begins at birth with rapid population occurring over the first months of life. The gut microbiota composition can differ significantly among individuals; however, within an individual, the gut microbiome composition is comparatively simple during early infancy, becoming complex after cessation of breastfeeding and remaining relatively stable during adulthood.[6, 7]

Probiotics are defined as live microorganisms that confer a health benefit on the host when administered in an adequate amount.[8] Probiotics consist of living yeast or bacteria and may contain a single microorganism or a mixture of several species. The term "probiotic" was first used in the 1960s and is derived from the Greek word meaning "*for life*". In the U.S., probiotics are generally sold as dietary supplements or foods, with the main probiotic products developed from two gram-positive genera: *Lactobacillus* and *Bifidobacterium*. Recently, due to the number of strains and substrains, the *Lactobacillus* genus has been reclassified into 25 genera including *Lacticaseibacillus*.[9] Probiotic products sold as dietary supplements are available as capsules, tablets, and sachets of packets in powder form. Foods that deliver probiotics are primarily fermented foods, such as yogurts and dairy-based milk products.[5, 10]

4.1 Summary of Potential Benefits of Probiotics

Probiotics exert their beneficial effects mainly through their interaction with the gut. The main outcomes observed with probiotics include lowering intestinal pH by organic acids, decreasing colonization and invasion of pathogenic bacteria, and modifying the host immune response.[5, 8, 11, 12] Understanding how probiotics exert their effects are still under investigation, however, it is known that the effects from probiotics tend to be specific to a particular strain. Therefore, the benefit of one strain should not be generalized to another strain.[5, 8, 13]

Lacticaseibacillus paracasei strain Shirota (LcS) is one of the most studied probiotics with clinical data suggesting it could improve abdominal symptoms and support immune system function. For example Koebnick *et al.* found that intake of LcS significantly reduced the incidence of hard or lumpy stools and reduced the severity of constipation in

patients with chronic constipation.[14] Matsumoto *et al.* showed that by ingesting LcS the frequency of defecation increased, stools were softer, and the number of indigenous bifidobacteria in the stools increased.[15] Sur D *et al.* reported that daily intake of LcS can play a role in prevention of acute diarrhea in Indian young children.[16] With respect to immune support, Gleeson M *at al.* found LcS decreased the frequency of upper respiratory tract infections in healthy athletes.[17]

4.2 Summary of Risks of Probiotics and LcS

Probiotic has been consumed by humans in one form or another for over 100 years with a generally good safety record. No known or potential risks are expected from the use of probiotics. Specifically, no pathogenic or virulence properties have been found for lactobacilli, bifidobacteria or lactococci.[9, 18-20] There is a concern over the use of bacteria due to possible transmission of drug resistance genes, which may be related to chromosomal, transposon, or plasmid located genes.[8] In certain conditions, some lactobacilli strains have been associated with rare cases of bacteremia, endocarditis, and sepsis; however, a recent epidemiological study of systematically collected lactobacilli bacteremia case reports in one country has shown that there were no increased incidences of frequency of bacteremia with the increased usage of probiotic lactobacilli.[13, 19, 21-23] Although generally considered safe and well tolerated, the most common adverse effects are the GI symptoms of bloating and flatulence, which are typically mild and subside with continued use of the probiotic. [13, 24]

Specific to this study, milk fermented by LcS has been consumed for more than 85 years and is sold in 40 countries/area around the world. LcS has Generally Recognized As Safe (GRAS) documentation and notification has been made to the U. S. Food and Drug Administration (FDA) with a response of no questions from the Agency (GRAS No. 429, Dec. 10, 2012).[25] The product is also available as a commercial product sold under the brand name Yakult.

4.3 Rationale of the Study

Probiotics are being used with increasing frequency in medicine and by the general population given the increasing evidence on the beneficial effects on human health. The probiotic efficacy relies on their ability to survive in the digestive system and then to proliferate in the gut. Factors related to survivability in the GI tract include the probiotic strain, as well as other intrinsic and extrinsic factors, and the ability to survive in the GI tract varies considerably among lactobacilli species. [26, 27] Ingested bacteria are exposed to adverse conditions starting as soon as they reach the stomach, with survival influenced by the time required to leave the stomach. The gastric emptying rate, therefore, is an important feature for the survival of bacteria. Factors such as type and composition of food consumed, lifestyle age, environment and race are known to influence the gastric emptying rate and, thus, influence the survival of the probiotic bacteria. The small intestine, especially its proximal part, contains hydrolytic enzymes and bile salts known to have a lethal effect on microorganisms. Thus, ability to pass easily through this compartment of the GI tract may also substantially affect the survival of ingested bacteria.[28-30]

The aim of this study is to investigate the survival of LcS in the intestine of generally healthy adults after intake of fermented milk (Yakult). Similar studies have been conducted by other investigators. For example, clinical studies conducted in Japan,[27] Vietnam,[31] China,[32] and the United Kingdom[33] have reported on the survival of LcS in the intestine in healthy adults. The study described in this proposal is intended to investigate whether the survival of LcS is the same or not in generally healthy adults in the U.S., who may have different ethnicities (e.g., genetic factors), as well as lifestyles, food habits, and environmental influences compared to these previous studies.

4.4 Rationale for Proposed LcS Serving Amount

There is no consensus regarding the minimum number of microorganisms that must be ingested to obtain a beneficial effect. Probiotic dosing varies depending on the product and specific indication. Typically, a probiotic contains several billion microorganisms (1-20 billion CFU).[7] A study conducted by Yuki *et al* showed that the consumption of 10 billion LcS for 3 days resulted in a mean live bacterial count of 10^7 LcS/g feces.[27] Other studies in Vietnamese and Chinese subjects have also shown the ability to detect 10^7 LcS/g feces after consumption of a fermented dairy beverage delivering 6.5 or 10 billion LcS for 14 days, respectively.[31, 32] The test product for this study is a fermented milk containing 8 billion LcS per 80 mL (10^8 CFU/mL), which will be produced by the Yakult U.S.A. Inc.

5. Objective

The objective of this study is to investigate the survival of LcS in the human GI tract after consumption of fermented milk.

6. Subjects

Subjects will be generally healthy adults, 18 to 40 years of age (inclusive), with a body mass index (BMI) $\geq$18.5 and $\leq$29.9 kg/m^2.

7. Study Design and Procedures

7.1 Study Product

The study product is a fermented milk containing 8 billion LcS per 80 ml (10^8 CFU/mL) produced by Yakult U.S.A. Inc.

7.2 Study Design

The study is a single-arm, open-label study with one screening visit (Visit 1; Day 0), one baseline visit (Visit 2; Day 14), two intervention visits (Visits 3 and 4; Days 21 and 28), and one post-intervention follow-up visit (Visit 5; Day 42).

After signing the informed consent, participants will be screened for eligibility. Screening will include collection of demographic, concomitant medication, and medical history information, as well as clinic procedures [measurement of height (Visit 1 only), body weight, BMI calculation (Visit 1 only), vital signs (heart rate and blood pressure), and where appropriate, last menses query]. All women will be required to take an in-clinic urine pregnancy test.

After review of inclusion/ exclusion criteria, eligible participants will then be enrolled in the study and start a 14-d run-in period. Eligible participant will be instructed to continue with their habitual diets and lifestyle patterns, with exception of excluding fermented dairy and non-dairy products (**Appendix 3**). A Stool Collection Demonstration will be conducted and participants will be provided a stool collection kit, along with ice packs, an insulated cooler and temperature measurement device as described in the Specimen Management Plan (SMP; **Appendix 4**). Participants will be instructed to collect a stool sample sometime after 7 pm the day before and prior to their next visit (Visit 2; Day 14). Participants will also be instructed on the electronic Daily Diary (**Appendix 5**), which asks compliance with study instructions to avoid fermented products as well as questions on concomitant medication intake.

Visit 2 (Day 14) is the end of the run-in and start of the ingestion period. At Visit 2, participants will arrive in the morning, undergo clinic procedures (measurement of body weight, vital signs (heart rate and blood pressure), last menses query where appropriate, and review of inclusion/exclusion criteria for potential protocol deviations). Stool samples will be collected (Day 14 samples), and the electronic Daily Diaries reviewed as appropriate. Additionally, participants will be queried about following the study instructions provided at Visit 1. The first serving of study product will be consumed during the clinic visit after participants have consumed breakfast (at home) and obtained and dropped off their stool samples. Participants will then be dispensed two 5-packs of study product for at-home daily consumption until the next visit (Visit 3; Day 21) and instructed to consume one bottle of the study product within the 30 minutes following breakfast, and to keep all dispensed product refrigerated prior to consumption. A new stool collection kit with fecal sample storage materials (i.e., ice pack, insulated cooler, temperature measurement device) will be provided and participants instructed to collect a stool sample sometime after 7 pm the day before and prior to their next visit (Visit 3; Day 21). Participants will be reminded to maintain their habitual dietary and lifestyle patterns and refrain from consumption of any other fermented dairy and non-dairy products, consume their study product daily, and complete their electronic Daily Diaries every day. The Daily Diary will contain the same questions as during the run-in plus queries on daily product consumption, which will then be used for compliance assessment.

Adverse events (AE) will be assessed by open-ended question at the beginning and end of Visit 2 (Day 14) and at the beginning of Visits 3, 4, and 5 (Days 21, 28, and 42, respectively). Participants will be counseled to contact the clinic with concerns or discomforts.

If participants miss consuming study product in the morning, they should be counseled to consume the product as soon as possible during that day; however, participants should not consume more than one serving of product per day. Therefore, if they miss

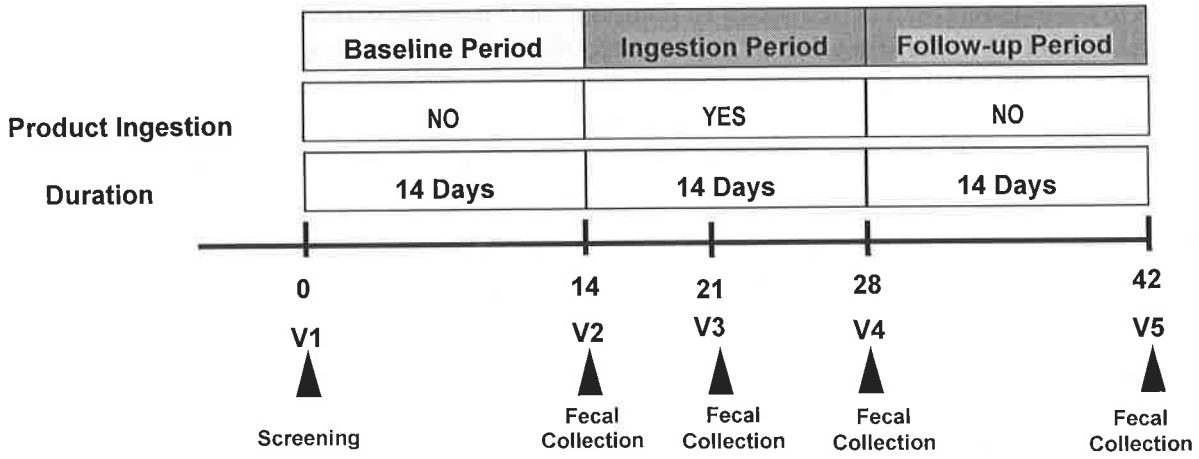
consuming the product during the entire day, they should document the lack of study product consumption and continue consuming one serving per day.

Following the first seven days of the ingestion period, participants will return for an interim visit (Visit 3; Day 21) to drop off their stool sample (Day 21 sample) and to obtain a new stool collection kit. Participants will be instructed to collect another stool sample sometime after 7 pm the day before and prior to their next visit (Visit 4; Day 28). The electronic Daily Diary will be reviewed, including product consumption compliance, and participants will be queried about following the study instructions. AEs will be assessed. Participants will return unused study product and be dispensed two new 5-packs of study product to continue at-home daily consumption, with reminder to consume one serving per day until the next visit. Participants will also be reminded to continue to maintain habitual dietary and lifestyle patterns while avoiding fermented dairy and non-dairy products, as well as to complete their electronic Daily Diaries every day.

At the end of the ingestion period, participants will return for Visit 4 (Day 28) to drop off their stool sample (Day 28 sample) and return unused study product. Clinic procedures will be performed, AEs assessed, and electronic Daily Diaries and compliance will be reviewed. At this point, participants will be instructed that the will no longer need to consume study product and will enter the follow-up period. Participants will be dispensed a new stool collection kit and instructed to collect a final stool sample sometime after 7 pm the day before and prior to their next visit (Visit 5; Day 42). Participants will also be reminded to continue to maintain habitual dietary and lifestyle patterns while avoiding fermented dairy and non-dairy products as well as to complete their electronic Daily Diaries every day.

Participants will return for their final visit (Visit 5; Day 42) after collection of their follow-up stool sample (Day 42 sample). Participants will drop off their final stool sample. Clinic visit procedures will be conducted, AEs assessed, and participants will be queried about following study instructions. Electronic Daily Diaries will be reviewed as appropriate. Participants will then be discharged from the study.

7.2.1 Figure 1. Study Design



7.2.2 Flowchart (n=25)

	Screening/ Start Run-in	End Run-in/ Start Study Product	Interim Study Visit	End Product/ Start Follow-up	End Follow-up
Visit [1]	**1**	**2**	**3**	**4**	**5**
Days	**0**	**14**	**21**	**28**	**42**
Informed Consent & HIPAA [2]	X				
Clinic Visit [3]	X	X		X	X
Medical History & Screening Questionnaires	X				
In-clinic Urine Pregnancy Test [4]	X				
Study Instructions & Query [5]	X	X	X	X	X
Review Daily Diary [6]	X	X	X	X	X
Dispense Study Product [7]		X	X		
Stool Collection Demonstration	X				
Dispense Fecal Collection Kit [8]	X	X	X	X	
Fecal Collection Kit Returned [8]		X	X	X	X
Compliance [9]			X	X	
Adverse Events [10]		X	X	X	X

Footnotes:

[1] A window of +1 d will be allowed for Visits 2, 3, 4, and 5 anchored to Visit 1, Day 0.

[2] Health Insurance Portability and Accountability Act (HIPAA). Signed document authorizes the use and disclosure of the subject's Protected Health Information by the Clinical Investigator and by those persons who need that information for the purposes of the study.

[3] Clinic visit procedures include measurement of height (Visit 1 only), body weight, BMI calculation (Visit 1 only), vital signs (heart rate and blood pressure), and where appropriate, a review of inclusion/exclusion criteria [for eligibility (Visit 1) and for potential protocol deviations at subsequent visits], concomitant medication/supplement use, and last menses query.

[4] To be completed on all women.

[5] Study instructions will be provided: maintain usual dietary and lifestyle patterns, with exception of avoiding fermented products (**Appendix 3**); completing the electronic Daily Diary.

[6] Participants will be instructed on completing an electronic Daily Diary (**Appendix 5**) at Visit 1. The Daily Diary will ask about illness and GI symptoms, consumption of fermented dairy products, and concomitant medications throughout the study, and include questions on compliance with the daily study product consumption between Visits 2 and 4. At Visits 2, 3, 4, and 5, participants will be queried about following study instructions and Daily Diaries will be reviewed, as appropriate.

[7] Study product will be dispensed. Participants will be instructed to store the product in the refrigerator. The first serving of study product will occur in the clinic during Visits 2, to be consumed within 1 h after consuming breakfast. Participants will be instructed to consume the rest of the products at one serving per day within 30 min after breakfast for the 14-day intervention period.

[8] Stool collection kit, including the fecal sample storage materials (ice pack, insulated cooler, temperature measuring device), will be provided for participants with instructions to collect stool samples at home

sometime after 7 pm the night before and prior to the subsequent visit, as described in the Specimen Management Plan (**SMP; Appendix 4).** The baseline fecal sample (Day 14), collected at Visit 2, should be collected before ingestion of the study product.

[9] Compliance will be determined by review of the electronic Daily Diary. Participants who do not drink the study product for 2 days or more during the ingestion period (Visits 2-4; Days 14-28) will be considered non-compliant. Unused study products will be collected at Visits 3 and 4; Days 21 and 28, respectively.

[10] AE inquiry will occur with an open-ended question at the beginning and end of Visit 2, and at the beginning of Visits 3, 4, and 5.

8. Study Sample

Each participant must meet all of the following inclusion criteria and none of the exclusion criteria in order to participate in this study.

8.1 Inclusion Criteria

1. Healthy men and women between 18-40 years old, inclusive at Visit 1.
2. BMI between $\geq$18.5 to $\leq$29.9 kg/m^2.
3. Regular bowel habits, by self-report, including consistently having a bowel movement every day, preferably in the morning.
4. Regular breakfast consumer by self-report.
5. Willing to consume the study product per the protocol instructions throughout the study intervention period (14 d).
6. Willing to maintain habitual dietary, lifestyle, and physical activity (with exceptions per study instructions) throughout the trial and to refrain from exclusionary medications, supplements, and products throughout the study.
7. Willing to limit alcohol consumption to $\leq$3 standard drinks/d and $\leq$7 standard drinks/wk throughout the trial.
8. Non-user of tobacco products or former user of any tobacco product (not used within 6 months) and has no plan to change nicotine habits during the study period. Tobacco products include tobacco, smoking products (including, but not limited to cigarettes, cigars, chewing tobacco, e-cigarettes), and nicotine products (e.g., nicotine gum and/or nicotine patches) within 6 months of Visit 1 (Day 0) and during the study period.
9. Non-user or former user of any marijuana or hemp products (not used within 6 months) of Visit 1 (Day 0) and during the study period and has no plans to use marijuana or hemp products during the study period. No washout is required for topical marijuana or hemp products, but subjects are required to abstain from these products during the study period.
10. No health conditions that would prevent him/her from fulfilling the study requirements as judged by the Clinical Investigator on the basis of medical history.

11. Understands the study procedures and signs forms providing informed consent to participate in the study and authorizes the release of relevant protected health information to the Clinical Investigator.

8.2 Exclusion Criteria

1. Any known food allergies, intolerances or sensitivities to dairy or to any of the study product ingredients (**Appendix 2**).
2. Presence of a clinically important GI condition that would potentially interfere with the evaluation of the study product (e.g., inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), gastric reflux, indigestion, dyspepsia, Crohn's disease, celiac disease, history of surgery for weight loss, gastroparesis, and clinically significant lactose or gluten intolerance or other food or ingredient allergies). IBS will be determined as recurrent abdominal pain or discomfort at least 3 d/mo in the last 3 mo associated with: (a) improvement with defecation, (b) onset associated with change in frequency of stool, and (c) onset associated with a change in form (appearance of stool).
3. Self-reported history (within 6 wks) or presence of functional constipation or diarrhea as defined by the Rome IV criteria and at the discretion of the Clinical Investigator.
   - Diarrhea is defined as loose or watery stools, without predominant abdominal pain or bothersome bloating, occurring in more than 25% of stools.
   - Constipation is defined as two or more of the following: (a) straining during more than ¼ (25%) of defecations; (b) lumpy or hard stools (Bristol Stool Form Scale 1-2) more than ¼ (25%) of defecations; (c) sensation of incomplete evacuation more than ¼ (25%) of defecations; (d) sensation of anorectal obstruction/blockage more than ¼ (25%) of defecations; (e) manual maneuvers to facilitate more than ¼ (25%) of defecations (e.g., digital evacuation, support of the pelvic floor); (f) fewer than 3 single bowel movements/ per week; (g) loose stools are rarely present without the use of laxatives.
4. Self-reported history (within 6 wks) or presence of abdominal pain, defined as continuous or nearly continuous pain in the abdominal area in which (a) no or only occasional relationship with physiological events (e.g., eating, defecation, menses), (b) some loss of daily functioning (pain limits activity at least some of the time), (c) the pain is not feigned, (d) the pain is not related to another GI disorder (e.g., epigastric pain syndrome, irritable bowel syndrome, anorectal pain).
5. Uncontrolled and/or clinically important pulmonary (including uncontrolled asthma), hepatic, renal (except history of kidney stones in participants who are symptom free for 6 months), cardiac (including, but not limited to, atherosclerotic disease, history of myocardial infarction, peripheral arterial disease, stroke), endocrine (including Type 1 and Type 2 diabetes mellitus), hematologic, immunologic, neurologic (such as Alzheimer's or Parkinson's disease), psychiatric (including depression and/or anxiety disorders) or biliary condition(s). Conditions which are well-controlled or resolved will be assessed by the Clinical Investigator on a case-by-case basis.

6. Uncontrolled hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg) as defined by the blood pressure measured at Visit 1 (Day 0). One re-test may be allowed on a separate day prior, with repeating of Visit 1, for subjects whose blood pressure exceeds either of these cut points at Visit 1 (Day 0), in the judgment of the Clinical Investigator. If taken, the repeat blood pressure measurement will be used to determine eligibility. Stable use of hypertension medication is allowed [defined as no change in medication regimen within 90 d of Visit 1 (Day 0)].
7. Weight loss or gain > 4.5 kg within 90 d of Visit 1 (Day 0), or currently or planning to be on a weight loss regimen or muscle-building/strengthening program during the study.
8. Signs or symptoms of an active infection of clinical relevance within 5 d of Visit 1 (Day 0). The visit may be rescheduled such that all signs and symptoms have resolved (at the discretion of the Clinical Investigator) at least 5 d prior to Visit 1 (Day 0).
9. Major trauma or any other surgical event within 90 d of Visit 1 (Day 0).
10. History or presence of cancer in the prior 2 y, except for non-melanoma skin cancer.
11. Use of proton pump inhibitors, H2 receptor antagonists, anticoagulants (with the exception of 81 mg aspirin), corticosteroids, antibiotics, antifungals, antiparasitics, antidiarrheals, laxatives, or regular (> 3 d/wk) use of NSAIDs within 30 d of Visit 1 (Day 0).
12. Exposure to any non-registered drug product within 30 days prior to Visit 1 (Day 0).
13. Subject is a female who is pregnant, planning to be pregnant during the study period, lactating, or is of childbearing potential and is unwilling to commit to the use of a medically approved form of contraception during the study period. The method of contraception must be recorded.
14. Recent history (within 12 mo of screening; Visit 1; Day 0) of alcohol or substance abuse. Alcohol abuse is defined as >14 drinks/wk (1 drink = 12 oz beer, 5 oz wine, or 1½ oz distilled spirits).
15. Recent (within 2 mo) participation in any other clinical study prior to Visit 1 (Day 0).
16. Has a condition the Clinical Investigator believes would interfere with his/her ability to provide informed consent, comply with the study protocol, which might confound the interpretation of the study results, or put the subject at undue risk.

8.3 Excluded Medications/Products and Foods

- Unstable use of hypertension medication [unstable use is defined as a change in medication regimen within 90 d of Visit 1 (Day 0)] and during the study.
- Tobacco, smoking products (including, but not limited to cigarettes, cigars, chewing tobacco, e-cigarettes), and nicotine products (e.g., nicotine gum and/or nicotine patches) within 6 mo of Visit 1 (Day 0) and during the study period.

- Marijuana or hemp products within 6 mo of Visit 1 (Day 0) and during the study period.
- Proton pump inhibitors, H2 receptor antagonists, anticoagulants (with exception of 81 mg aspirin), corticosteroids, antibiotics, antifungals, antiparasitics antidiarrheals, or laxatives within 30 days of Visit 1 (Day 0) and during the study.
- Regular (>3 d/wk) use of NSAIDs within 30 d of Visit 1 (Day 0) and during the study.
- Non-registered drug product within 30 d prior to Visit 1 (Day 0) and during the study.
- Fermented dairy and non-dairy food or beverage products (see **Appendix 3**)

A list of exclusionary medications/products/foods can be found in **Appendix 1.**

8.4 Randomization

Not applicable

9. Study Products

9.1 Description

The study product is a fermented milk containing 8 billion LcS per 80 mL (10^8 CFU/mL) produced by the Yakult U.S.A. Inc. The study product is also available as commercial product with brand name Yakult. Study products will be ready-to-use and provided in individual serving containers, with 5 individual servings per package of product. Participants will be provided two 5-packs of study product at Visits 3 and 4.

Study products are for oral use only. Participants will be instructed to drink one bottle of study product every day for 14 consecutive days (Visits 2-4; Days 14 to 27). The study product is to be consumed within 30 min after consuming breakfast on all days, with the exception of the first day of consumption (during Visit 2; Day 14), where the study product will be consumed in the clinic, after the stool sample has been obtained. Participants should have consumed breakfast before attending the study visit (Visit 2; Day 14).

9.2 Labeling and Packaging

Bulk study products will be labelled in accordance with applicable laws and regulations. Study products will be over-labeled with the protocol number, product identification, expiration date, batch or lot number, and the statement "For Investigational Use Only" by Biofortis

Subjects will be instructed to maintain study product under refrigerated conditions (<10°C).

9.3 Shipment and Storage of Bulk Study Products

Shipments of products will include a letter of receipt, and upon delivery, all products will be checked for completeness and possible damage, with a confirmation of receipt sent to the Sponsor.

The study product will be stored under refrigerated conditions (<10°C). Study product disposition and return will be recorded on an accountability log. Study product supplies are to be used only in accordance with this protocol and under the supervision of the Clinical Investigator. All records must be available for inspection by the Sponsor and are subject to regulatory agency inspection at any time. A written explanation from the study staff will be required for any missing study product.

At the end of the study, all products will either be destroyed on site with adequate documentation or returned to Sponsor for destruction.

9.4 Access to Randomization Code

Not applicable

10. Clinical Measurements

10.1 Clinic Visit Procedures

Clinic visit procedures include measurement of height (Visit 1; Day 0 only), body weight (Visits 1, 2, 4 and 5), calculation of BMI (Visit 1; Day 0 only), vital signs measurements (see below), and a review of inclusion/exclusion criteria, and last menses query (as applicable). Concomitant medication/ supplement use will also be collected at Visit 1 (Day 0) and changes obtained on the electronic Daily Diary, with queries at clinic visits, as appropriate.

Standardized vital signs measurements will be assessed at each clinic visit and will include heart rate and resting blood pressure measured using an automated blood pressure measurement device. Blood pressure will be obtained after the subject has been sitting for at least five min. Systolic and diastolic pressures will be measured once using an appropriately sized cuff (bladder within the cuff must encircle ≥80% of the arm). Clinic staff may take a repeat measurement, if warranted; the second measurement will be recorded in the eCRF.

10.2 Laboratory Measurements

No blood or urine clinical laboratory measurements are included in this study, with exception of an in-clinic pregnancy test.

10.3 Study Instructions/Query

The following study instructions will be provided prior to each visit as indicated. A query on the compliance to these instructions will be performed at each visit.

- Maintain usual body weight

- Maintain habitual dietary and lifestyle patterns
- Abstain from fermented dairy and non-dairy products (foods, beverages, and supplements)

10.4 Daily Diary

Participants will be counseled to complete an electronic Daily Diary (**Appendix 5**) which will assess compliance with instructions to avoid fermented dairy and non-dairy foods and beverages and changes to concomitant medications/supplements. The Daily Diary will also assess compliance with study product consumption from Visit 2 (Day 14) to Visit 4 (Day 28). Clinic staff will review the diaries with participants at each visit, as appropriate.

Participants will receive an email each study day with a link to the electronic Daily Diary appropriate to the current phase of the study.

10.5 Stool Collection

Participants will be instructed to provide a stool sample, to be obtained immediately prior to (i.e., collected after 7 pm the day before until the time of clinic visit, as appropriate) Visits 2, 3, 4, and 5 (Days 14, 21, 28, and 42, respectively). Participants will be provided with storage containers for maintaining stool samples at cool temperature until drop off at the clinic, as defined in the SMP (**Appendix 4**). The baseline sample (Sample Day 14) must be obtained prior to the participant consuming the first serving of study product.

10.6 Adverse Events Assessment

Inquiry about AEs will occur with an open-ended question at the beginning and end of Visit 2, and at the end of Visits 3, 4, and 5.

11. Procedures at Each Clinic Visit

Procedures listed are not necessarily performed in the order below.

11.1 Screening/ Start of Run-in (Visit 1; Day 0)

- Informed consent/HIPAA
- Medical history
- Screening demographics
- In-clinic pregnancy test (women only)
- Clinic visit
    - Height
    - Body weight
    - BMI calculation
    - Vital signs
    - Last menses query (as applicable)
    - Assess concomitant medication/supplement use
- Review inclusion/exclusion criteria for study enrollment

- Provide study instructions
    - Maintain habitual dietary and lifestyle patterns
    - Avoid fermented dairy products (foods and beverages)
- Stool Collection Demonstration
- Provide stool collection kit
- Instruct on electronic Daily Diary use

11.2 End of Run-in/ Start Product Consumption (Visit 2; Day 14)

- Clinic visit
    - Body weight
    - Vital signs
    - Assess concomitant medication/supplement use
    - Review inclusion/exclusion criteria for protocol deviations
    - Last menses query (as applicable)
- Study instruction query/review
- Receive baseline fecal collection (Labeled Sample Day 14)
- Provide new stool collection kit
- Review Daily Diary (as appropriate)
- Provide one serving of study product for in-clinic consumption (consume first product in-clinic, to be consumed within 1 h of breakfast)
- Dispense two 5-packs of the refrigerated study product for consumption at home and instruct participants to consume one bottle within 30 min following breakfast every day and return unused product at the next visit
- Assess AEs at the beginning and end of the visit

11.3 Interim Visit (Visit 3; Day 21)

- Drop-off fecal collection (Labeled Sample Day 21)
- Compliance check/ Study instruction query/review
- Receive unused study product
- Provide new stool collection kit
- Review Daily Diary (as appropriate)
- Dispense two 5-packs of the refrigerated study product for consumption at home and instruct participant to consume one bottle within 30 min of breakfast every day and return unused product at the next visit
- Assess AEs at the beginning visit

11.4 End Product Consumption/ Start Follow-up (Visit 4; Day 28)

- Clinic visit
    - Body weight
    - Vital signs
    - Assess concomitant medication/supplement use

    - Review inclusion/exclusion criteria for protocol deviations
    - Last menses query (as applicable)
- Compliance check/ Study instruction query/review
- Receive unused study product
- Receive fecal sample (Labeled Sample Day 28)
- Provide new stool collection kit
- Review Daily Diary (as appropriate)
- Assess AEs at the beginning of the visit

11.5 End of Follow-up (Visit 5; Day 42)

- Clinic visit
    - Body weight
    - Vital signs
    - Assess concomitant medication/supplement use
    - Review inclusion/exclusion criteria for protocol deviations
    - Last menses query (as applicable)
- Study instruction query/ Review Daily Diary (as appropriate)
- Receive fecal collection (Labeled Sample Day 42)
- Assess AEs at the beginning of the visit

11.6 Early Termination Procedures

The term "Early Termination" refers to a subject's non-completion of the study. Should a subject decide to withdraw, all efforts will be made to complete and report observations as thoroughly as possible. In the event that a subject is withdrawn from the study, the reason for the withdrawal and the party who initiated the withdrawal (subject or Clinical Investigator) will be documented. Should the subject decide to withdraw, documentation of early termination and any AEs and concomitant medication use should be recorded.

The primary reason for a subject withdrawing prematurely should be selected from the following standard categories:

Adverse Event – event which results in discontinuation of the study product by the subject or that in the judgment of the Clinical Investigator for the best interest of the subject requires discontinuation of study product (includes all categories of study product relatedness; Not Related, Unlikely, Possibly, Probably, and Definitely).

Death – death of the subject.

Withdrawal of Consent – subject desires to withdraw from further participation in the study in the absence of a medical need to withdraw determined by the Clinical Investigator.

Lost to Follow-Up – subject did not return for one or more follow-up visit(s) following dispensing of study product and could not be contacted thereafter. The reason for withdrawal was unknown and could not be documented.

Other – causes of premature termination from the study other than the above, such as theft or loss of study products, termination of study by Sponsor, etc.

12. Data Analysis and Statistical Methods

12.1 Outcome Variables

12.1.1 12.1.1 Primary Outcome Variable

The primary outcome variable will be the change in LcS number (CFU/g feces) from baseline (Visit 2; Day 14) to the end of the ingestion period (Visit 4; Day 28).

12.1.2 12.1.2 Secondary Outcome Variables

- Change in LcS number (CFU/g feces) from baseline (Visit 2; Day 14) to during the ingestion period (Visit 3; Day 21).
- Change in LcS from the end of the ingestion period (Visit 4; Day 28) and to the end of follow-up (Visit 5; Day 42)

12.1.3 12.1.3 Safety Outcome Variables

- Overall incidence of product-emergent AEs
- Number, types, and duration of Serious Adverse Events (SAEs)

12.2 Sample Size

Sample size was determined based on previous studies, which indicate enrolling a sample size of N=25 will provide sufficient data for a significant effect. [29, 30]

No subjects will be replaced in the event of early terminations.

12.3 Statistical Analysis

Statistical analysis details for this study will be provided in a Statistical Analysis Plan. Briefly, all statistical analyses will be conducted using SAS for Windows (version 9.4, or higher, Cary, NC) and/or R (R Core Team 2022). Analysis populations will include intent-to-treat (ITT; all subjects randomized in the study who have consumed at least one serving of the study product) and per protocol (PP; all subjects completing the study in compliance). Safety outcomes will be evaluated on the ITT population. The PP population will serve as the primary analysis population for efficacy outcomes.

Subjects may be excluded from the PP population for non-compliance, which includes but not limited to:

- Missing consumption of product for two or more days during study product ingestion period
- Not consuming the study product the day before stool collection
- Missing appointments
- Use of prohibited drugs or any products thought to alter the primary outcome variable during the study

- Not adhering to instructions as outlined in the protocol

All decisions regarding subject population and data inclusion will be documented prior to database lock.

12.3.1 12.3.1 Baseline Characteristics

Descriptive statistics [number of subjects, mean, standard deviation (SD), standard error to the mean (SEM), median, interquartile limits, minimum and maximum or frequency counts] will be presented for subject demographics and anthropometric measurements at screening/baseline for all analysis populations.

12.3.2 12.3.2 Outcome Analysis

Bacterial count will be conducted at Institute for Food Safety and Health (IFSH; Bedford Park, IL) using the culture method (FOM-LLV) and PCR approaches, and reported as CFU/g feces. The LcS number will be evaluated with a repeated measures model. The LcS number measured at each time point will be included in the response vector. The absolute change from baseline to each measured time point will be estimated along with the corresponding 95% confidence interval. Non-detectable levels will be replaced by zero. In the event model assumptions are violated (i.e. constant variance and/or normality of residuals), a rank-based transformation or log transformation (if non-zero counts) will be considered.

12.3.3 12.3.3 Safety Analysis

All AEs will be collected; however, safety will be assessed by SAEs and product-emergent AEs only as reported by the subjects, as well as an assessment of vital signs.

12.3.4 12.3.4 Missing or Incomplete Data

Missing data will not be imputed and only observed data will be included in the analysis. Statistical models that account for unbalanced/missing data will be used.

13. Study Monitoring

13.1 Concomitant Medication/Supplements and Treatment

All concomitant medications/supplements used 90 days prior to Visit 1 (Day 0) and during the study will be reported to the study personnel for assessment and recorded in the subject's eCRF.

Use of the medications/supplements described in the "Exclusion Criteria" section and **Appendix 1** is not allowed during this study. If a participant requires any of these medications/supplements, the participant may not enter the study. If a participant uses any exclusionary medications/supplements once enrolled, a protocol deviation will be documented.

13.2 Compliance Monitoring

Compliance with the study product will be recorded in the participant's source document. Non-compliance will be determined as: (1) participants not consuming a full product serving for ≥2 d during the product ingestion period (between Visits 2 and 4; Days 14-28), or (2) participants not consuming a serving of product the day before stool collection. Compliance will be determined by the electronic Daily Diary.

13.3 Adverse Event Monitoring

AEs are defined as any untoward medical occurrence or undesirable clinical experience in a participant in a clinical trial, whether or not considered related to the study. This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures (including laboratory test abnormalities where applicable). Therefore, clinical observations, including responses to the question, "Have there been in any changes in your health or medications since you were last asked?" will be collected in-clinic at the beginning and end of Visit 2 (Day 14), and at the beginning of Visits 3, 4, and 5 (Days 21, 28, and 42, respectively). These observations will be reviewed for assessment of AE, including severity and potential relationship to the study as determined by the Clinical Investigator. Only product-emergent AEs reported following study product consumption will be evaluated and reported in the Study Report.

Events should be considered AEs if they:

- Result in discontinuation from the study,
- Require treatment or any other therapeutic intervention,
- Require further diagnostic evaluation (excluding a repetition of the same procedure to confirm the abnormality),
- Are associated with clinical signs or symptoms judged by the Clinical Investigator to have a significant clinical impact.

13.3.1 Grading and Severity

The Clinical Investigator will evaluate all AEs with respect to their severity, and record the outcome and action taken on the AE eCRF. AEs will be graded as:

Mild: Awareness of symptoms but easily tolerated

Moderate: Discomfort enough to interfere with but not prevent daily activity

Severe: Unable to perform usual activity

13.3.2 Relationship

The Clinical Investigator will also judge the likelihood that the AE was related to the study product and document this on the appropriate eCRF as:

NOT RELATED	This category applies to those adverse experiences which, after careful consideration, are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).
UNLIKELY	In general, this category can be considered applicable to those experiences that after careful medical consideration at the time they are evaluated, are judged to be, unlikely related to the study product administered.
POSSIBLY	This category applies to those adverse experiences for which, after careful medical consideration at the time they are evaluated, a connection with the study product administration appears possible but cannot be ruled out with certainty.
PROBABLY	This category applies to those adverse experiences that, after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be related to the study product.
DEFINITELY	This category applies to those adverse experiences which, the Clinical Investigator feels are incontrovertibly related to the study product.

Appropriate therapeutic action and follow-up measures will be performed by the Clinical Investigator in accordance with good medical practice.

13.4 Serious Adverse Event Definition/Qualification

A SAE is defined as an AE that results in any of the following outcomes:

- Death (note that death is the outcome of a SAE and the cause of death should be listed as the AE)
- Life-threatening event
- In-patient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- Congenital anomaly or birth defect
- Any other important medical event that may not result in death, be life-threatening, or require hospitalization, may be considered a SAE when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

In the event of a SAE, the participant may be dropped from the study if the Clinical Investigator deems it necessary.

13.4.2 Serious Adverse Event Reporting Instructions

If in the opinion of the Clinical Investigator the event meets the criteria of a SAE the following procedures will be followed:

- The Clinical Investigator will report the SAE to Biofortis Innovation Services immediately upon becoming aware of the event.
- In addition, the initial SAE report should be submitted with other applicable information (such as medical history, concomitant medications, AEs) to Biofortis Innovation Services within 24 hours of reporting the event to the attention of:

  Linda Derrig, MA, CCRA
  Director, Clinical Project and Data Management
  Biofortis Innovation Services
  800A South Rohlwing Road
  Addison, IL 60101
  Phone: +1 (630) 341-6312
  E-mail: linda.derrig@mxns.com

- The Clinical Investigator will notify the IRB of the event within the parameters and timeframe specified under the IRB Standard Operating Procedures (SOP) after becoming aware of the SAE. An initial report followed promptly by a complete report will be forwarded to the IRB, when applicable.
- Follow-up information relating to a SAE must be submitted to Biofortis Innovation Services as soon as additional data related to the event are available.
- If a participant is hospitalized or hospitalization is prolonged due to the SAE, the hospital discharge summary will be obtained if possible, when it becomes available.
- If a death occurs and an autopsy is performed, a copy of the autopsy report will be obtained if possible when it becomes available. All efforts must be undertaken to obtain follow-up information promptly.

13.4.3 Electronic CRF Recording of Adverse Events

All AEs and SAEs will be recorded on the AE eCRF page. For participants who have an ongoing AE and SAE at their final study visit, a follow-up will be completed after 30 days.

13.4.4 Serious Adverse Event Follow-Up

For all ongoing SAEs occurring during the study, the Clinical Investigator must submit follow-up reports to Biofortis Innovation Services regarding the participant's subsequent course. All SAEs that are ongoing at the end of the study or upon discontinuation of the participant's participation must be followed until either:

- The event resolves, or
- The event/condition has stabilized (e.g., in the case of persistent impairment), or
- The event returns to baseline, if a baseline value is available, or
- The participant dies, or
- The event can be attributed to other than the study treatment, or to other than the study conduct.

13.5 Pregnancy

The outcome variables measured may be affected by pregnancy and lactation. All female participants will undergo in-clinic urine pregnancy testing at Visit 1 (Day 0). Pregnant or lactating women will be excluded from the study and women of childbearing potential will be required to use appropriate contraceptive methods to avoid pregnancy. Documentation of contraception method must be recorded in the source chart.

14. Conduct of the Study

14.1 Ethics and Regulatory Considerations

This study will be conducted according to ICH (E6) Good Clinical Practice (GCP) Guidelines, the Declaration of Helsinki (2013), and US 21 CFR. Signed written informed consent for participation in the study will be obtained from all participants before protocol-specific procedures are carried out. Participants will be informed of their right to withdraw from the study at any time.

14.2 Institutional Review Board

The Clinical Investigator will ensure that an appropriately constituted IRB, in compliance with the requirements of 21 CFR 56, reviews and approves the clinical study. Before the study is started, the Clinical Investigator will forward copies of the protocol and consent form for this study to the IRB for review and approval. IRB approval must refer to the study by exact protocol title and number, identify the documents reviewed, and state the date of review. The IRB must be informed of all subsequent protocol amendments. No alterations, modifications to IRB-approved documents, including the protocol, protocol summary, consent form, recruitment materials and questionnaires will be allowed. The IRB must also be informed of all SAEs and of unexpected AEs as outlined in the IRB's SOPs or reporting guidelines. In addition, the Clinical Investigator will immediately forward copies of all correspondence with the IRB to Biofortis Innovation Services.

14.3 Informed Consent and Protected Health Information

The study will be explained verbally as well as on the informed consent document. Each participant will be given ample opportunity to inquire about details of the study and to read and understand the consent form before signing it.

Consent must be documented by the dated signature of the participant. Each participant's signed informed consent document must be kept on file by the Clinical Investigator for possible inspection by regulatory authorities or by the Sponsor. The participant should receive a copy of the written informed consent document once he/she has signed it.

The Sponsor recognizes the importance of protecting the privacy of participant data. Therefore, for study sites within the United States, the informed consent form will incorporate, or be accompanied by, a separate document incorporating HIPAA-compliant wording, by which participants authorize the use and disclosure of their Protected Health

Information by the Clinical Investigator and by those persons who need that information for the purposes of this study.

A participant may not be admitted to the study unless informed consent of the participant (or his/her legally authorized representative) has been obtained.

14.4 Participant Confidentiality

The Clinical Investigator is responsible for ensuring that participants' anonymity will be maintained. The eCRFs and other documents will identify participants by initials, number, or code, and not by name. The Clinical Investigator will keep a separate log showing codes, names, and addresses. All documents showing the participants' identity will be kept in strict confidence by the Clinical Investigator. However, the Clinical Investigator agrees that the Sponsor, its employees or agents, the IRB, as well as representatives of the FDA, will have the right to audit and review pertinent medical records relating to this clinical trial and that the participants will provide written informed consent to this effect.

14.5 Withdrawal of Participants from the Study

Participants may be removed from the study for any of the following reasons:

- A participant requests discontinuation;
- The Clinical Investigator initiates removal for medical or compliance reasons;
- Occurrence of any AE or condition that could, in the Clinical Investigator's opinion, interfere with the evaluation of the treatment effect of the study product or put the participant at undue risk.

It is understood by all concerned that an excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawal of participants should be avoided. Should a participant decide to withdraw, all efforts will be made to complete and report observations as thoroughly as possible. In the event that a participant is withdrawn from the study, the reason for the withdrawal will be documented in the eCRF.

15. Administrative Matters

All references to the Sponsor in this section include all designees e.g., Contract Research Organizations or Consultants acting on behalf of the Sponsor.

15.1 Changes to the Protocol

All changes to the protocol must be documented by amendments to the protocol signed by the Sponsor and the Clinical Investigator. The amended protocol and a revised informed consent form will be submitted for approval to the IRB. A copy of the approval will be provided to Biofortis Innovation Services and the Sponsor. Where the local IRB regulations regarding protocol amendments differ from this policy, the local regulations will apply.

The above-mentioned requirements do not preclude any immediate action from being taken in the interests of participants' safety.

15.2 Protocol Deviations and Violations

A protocol deviation is a minor departure from the protocol that is approved by the Project Manager prior to implementation and does not compromise participant safety or the integrity of the data. Any deviation from the inclusion/exclusion criteria requires an approved waiver from the Sponsor or authorized designee prior to enrollment in order to enroll that participant into the study. The site should accurately document the deviation and approval in the source document and complete the protocol deviation/violation eCRF.

A protocol violation is a divergence from the IRB-approved protocol that is not approved by the Sponsor or authorized designee prior to implementation. A violation can be classified as major or minor. A major violation compromises the safety of the participant or the integrity of the data collected. A minor violation is a less-significant departure from the protocol that, though not pre-approved, does not compromise the safety of the participant or the integrity of the data collected. The site should accurately document the violation in the source document and complete the protocol deviation/violation eCRF. Violations that could significantly influence participant safety will be reported to the IRB.

15.3 Participant Data Capture

Clinical data (including adverse events (AEs) and concomitant medications) will be entered into the Medrio electronic data capture platform, a 21 CFR Part 11-compliant data capture system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

All information required by the protocol should be documented in source records. The majority of source data will be collected and recorded via the Medrio Direct Data Capture (DDC) platform. Additional data, including paper questionnaires and forms, may be entered directly into the eCRF by site staff from paper source or recorded directly by participants using Biofortis tablets in-clinic. Additional data will be captured directly from participants at home via Medrio's electronic Patient Report Outcomes platform. Regardless of the means, all data captured will be documented in an anonymous fashion (e.g., the participant will be identified only by their study number and initials.)

The Clinical Investigator must agree to complete and maintain source records for each participant participating in the study. An explanation must be given for any omissions. Any eCRF entry must be completed as soon as possible after the participant's visit, in order that the monitor may verify the validity and completeness of the data. The Clinical Investigator will review and sign (as required) all eCRFs for completeness and accuracy. All information captured in the eCRF must be traceable back to the source records.

15.4 Clinical Monitoring

An initiation meeting will be conducted by the Sponsor or an approved representative. At this meeting, the protocol, eCRFs, and pertinent aspects of GCP will be reviewed with the Clinical Investigator and all study staff.

On-site monitoring visits will be conducted during the study, focusing on human participant protection and data integrity risks of the trial. A clinical monitoring plan will be developed which identifies specific risk-based monitoring focal points. These may include:

- Informed consent
- Eligibility criteria
- SAEs
- Serious protocol violations
- Stool collections
- Study product dispensing
- Accountability

No data disclosing the identity of participants will leave the study center, except as described in Section 9 and 11. Biofortis Innovation Services and any designees will maintain confidentiality of all participant records.

The Clinical Investigator must ensure that access to the eCRF is secure and that other study documentation is stored in a secure location. During the course of the study, the responsible Biofortis Innovation Services staff will be available to discuss any matters relating to the conduct of the study.

15.5 Auditing Procedures

In addition to the monitoring visits outlined above, an investigational site may undergo a quality assurance audit. The Sponsor representatives or a regulatory agency may conduct the audit. If a regulatory agency requests an audit of the study site, the Clinical Investigator is required to inform the Sponsor and Biofortis Innovation Services immediately.

15.6 Records Retention

All study documentation generated in connection with this study will be retained for the greater of 5 years or time required by applicable federal regulations, and to allow for inspection by Sponsor's authorized representatives and/or governmental or regulatory authorities of all such records including the Research Participants medical records. Sponsor may at any time prior to disposal or destruction, request to acquire such records from Biofortis. At the end of the required retention period, Biofortis will dispose of or destroy all Study records, unless Sponsor provides notice to Biofortis in writing of its intent to acquire such records. The study documents include IRB approvals for the study protocol and all amendments, all source documents and laboratory records, eCRF records, signed participant informed consent forms, and any other pertinent study document.

16. Termination of Study

The Sponsor and the Clinical Investigator reserve the right to terminate the study at any time. In terminating the study, the Sponsor and the Clinical Investigator will assure that adequate consideration is given to the protection of each participant's interest.

17. Disclosure

By conducting this study, the Clinical Investigator agrees that all information provided will be maintained by the Clinical Investigator and his/her staff in strict confidence. Such information may be communicated to the Sponsor representative(s) and/or IRB under a similar, appropriate understanding of the confidential nature of the information. Study documents provided (protocols, other material, as necessary) will be stored appropriately to ensure their confidentiality. It is understood that the confidential information provided to the Clinical Investigator will not be disclosed to others without written authorization, except to the extent necessary to obtain informed consent from those participants who are eligible and choose to participate in the study. Such information will not be provided to potential participants or participants by telephone or to any other individual. Conflicts of interest for key study personnel will be reported to the Sponsor.

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Appendix 1: Examples of Exclusionary Medications, Supplements, and Foods

This list is not intended to be comprehensive.

CLASS OF DRUG/PRODUCT	GENERIC/BRAND NAME	
Excluded within 6 months of Visit 1 (Day 0) and during the study		
Marijuana or hemp products	Products containing cannabinoids (CBD) Hemp-derived foods, beverages, or supplements (e.g., seed, oil, protein, tea, energy bars, etc.) Marijuana-containing foods, beverages, or supplements	
Nicotine-containing products	Cigarettes, cigars, pipe, chewing tobacco, tobacco pouch, hookah, e-cigarettes	
Unstable use of hypertension medication [unstable use is defined as a change in medication regimen within the 3 months prior to Visit 1 (Day 0)].		
Proton pump inhibitors	Aciphex Nexium Omeprazole Prevacid Prevacid NapraPAC Prevacid Solu Tab	Prevpac Prilosec Prilosec OTC Protonix Zegerid
Histamine H2 receptor antagonists	Axid Axid AR Cimetidine Famotidine Fluxid Nizatidine	Pepcid Pepcid AC Pepcid RPD Ranitidine Tagamet Zantac
Corticosteroids	Oral or systemic use of: Betamethasone Budesonide Cortisone Dexamethasone Hydrocortisone Methylprednisolone Prednisolone Prednisone Triamcinolone	Systemic use of: Fludrocortisone acetate (Florinef) Non-oral (inhaled, intranasal or dermal) use of more than 1500 µg/d of any corticosteroid
Regular (>3 times/wk) use of NSAIDs	Celecoxib (Celebrex)	Ketorolac (Toradol) Nabumetone (Relafen)

Occasional NSAID use is allowed	Diclofenac (Cambia, Cataflam, Voltaren-XR, Zipsor, Zorvolex) Diflunisal (Dolobid) Etodolac (Lodine) Ibuprofen (Motrin, Advil) Indomethacin (Indocin) Ketoprofen (Orudis)	Naproxen (Aleve, Anaprox, Naprelan, Naprosyn) Oxaprozin (Daypro) Piroxicam (Feldene) Salsalate (Disalsate) Sulindac (Clinoril) Tolmetin (Tolectin)
Cephalosporin antibiotics	Cefaclor (Ceclor) Cefadroxil (Duricef) Cefamandole (Mandole) Cefazolin (Ancef) Cefepime (Maxiprime) Cefdinir (Omnicef) Cefoperazone (Cefobid)	Cefotaxime (Claforan) Cefprozil (Cefzil) Ceftazidime (Fortum) Ceftriaxone (Rocephin) Cefuroxime (Ceftin) Cephalexin (Keflex)
Penicillin antibiotics	Amoxicillin (Novamox) Ampicillin Flucloxacillin	
Sulfonamide antibiotics	Trimethoprim-Sulfamethoxazole (Bactrim)	
Macrolide antibiotics	Azithromycin (Zithromax) Clarithromycin (Biaxin) Erythromycin (E-Base, E-Mycin, E.E.S., Ery-Tab, ERYC, EryPed, Erythrocin, Ilosone)	
Ketolide antibiotics	Telithromycin (Ketek)	
Quinolone antibiotics	Cipro Floxin Levaquin	
Tetracycline antibiotics	Declomycin Doxycycline Periostat Vibramycin	
Antifungals	Amphotericin B (Abelcet, AmBisome) Anidulafungin (Eraxis) Atovaquone (Mepron) Caspofungin (Cancidas) Clotrimazole (Lotrimin, Mycelex) Fluconazole (Diflucan) Flucytosine (Ancobon) Griseofulvin (Grifulvin V, Gris-PEG) ibrexafungerp (Brexafemme)	

	Isavuconazonium sulfate (Cresemba) Itraconazole (Sporonox, Tolsura) Ketoconazole (Extina, Nizoral, Xolegel) Micafungin (Mycamine) Miconazole (Oravig) Nystatin (Mycostatin, Nystat) Posaconazole (Noxafil) Terbinafine (Lamisil) Voriconazole (Vfend)
Antiparasitics	Albendazole (Albenza) Atovaquone (Mepron) Benznidazole (same name for trade name) Dapsone (Aczone) Ivermectin (Stromectol) Mebendazole (Emverm, Vermox) Metronidazole (Flagyl) Miltefosine (Impavido) Nitazoxanide (Alinia) Nifurtimox (Lampit) Paromomycin (Humatin) Praziquantel (Biltricide) Pyrimethamine (Daraprim) Pyrantel (Reese's pinworm) Sulfamethoxazole/Trimethoprim (Bactrim DS, Septra DS) Tinadazole (Tindamax) Triclabendazole (Egaten)
Excluded throughout the study (Visits 1-5; Days 0-42)	
Fermented Foods and Probiotic supplements	• Yogurt (dairy and plant-based) with or without probiotics • Kefir • Cultured buttermilk • Acidophilus milk • Cultured sour cream • Soured milk • Kombucha • Kimchi • natto • Probiotic supplements (e.g., Lactobacillus, Lactococcus, Bifidobacteria, Leuconostoc)
Excluded during the study period (Visits 1 through 5; Days 0-42)	
Topical marijuana or hemp products	Lotion, rubs, oils, balms containing cannabinoids (e.g., THC, CBD)

Appendix 2: Study Product Ingredients

Ingredients: Water, Sugar, Nonfat milk, Glucose, Natural flavors, *Lacticaseibacillus paracasei* strain Shirota

Appendix 3. Abstain from fermented dairy food products

For this study, you are required to abstain from fermented dairy and non-dairy food products throughout the study.

To identify fermented dairy and non-dairy food products, look for the following ingredients:

- Lactobacillus
- Lactococcus
- Probiotics
- Bifidobacteria
- Leuconostoc

Examples of fermented dairy food products include:

- Probiotic supplements
- Yogurt with or without probiotics
- Kefir
- Cultured buttermilk
- Acidophilus milk
- Cultured sour cream
- Soured milk
- Unpasteurized, raw or mold-ripened soft cheese*
- Home fermented foods or beverages
- Créme fraiche

Examples of fermented dairy food products include:

- Plant-based yogurt
- Kombucha
- Kimchi
- Natto

Sometimes, you will find fermented food products as part of regular products, such as baked goods or certain ethnic foods. For example, yogurt is part of a baked good recipe or a salad dressing or sauce. Because the amount of the fermented dairy food may be very low, it is ok to consume these products. However, these foods should be noted on the Daily Diary.

If you are not sure if you can consume a regular product that is part of your habitual diet but lists a fermented dairy food as an ingredient, please check with us!

*Based on CDC recommendations for pregnancy to avoid risk of live microbes. 10 Tips for Preventing Infections Before and During Pregnancy | CDC CDC indicates to avoid soft cheeses (e.g., feta, brie, camembert, blue-veined cheeses and Mexican cheeses like queso, blanco, fresco, and panela) unless they have labels that say they are pasteurized or cooked until bubbly. Hard cheeses, such as parmesan, provolone, Romano, asiago and aged Gouda are pasteurized are okay to consume.

Appendix 4: Specimen Management Plan

Yakult: BIO2208 ***SMP v4.0***

Total number to randomize: 25

Total number of screen fails allowed: 15

Subject Activity and scheduling

Visit 1: Screening of all subjects will occur in August 2022

Collection Visits (Visits 2-5):

V2	V3	V4	V5			# of Subj.	Time window for fecal sampling	Backup for	Time window for fecal sampling
9/19	9/26	10/3	10/17	Mon	Gr. 1	7	Sun 7pm to Mon AM		
9/20	9/27	10/4	10/18	Tue	Gr. 2	6	Mon 7pm to Tue AM	Gr. 1	Mon PM to Tue AM
9/21	9/28	10/5	10/19	Wed	Gr. 3	6	Tue 7pm to Wed AM	Gr. 2	Tue PM to Wed AM
9/22	9/29	10/6	10/20	Thu	Gr. 4	6	Wed 7pm to Thu AM	Gr. 3	Wed PM to Thu AM
9/23	9/30	10/7	10/21	Fri				Gr. 4	Thu PM to Fri AM

Figure 1: Cohort visit schedules

Subject Specimen Collection

Visits 1-4 Collection Materials Dispensed:

Subjects will be dispensed a stool collection kit at Visits 1 through 4, along with ice packs, an insulated cooler and temperature measurement device (see Figure 2). A demonstration will be conducted to illustrate for subjects both how to collect stool specimens, how to set the temperature logger, and how to transport specimens to the clinic site.

Subjects will be assigned to one of four collection groups and subsequently assigned a collection date (see Figure 1). Subjects will also be instructed to begin a 14 day run-in period tied to their Visit 2 date. Run-in instructions include a consistent diet and habitual exercise and lifestyle as well avoidance of fermented dairy and non-dairy products.

An alternate or back up collection date for each visit will allow subjects who cannot produce a specimen on their collection date to continue in the study. Subjects will be instructed to continue consuming study product as per the protocol through their clinic visit (during the study product ingestion period—Visits 3 and 4).

Checklist of items to be provided to subjects:

Stool collection and storage kit contains the following:

- Cooler or insulated shipping kit
- 4 ice packs
- 1 stool collection system (container with lid and toilet bowl holder) **Figure 3**
- 2 collection tubes (see **Figure 4**); pre-labeled with subject screen number
- 1 temperature logger device (see **Figure 2**) *The temperature logger will be programmed by site staff prior to dispensation to subject.*
- 1 clear sandwich-size sealable aluminum foil bag

Figure 2. Elitech RC 5 + Temperature logger

Subject Instructions for stool collection and handling:

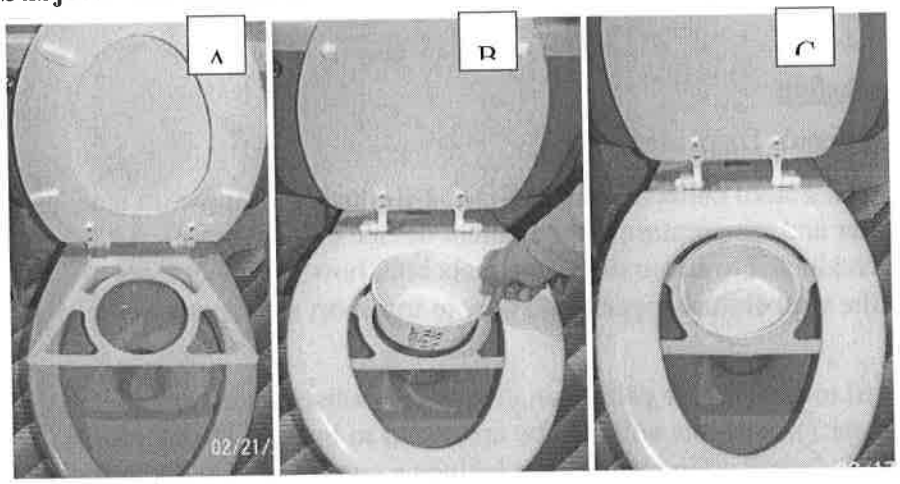

Figure 3. Lift up the inner toilet seat ring and place the holder (A) followed by the stool collection container (B) under the inner toilet seat ring in the center of the back of the toilet bowl. Close the inner toilet seat ring to hold the stool specimen collection system in place (C).

1. **IMMEDIATELY** put the ice packs provided into your freezer.
2. Please collect a stool sample within the timeframe provided to you by the clinic staff.
3. Before having a bowel movement, empty your bladder of urine completely. The stool specimen collection system is meant to be used for collection of stool samples only. Every effort should be made to avoid contamination of the stool sample with urine.
4. After emptying your bladder, lift up the inner toilet seat ring. Remove the lid from the container. Place stool specimen collection system under the inner toilet seat ring in the center of the back of the toilet bowl (**Figure 3A and 3B**).
5. Close the inner toilet seat ring to hold the stool specimen collection system in place (**Figure 3C**).
6. Complete your bowel movement into the container, ensuring that your stool goes into the container.
7. Remove the collection container from the holder by placing the entire stool specimen collection system on a flat surface and lifting the collection container upward.
8. Place the collection container on a stable and flat surface (e.g., bathroom floor or counter)
9. Using the stool collection tube with scoop (**Figure 4**), collect one scoop of stool (the amount should be level with the spatula) and place inside the collection tube. Screw the cap back on until finger tight. Then, repeat with the second collection tube.

Figure 4. Stool Collection Tube

Subject Specimen Storage and Transport:

1. Place the two, sealed collection tubes containing your stool samples into the provided sealable aluminum foil bag. Gently push out as much air out of the bag as possible and seal the bag completely.
2. Record the time and date of your bowel movement on the label affixed to the aluminum foil bag.

3. If your stool sample is collected the morning of your visit, place the aluminum bag inside the cooler or insulated shipping kit. Start the temperature logger and insert the logger, placing it on top of the aluminum foil bag.

4. If your stool sample is collected the day prior to your visit, please store the aluminum bag and temperature logger in your refrigerator until departing for the clinic. Start your temperature logger as soon as the specimen is placed in the refrigerator. Then follow the remaining instructions

5. With the aluminum foil bag and temperature logger placed in the cooler/shipper, add the two ice packs—one at the bottom of the shipper/cooler and one on top.

6. All specimens should be brought to the clinic no later than 10 a.m. on your scheduled collection date.

All collection, storage and transport instructions will be reviewed in detail with subjects during each subject's screening visit (Visit 1).

Biofortis Specimen Receipt

All subject specimens should be received at Biofortis on the scheduled collection dates as early as 7:00 a.m. but no later than 10 a.m. for the scheduled 10:30 a.m. courier pick up.

On the scheduled collection date:

1. Open the cooler/shipper bag and confirm the presence of two collection tubes containing stool samples, frozen ice pack and a **running** temperature logger device.
2. The collection time recorded on the aluminum foil bag and/or on the subject's diary should be checked to confirm the specimen was collected no earlier than 7 p.m. in the evening prior to drop off. Collection time should be recorded in the DDC/eCRF data management platform. A QC is required.
3. Record the subject's collection information in the daily specimen shipping log (see Biofortis specimen tracking).
4. The temperature logger should be removed from the cooler/shipper and the stool specimen stored in the laboratory refrigerator until packaged for the courier.
5. Lab personnel will download temperature data from each returned logger as per instructions included with the Elitech RC 5 + Temperature logger. The data file will be shared with Biofortis Data Management.
6. A courier has been scheduled to pick up specimens at 10:30 a.m. for drop off on or before 11:30 a.m. on each collection day (see Figure 1).
7. If no specimens have been dropped off by 10 a.m. on any of the scheduled collection days, please notify the contracted courier and project manager (see contact info below) no later than 10:15 a.m. It is the responsibility of the project manager to ensure the Yakult researchers are notified no later than 11:00 a.m.

8. Subjects who fail to drop off a specimen on their scheduled collection date will be contacted and reminded to provide a specimen the following morning. This contact must be documented.
9. Approximately 10 minutes before the courier's scheduled arrival, the lab should pack each of that day's collected specimens in an insulated shipping box. Sufficient ice packs should be added, along with a new temperature logger. The logger's tracking mechanism should be initiated as soon as specimens are put into the insulated shipper.
10. If the courier has not arrived by 10:40 a.m. on a scheduled collection date, please notify the contracted courier and the project manager as soon as possible. (see contact info below) It is the responsibility of the project manager to alert the Yakult researchers regarding missed or delayed courier pick up.

    MATT TJARDES
    ROYAL EXPEDITING, INC. MC-741963
    ROYAL COURIER, INC. MC-667323-C
    Ph: 630-628-8900
    Fax: 630-628-8920

11. Shipping address (Royal Courier has the shipment address):

    Indika Edirisinghe
    6502 S Archer Rd
    Bedford Park, IL 60501
    Phone: 708 308 0178
    Email: iesirisi@iit.edu

Biofortis Specimen Tracking

1. Biofortis lab manager will maintain a specimen shipping log for each courier pick up.
2. The shipping log will be included with the specimen transport materials.
3. A copy of the shipping log will also be emailed same day to the Biofortis Project Manager and Yakult researchers.

IFSH Specimen Receipt

1. Specimens should be received at IFSH no later than 11:30 a.m. on each scheduled collection date (**Figure 1**).
2. Drop off location is behind the building. A manned security gate can provide direction to the receiving dock.
3. IFSH phone number for delivery concerns or issues: 708 308 0178.

4. Yakult researchers to provide Biofortis with daily confirmation of courier receipt, as appropriate. Confirmation should include accountability of the specimens received against the shipping log. Any discrepancies should be immediately reported to the Biofortis Project Manager.
5. Yakult researchers to remove the temperature logger from shipping boxr and download temperature data as per instructions included with the Elitech RC 5 + Temperature logger.
6. Yakult researchers will be responsible for notifying Biofortis Project Manager of any specimens received out of temperature range and determination of secondary collection will be considered.
7. Yakult researchers will be responsible for disposal of used shippers/coolers and temperature logs.
8. Specimens should be received at IFSH no later than 11:30 a.m. on each scheduled collection date (**Figure 1**).
9. Drop off location is behind the building. A manned security gate can provide direction to the receiving dock.
10. IFSH phone number for delivery concerns or issues: 708 308 0178.
11. Yakult researchers to provide Biofortis with daily confirmation of courier receipt, as appropriate. Confirmation should include accountability of the specimens received against the shipping log. Any discrepancies should be immediately reported to the Biofortis Project Manager.
12. Yakult researchers to remove the temperature logger from each subject shipper/cooler and download temperature data as per instructions included with the Elitech RC 5 + Temperature logger.
13. Yakult researchers will be responsible for notifying Biofortis Project Manager of any specimens received out of temperature range and determination of secondary collection will be considered.
14. Yakult researchers will be responsible for disposal of used shippers/coolers and temperature logs.

Project Manager:

Linda Derrig
Biofortis Innovation Services
800 S. Rohlwing Road
Addison, IL 60101
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Appendix 5. Daily Diary

Note: Diary will be converted to an electronic format that will prompt for collecting the information below on a daily basis.

NAME	:		
SUBJECT NO.	:		
PERIOD	:	☐ **Baseline**	☑ **Ingestion**
		☐ **Follow-up**	

PLEASE BRING THIS DIARY on Oct 3rd (7 ~11:00am) WITH YOUR FECAL SAMPLEs TO Biofortis

If you have any question related to the study or in case the visits cannot be scheduled within the submitted time, please contact the Investigators:

XXXXXXXXXXX

phone: XXXXXXXXXXX

Thank you for your attention and cooperation

DAILY RECORD (Compliance will be assessed from Visit 3-Visit 5 only)

Compliance:

Please drink test product ONE a day and do NOT drink two or more even you miss drinking the day before.

Scheduled Date (days from period start)	Date Day (days from start)	Date Day (days from start)	Date Day (days from start)	Date Day (days from start)	Date Day (days from start)	Date Day (days from start)	Date Day (days from start)
Record of Test Product Consumption (Only for Ingestion Period)							
Consumed product on:	/	/	/	/	/	/	/
Consumed product at:	: am pm	: am pm	: am pm	: am pm	: am pm	: am pm	: am pm

Compliance: Please refrain from consuming other probiotic supplements and fermented products during the study. Please confirm your compliance by answering the questions below.

	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
Fermented products (foods/beverages containing viable bacteria, such as Yoghurt, Natural cheese, kombucha, *etc.*)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
If **Yes**, write the name of foods/beverages consumed														
If **Yes**, write the amount consumed														
Probiotic supplements	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
If **Yes**, write the name of the supplement consumed														

Scheduled Date (days from period start)	Date Day (days from start)		Date Day (days from start)		Date Day (days from start)		Date Day (days from start)		Date Day (days from start)		Date Day (days from start)		Date Day (days from start)	
Record of Drugs Taken														
	Yes	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
Antibiotic/Antifungal drugs	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Laxative drugs	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Antidiarrheal drugs	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other drugs	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
If **Yes**, please write the name of drugs														
Check point	☐ Put ice packs in the freezer ☐ Put products in the fridge													
Memo in case the specimen is NOT obtained.														