Safety of *Lactobacillus reuteri* in healthy children aged 2-24 months

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STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by ICH E6; 62 Federal Register 25691 (May 9, 1997) and laws governing the conduct of clinical trials in Peru under the authority of Peru's National Institute of Health (INS) in accordance with XV article 28 of law 26842 and the oversight of the National Institutes of Health of Peru (INS) as specified by articles 4 and 5 of Decree. 017-2006-SA in accordance to current guidelines described in the Guidelines for Clinical Trials in Peru, 20 August 2011 (Reglamento de Ensayos Clinicos en el Peru, Lima 2011, integra: El DS 0-17-2006-SA, El DS 006-2007-SA y El DS 011-2007-SA).

All key personnel and persons involved in human subjects contact, contact with CRFs and source documents and data management for the project have completed Human Subjects Protection Training.

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LIST OF ABBREVIATIONS

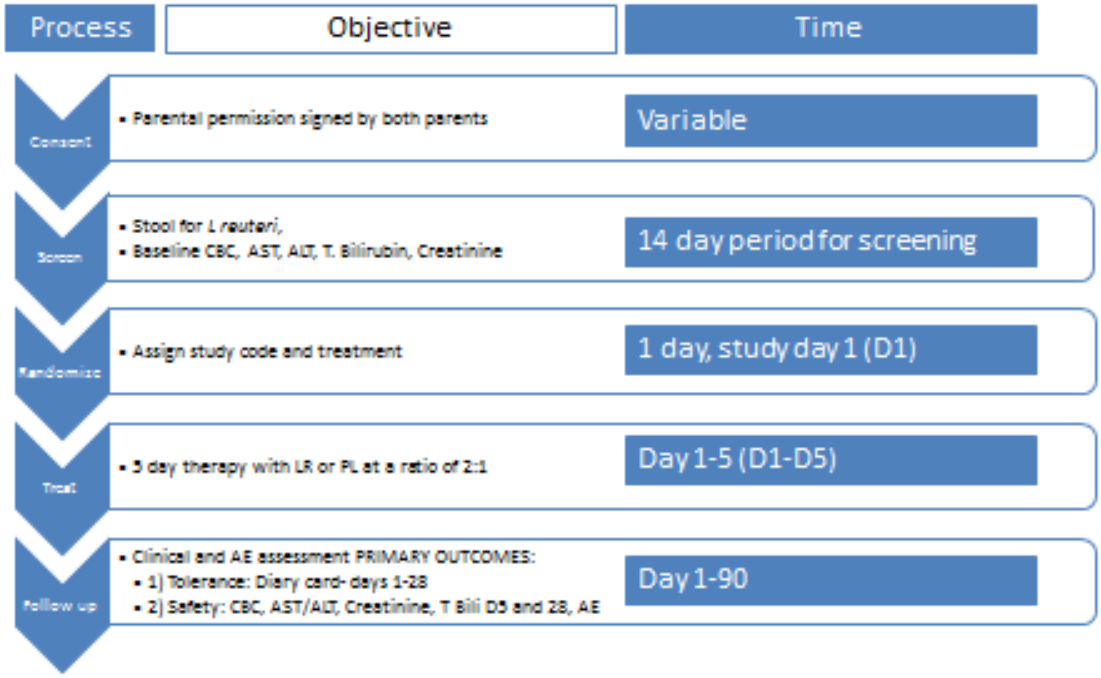
AE	Adverse Event
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
EC	Ethics Committee
FWA	Federal-Wide Assurance
GCP	Good Clinical Practice
IATA	International Air Transit Association
ICH	International Conference on Harmonisation
IND	Investigational New Drug Application
INS	Peruvian National Institute of Health (Instituto Nacional de Salud)
IRB	Institutional Review Board
CITI	Collaborative Institutional Training Initiative
LR or Lr	Lactobacillus reuteri
MOP	Manual of Procedures
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OHSR	Office of Human Subjects Research
PI	Principal Investigator
PID	Personal Identifying Code
PL	Placebo
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SID	Specimen identification code
SOP	Standard Operating Procedure
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	Safety of *Lactobacillus reuteri* in healthy children aged 2-24 months
Précis:	Phase I double blinded randomized trial of the safety and tolerability of *Lactobacillus reuteri* DSM 17938 given for five successive days in healthy children. Sixty children will receive study product at a treatment to placebo ratio of 2:1 and followed for 3 months for safety outcomes.
Objectives:	Primary: To assess the safety and tolerability of Lactobacillus reuteri (Lr) strain DSM 17938 in healthy infants and children. Safety will be assessed through the evaluation of adverse effects in both study arms for 90 days following product administration and by laboratory monitoring of CBC, AST, ALT, T. bilirubin, BUN and creatinine prior to product or placebo administration with follow-up assessment at 5 and 28 days. Tolerability will be assessed based on parental report of subjective symptoms of well-being during product administration and thru D28 of the study. Secondary: To evaluate the duration of shedding of Lactobacillus reuteri (Lr) strain DSM 17938 following administration. This will be determined by and endpoint PCR assay for *L. reuteri* done on stool collected on D3, D5, D12, D15, D18, D24, D28, D36 on all participants.

Population:	The sample size of sixty is expected to include an equal number of healthy boys and girls 2-24 months of age at the time of enrollment from a rural community in Loreto Peru where chronic undernutrition and diarrheal disease are both highly prevalent.
Phase:	I
Number of Sites:	Single site- Loreto, Peru
Study Duration:	7 months
Subject Participation Duration:	3 months
Description of Agent or Intervention:	**1. Active Product: Description** *Lactobacillus reuteri* 17938 suspended in sunflower oil, medium chain triglyceride oil, silicone dioxide. Total viable count of *L. reuteri* 17938 1 x 10^8 CFU/5 drops per day for 5 consecutive days **2. Placebo: Composition** Sunflower oil, medium chain triglyceride oil and silicon dioxide. Total viable count of L. reuteri is zero CFU/ 5 drops.
Estimated Time to Complete Enrollment:	4 months

Schematic of Study Design:



1 KEY ROLES

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2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Probiotic therapy is intrinsically inexpensive and readily applicable in resource-poor developing countries. Because of already published trials showing efficacy in the treatment of diarrheal diseases, probiotic organisms such as *L. reuteri* are already in clinical use for the treatment of acute infectious diarrhea[1,2], infantile colic,[3,4] and atopic disease.[5] However, the avoidance of the formal IND process and FDA observed clinical trials has compromised the credibility of many of these reports. There is substantial evidence that the quality of preparations in distribution is highly variable and labels do not always reflect true contents of marketed materials.[6-8]

Our long term goal is to develop *Lactobacillus reuteri* DSM 17398 as a safe and effective therapy for acute infectious diarrhea in children. We have completed a Phase 1 trial in adults[9] ((NCT00774163) and are in the process of completing a Phase 1 study of children ages 2-5 years with this product under IND ((NCT020124122). This study is designed to provide evidence of the safety of the study product in well children in Peru under demanding international quality standards. The study will not be done under IND but will be done with extensive oversight by the study team in collaboration with NIDDK and a DSMB. If shown to be efficacious in phase II/III trials we believe this will allow for the greater use and acceptance of this product as part of the public health response to the management of acute infectious diarrhea in children.

2.2 Rationale

We hypothesize that *L reuteri* given at a dose of 1 x 10^8 CFU/ day is safe in infants in children and likely to be beneficial in shortening the duration of episodes of diarrhea in children in resource constrained settings.

BioGaia has chosen the dose level of 1x10^8 CFU/day for *L. reuteri* in man as a safe and efficacious dose. The same dose is recommended for all ages.
Unlike pharmaceuticals, where doses are absorbed and metabolized, *Lactobacillus reuteri* consists of live cells that become active and multiply along the entire digestive tract. Whether in adults, children or infants, *Lactobacillus reuteri* grows from the initial dose to colonize the available gut mucosa and thus, even though the surface area of the GI tract differs with age, the substrates for multiplication are not limiting and thus the same dose is effective.

The recommended daily dose of $1x10^8$ CFU has shown efficacy in 10 studies on prevention or treatment of symptoms.[3,10-13]

Doses in the range of $10^8 – 10^9$ CFU/day have shown efficacy in studies by: Eom 2005 ($2x10^8$),[14] Valeur 2004 ($4x10^8$),[15] Ouwehand 2002 ($7x10^8$).[16]

Some early studies in humans used higher doses up to 10^{10-11} CFU/day,[1,2,17] primarily in safety studies in young children, adults and immunocompromised adults (HIV-positive). Blood, urinary, fecal and clinical parameters showed such higher levels to be safe. The only possible side effect observed was a mild, and temporary, increase in flatulence.

Studies performed with lower than the recommended $1x10^8$ CFU dose demonstrated gut colonization, but some of studies demonstrated less optimal effect for prevention or treatment of diarrhea. The $1x10^5$ and $1x10^7$ dosages both colonized the gut and had significant effect on watery stools in newborns. $1x10^7$ CFU/day had significant effect on frequency of diarrhea and close to significant effect on duration of watery diarrhea in rotavirus gastroenteritis.[2]

The five day treatment period is based on clinical studies that generally showed biologic response with 24-48 hours. Shorter duration of therapy is preferred as it is more practical to implement and limits cost per treatment making this treatment a feasible option for a common important public health problem.

2.3 Potential Risks and Benefits

Potential Risks

There are slightly greater than minimal risks associated with study participation. Blood draws have a slight risk of bruising or infection. Health workers are trained in phlebotomy. The prospective surveillance portion involves the limited inconvenience of daily interviews to obtain symptom and illness histories. There also exists a small risk that the information collected may not remain confidential, however, the data collected is not of a sensitive nature and would be unlikely to have important social or legal consequences for the participant and his or her family. Each enrollment in the study involves the risk associated with the possible consumption of the probiotic *Lactobacillus reuteri*. Although there is extensive worldwide use of these products, it is possible that their ingestion could lead to complications, including sepsis.[18,19] As we have cited numerous studies where these probiotics have been used for both treatment and prophylaxis of diarrhea in children without serious adverse effects, we believe the risk is very small. We will monitor closely for adverse events and address them promptly with appropriate medical interventions, which will be supplied by the study.

Benefits

It is not believed that subjects will receive any health benefit related to participation in the study. Parents may only perceive that they are contributing to the evaluation of a treatment of diarrhea that may be important in the future.

2.3.1 Potential Risks

The major potential risk associated with the administration of probiotics is the rare disseminated bloodstream or deep tissue infection with the probiotic strain or the contamination of the probiotic product with a pathogen or toxin. Bloodstream infection, liver abscesses, and joint infections have occurred with probiotic bacteria, predominantly Lactobacillus rhamnosus GG, although there have been reports of *L. acidophilus* bacteremia associated with product ingestion.[18,20-23] Additionally recently intestinal mucormycosis caused by the contamination of probiotic preparation ABC Dophilus powder which contains *B. lactis*, *S. thermophilus*, and *L. rhamnosus* was found to be contaminated with *Rhizopus oryzae* which lead to the death of one infant in Connecticut in 2014.[24]

To mitigate this risk we have taken several precautions. We have chosen a product that was isolated by the current provider who can trace the origin and any changes in the product, a standard almost no other product with demonstrated efficacy in treating acute enteric infections can meet. This product was derived from a strain isolated in human breastmilk in Peru by scientists at BioGaia who have held this reference strain since that time.[25] We have also chosen a product that has no documented cases in Pubmed (Reviewed Feb 20th, 2015) of disseminated bloodstream infections or deep tissue infections despite the distribution of the product in the US and Europe since 2007. The derivative strain *Lactobacillus reuteri* 55730 (different from the product strain only because it had additional plasmids that contained antibiotic resistance genes) was previously distributed for 10 years and taken together millions of doses of these two highly related products have been sold with no reports in the literature of bacteremia or deep tissue infection and no product contamination concerns. This product has been evaluated in several clinical trials[26,27] including ones done in infants, with no safety concerns, including studies that gave it at the study dose daily to prevent necrotizing enterocolitis in low birth weight newborns,[28,29] and in infants with colic.[30-35] Furthermore, as detailed in our product assurance plan, we will rigorously run independent assessments of the viability and identity of the product provided by our probiotic supplier which will further mitigate risk to subjects in compliance with CBER guidance on early clinical trials and provide a high standard for the evaluation of probiotic products by other investigators.

Evidence on the safety and history of the product strain are available in Appendix F.

2.3.2 Known Potential Benefits

There are no direct benefits to subjects who participate in this study. Potential benefits of this product in other studies have shown it efficacious in the treatment of infantile colic,[4] the prevention and treatment of acute infectious diarrhea,[2] and the prevention of necrotizing enterocolitis in low birth weight babies.[29]

3 OBJECTIVES

3.1 Study Objectives

Conduct a Phase 1 study to document safety and tolerability of *Lactobacillus reuteri* 17938 in children 2-24 months of age.

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

There are three major areas of safety and tolerability that will be assessed in this phase I trial. The outcome measurements will be compared between the two treatment groups (Intervention v placebo) with intent to treat analysis.

1. **Evidence of invasive infection resulting from the administration of L. reuteri DSM 17938**. Baseline and daily temperature measurements will be made during the study period. Complete hemogram with differential count obtained at baseline, on day 5, and on day 28. Vital signs will be monitored daily during the administration of the treatment (days 1-5). The evaluation of all febrile episodes will be done and include a physician exam and the evaluation of blood smears for malaria and blood cultures for the evaluation of possible bacteremia.

   Specific outcome parameters in this category include:

   a. Mean daily temperature
   b. Incidence of fever on study days 1-5
   c. Frequency of temperature readings categorized as fever (adjusted for number of subjects per group)
   d. Number of episodes of *L. reuteri* bacteremia

2. **Clinical tolerance.** The inclusion of a placebo group will allow for comparisons of daily symptom measurements to be compared between the treatment and placebo group. The symptoms will be monitored for at household visits, and will be graded as adverse events according to defined criteria. Symptoms monitored as AEs include fever, nausea/upset stomach, vomiting, diarrhea, irritability, rash, pruritis, and wheezing or bronchospasm. Diagnostic microbiology will be performed on all stool specimens from subjects who develop diarrhea during the 90 days following initiation of therapy.

   Specific outcome parameters in this category include:

a. Frequency and severity of symptoms monitored as AEs (adjusted for number of subjects per group)
b. Frequency of all AEs (adjusted for number of subjects per group)
c. Frequency of SAEs (adjusted for number of subjects per group)

3. Evidence of toxicity resulting from the L. reuteri DSM 17938 preparation.

Participants will have baseline, day 5, and day 28 assessment of hematologic status and renal and hepatic function. Assessment of renal function will include blood urea nitrogen (BUN) and creatinine (Cr). Assessment of hepatic toxicity will include transaminases (AST, ALT) and total bilirubin. Hematologic status will be determined by CBC to measure hemoglobin and absolute leucocyte count, as mentioned above.

Specific outcome parameters in this category include:

a. Mean values for laboratory tests monitored (adjusted for number of subjects per group). These are listed in Appendix D.
b. Frequency of out of range values for laboratory tests monitored (adjusted for number of subjects per group). These are listed in Appendix D.

3.2.2 Secondary Outcome Measures

Secondary outcomes are restricted to:
1) The assessment of the duration of shedding of *Lactobacillus reuteri* (Lr) strain DSM 17938 following administration. This will be determined by and endpoint PCR assay for *L. reuteri* done on stool collected on D3, D5, D12, D15, D18, D24, D28, D36 on all participants.

4 STUDY DESIGN

The study is a single center Phase 1 double blind placebo controlled trial of *Lactobacillus reuteri* DSM 17938 in children aged 2-24 months. The study population is based in a community with a registered clinical trial center in rural Peru where this protocol can be done in a highly controlled setting. The study is intended to enroll healthy children living in a resource constrained setting where chronic undernutrition and diarrheal disease is common, as this is the population that is most likely to receive benefit from the use of this and or other probiotic products for the treatment of acute infectious diarrhea and environmental enteropathy.

Up to 100 subjects will be enrolled in order to obtain the randomization of 60 subjects, who will receive study product or placebo at a ratio of 2:1. Participants will be enrolled between the ages of 8 weeks and 24 months. There will be no age stratification. Participants will be screened with a baseline history and physical examination and laboratory tests to determine the HIV status of the child, as well baseline laboratory tests to evaluate occult infections, hepatic function, and renal function. Upon randomization subjects will be screened and randomized to a treatment arm. Product will be administered under the direct supervision of study staff daily for 5 days. The primary outcome is the safety and tolerability of the product at this dose and formulation. Safety will be assessed by the monitoring of baseline laboratory tests at 5 and 28 days following the initiation of therapy and by AE monitoring. Tolerability will be evaluated by analyzing 28 day diary cards that are distributed to all randomized subjects on which parents record their child's symptoms (specifically fever, anorexia/oral intake, vomiting, diarrhea, irritability, rash, wheezing, or open fields which allows them to describe any issue their child may experience and grade its severity) . Secondary outcomes include:
1) The assessment of the duration of shedding of *Lactobacillus reuteri* (Lr) strain DSM 17938 following administration. This will be determined by and endpoint PCR assay for *L. reuteri* done on stool collected on D3, D5, D12, D15, D18, D24, D28, D36 on all participants.

The planned enrollment period is four months and the participation of individual subjects is for 3 months. The study should be completed in 7 months. Safety monitoring by treatment group, i.e., infants assigned to active or placebo study product will be conducted by NIDDK appointed DSMB under the direction of the NIDDK Program

Official.

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5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Subject Inclusion Criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Have a parental permission form signed by both parents
- Be between 8 weeks to 24 months of age with no preexisting exclusion criteria
- Have parents who are willing to comply with all planned study procedures and be available for planned study visits for 3 months.

5.2 Subject Exclusion Criteria

1) No enrollment of family members in households where any of the following are present:

a. Another study participant in the household

b. Presence of immune suppressed individuals or use of immunosuppressive agents (including but not limited to corticosteroids, methotrexate, etc.) by any household member

c. Presence of a serious congenital anomaly or chronic medical condition that in the opinion of the investigators would contraindicate participation in any household member, including history of gastrointestinal surgery, chronic gastrointestinal illness, abnormal intestinal anatomy, or abnormal bowel functionality

The following risk factors are at the level of the individual child:

2) Allergy to penicillin, cephalosporins, clindamycin or gentamicin
3) History of antibiotic use in the last 30 days
4) Use of probiotic products within the past 30 days, including masato (local product with fermenting bacteria) and yogurt products containing live bacterial cultures.
5) History of diarrheal illness within the past 30 days (See definition in Protocol Appendix B)
6) Presence of fever or a pre-existing adverse event monitored in the study (See Protocol Appendix B Definitions of AEs for specific adverse events monitored in the study)
7) Positive results on serum diagnostic tests for antibodies to HIV.

8) Presence of severe anemia, defined as serum hemoglobin $\leq$ 7 gm/dL

9) Out of range laboratory values for total leucocyte count, BUN, Creatinine, AST, ALT, and total bilirubin monitored as potential adverse events, as described in Appendix E.
10) Pre-enrollment stool sample (collected within 14 days of day 1 of the study) is positive for *L. reuteri* by PCR.

5.3 Strategies for Recruitment and Retention

The community where recruitment will occur has been a research site for the current group for the last fifteen years. During this time we have had successful recruitment and retention for intensive birth cohort studies and have recruited and trained nurses and health care workers and a study team from the area. The community is experienced in research participation which aids recruitment and retention of study subjects.[9,36-42]

5.4 Treatment Assignment Procedures

Treatment assignment is based on the order in which subjects complete screening and are assigned a study code. This study code is the same as the treatment vial ID number.

5.4.1 Randomization Procedures

Randomization. Upon satisfactory completion of screening, participants will be assigned a study code (different from the PID that accompanied screening data) that is associated with a list completed by the project biostatistician and shared with the individual at BioGaia preparing the treatment vials. This sequential code is associated with treatment vials randomized to one of two treatment groups (T or P) on a 2:1 (T:P) basis with the first randomized subject receiving the first study vial, etc. All vials are identical (see labelling section below) and the Treatment ID on the vial matches the study code number.

5.4.2 Masking Procedures

This is a double masked trial. Only the project biostatistician has the table linking treatment assignment to randomization code. Unmasking of the rest of the study team will occur only when the database is locked for final analysis or if a clinical situation arises in which the PI feels unmasking is necessary for the treatment of a SAE.

5.4.3 Reasons for Withdrawal

A study subject will be discontinued from participation in the study prior to completing 5 days of study product administration if:

A subject may be discontinued from the study for the following medical or administrative reasons:

1) Occurrence of an AE, which in the judgment of the investigator suggests an unacceptable risk to the subject to receive a test product (The investigator will follow the subject until satisfactory resolution of the AE or the AE is determined to be stable.) A likely example in this community might be the development of severe falciparum malaria during the product administration period.
2) Development of ANY condition fulfilling one of the exclusion criteria (except for out of range laboratory values);
3) Subject/parental request.

5.4.4 Handling of Withdrawals

The study is voluntary and allows for the withdrawal of participation at any point in the study. Despite this, we have a very low attrition rate in our studies. In general withdrawal from the study is partial- most frequently related to blood draws. In all cases, the reason for the withdrawal of consent will be discussed at length with parents. We will ask that the parents of the participant to continue to allow visits to monitor vital signs and illness history at the least. In most cases our experience informs us that parents will allow stool collection to continue. Blood draws are the most problematic- we will allow the family to opt for a fingerstick sample if the venous blood draw experience is that which is underlying the withdrawal. We also would allow for extended windows (i.e., blood draw at 35 days rather than 28 if the parent feels more time between blood draws would allow for their continued complete monitoring, although in this case a protocol deviation form would be included with the values. In short, every attempt will be made to obtain the most complete safety and tolerability data possible while respecting parental autonomy and their right to cease participation in a voluntary study. No replacement of subjects is planned, and we expect the number of consent withdrawals and missed study visits or missed study procedures to be low.

5.4.5 Termination of Study

This study may be prematurely terminated if, in the opinion of the PI or sponsor there is sufficient reasonable cause for termination. An example of a reason underlying termination would be NIDDK concurrence with DSMB recommendation to end study early, new findings that alter the safety profile of the product, discontinuation of study product for active and matching placebo production or impending market unavailability, the discovery of important protocol violations that may undermine the integrity and conclusions of the study.
If the study is prematurely terminated or suspended, the sponsor will promptly inform

the investigators/institutions so that the investigators can inform the U.S. and Peruvian regulatory authority(ies) of the termination or suspension and communicate the reason(s) for the termination or suspension. The IRB/IEC will also be informed promptly and provided the reason(s) for the termination or suspension by the sponsor or by the investigator/institution.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

6.1.1 Acquisition

The product will be obtained from BioGaia (Stockholm,Sweden). It is likely that a single shipment will be sufficient to cover the needs of the phase 1 infant trial as both product and placebo have a shelf life of 18 months. If unanticipated circumstances delay the start of phase 1 infant trial , a repeat shipment will be requested no later than 4 months prior to the expiration date of the product due to expire. It is possible that subsidiaries or agencies contracted by BioGaia packaging and preparation of product or placebo.

6.1.2 Formulation, Packaging, and Labeling

Formulation of test product and placebo are the following:

1. Active Product: Description *Lactobacillus reuteri* 17938 suspended in sunflower oil, medium chain triglyceride oil, silicone dioxide. Total viable count of *L. reuteri* 17938 1 x 10^8 CFU/5 drops. Study products supplied generally contain 2-8 x 10^8 CFU/5 drops from the manufacturer, in order to guarantee a potency of at least 1 x 10^8 CFU/5 drops by the product expiration date.
2. Placebo: Composition Sunflower oil, medium chain triglyceride oil and silicon dioxide. Total viable count of *L. reuteri* is zero CFU/ 5 drops.

The product is packaged in a single multidose amber glass vial per participant at a volume several times that required for the 5 day dose. The individual vials are packed in reinforced boxes for shipping with a temperature logging.

The label contains the following information
Name of Study: Safety of Lactobacillus reuteri in healthy children ages 2-24 months in Peru
BioGaia Drops Dose: 5 drops per dose Store at 2-8C Shake well before using
Test article code: xxxxxxx Discard 10 days after opening Exp date: yyyy Batch No: zzzz
Responsible MD: Margaret Kosek, Johns Hopkins University
Manufactured for BioGaia AB, Stockholm Sweden
Caution: New Drug- Limited to investigational use

6.1.3 Product Storage and Stability

The study product is to be stored at 2-8ºC in multidose subject specific amber vials.

Once opened, the contents of the vial should be dispensed in 10 days.

The product is administered in the clinical trial center by a member of the study staff in a designated product administration space. This space is adjacent to the dedicated product storage space in the clinic. The dosing is done once daily for 5 days and programmed based on participant and staff convenience. Details of product prescription and vial treatment confirmation upon each dose administration are described in the Manual of Procedures.

6.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product

The individual treatment vial is prepared and labelled by BioGaia. No reconstitution or dilution is needed. The vial will be inverted gently 5 times to assure homogenization and five drops of the suspension will be administered orally to the study subject by study staff that is blinded as to the treatment status of the individual.

6.3 Modification of Study Intervention/Investigational Product for a Subject

If the child vomits or spits up within 20 minutes of administration, the dose will be re-administered. If a subject has a SAE during the 5 day treatment window or if the child has blood cultures that are positive for *L. reuteri* product administration will be discontinued in that individual. All safety and planned protocol follow-up will continue.

6.4 Accountability Procedures for the Study Intervention/Investigational Product(s)

The study PI is accountable for the investigational product and the appropriate conduct of the clinical trial in compliance with ICH GCP guidelines and Peruvian laws and regulations.

Details on product accountability, including initial certificate of analysis, product accountability including the documentation of international cold chain transport and evaluation of product viability will be held onsite for evaluation by external monitors. The trial product will be shipped from the provider BioGaia and or suppliers by World Courier to Peru with temperature monitoring. Procedures for the temperature controlled movement and tracking of study product between the laboratory and the clinic is outlined in the MOP. Tests for product viability and the detection of possible contaminants are detailed in laboratory SOPs and detailed in the product assurance plan. At the end of the study all unused product will be autoclaved prior to disposal.

6.5 Assessment of Subject Compliance with Study Intervention/Investigational Product

All treatments are directly administered by study staff. Missed dosages will be documented. In the case that a subject is not available on a study day a 1 day window will be tolerated for the administration of a single missing dose. The daily dose is restricted to a single dose per day, so in this case, the dosing interval would occur over a 6 day period. Follow-up schedule would not be altered from the programmed plan. No more than a single dose will be given this window- if a second dose were to be missed, it would be documented in a protocol deviation form and analyzed per group treatment assignment. Subsequent doses are done at 24 hour intervals from the last dose, but visit scheduling cannot be altered after the first dose is administered.

6.6 Concomitant Medications/Treatments

All concomitant medications, including herbal or natural therapies are documented at

baseline or that are taken by the subject during the follow-up period will be documented on CRFs. Information on concomitant medications is solicited at baseline, upon each dosage with study product (D1-D5), D8, D12, D15, D18, D24, and D28.

Treatment for sepsis will be standard weight based doses of ceftriaxone and gentamicin. If the project clinician suspects that the product is a likely source of the sepsis (no source of lower respiratory tract infection, urinary tract infection, or diarrheal illness noted) clindamycin will be added to the treatment. Treatment guidelines are reviewed with study physicians and treatment is available on site to facilitate the rapid treatment of sepsis.

7 STUDY SCHEDULE

7.1 Recruitment process

A town meeting will be held to explain the research project to the community. Volunteers will be registered at this meeting or by contacting community health workers. A consent form, approved by the institutional review boards of Tulane, Johns Hopkins School of Public Health (JHSPH), and AB PRISMA as well as the Peru Instituto Nacional de Salud (INS; Peruvian government agency responsible for regulating clinical research) and written at the 8th grade level will be read aloud in Spanish to parents of potential participants after an informal explanation of the project by CITI trained study staff as per GCP guidelines. Parents of potential participants who agree to participate will then be asked to a private consent meeting where the process is repeated and they are given individualized attention in an environment that allows them to ask questions more freely by study staff, all of whom are CITI certified and have experience in conducting research in this community. They will then be asked to sign the form and be given a copy of the consent form with information that allows them to contact the Principal Investigator and the director of the IRB at AB PRISMA should the need arise prior to or during their child's participation in the study.

7.2 Consent Process and Documentation

Consent will be obtained from CITI certified employees of PRISMA with experience in biomedical research and community based research. Both parents must sign the consent form for children of this age to participate in clinical trials in Peru. The discussion will begin in a public forum where interested parties are invited by public loudspeaker to a community center. Individuals who retain interest in having their child participate after this forum will be invited to the study center where the process will be repeated in a private setting.

The study involves young children. To ensure the parental permission to have the child participate is valid, the study will be explained in detail to ensure that parents understand the risks and benefits for their particular child. The study team has extensive experience working with young children and their parents and the stress of study participation to the child participants will be minimized in part by clinical rapport gained from this experience.

A standard signed consent document written in Spanish at the 8th grade reading level will be used. Children of guardians lacking signatory capacity will not be allowed to participate.

7.3 Screening

1) The screening period may take up to 14 days, all days are categorized as D0 (day 0). repeat measures of biochemistry and hematology will be allowed in a one month time frame.

2) If the participant meets all criteria that do not require blood tests and both parents sign the informed consent, basic demographic data and clinical data, including use of medications and use of herbal or homeopathic treatments, will be recorded. A stool specimen will then be collected to test for presence of *L. reuteri* by PCR per Laboratory SOP (xxx). Potential participants must not currently be experiencing any of the monitored clinical adverse events as specified in Protocol Appendix C (Clinical adverse events to be monitored).

3) Following this step, blood will be collected for baseline (day 0) CBC, AST, ALT, total bilirubin, creatinine and BUN. Blood collection may be done by fingerstick, heelstick, or venipuncture, with method based on subject and parent/guardian preference and technical considerations. A total volume of 1.5 milliliters of blood is the minimum required volume to perform all of the assays listed above and a sample volume of 3.0 ml is the goal to ensure specimen adequacy for primary safety outcomes. Due to the time required for testing, reporting, and review of stool PCR and lab tests that would potentially exclude a participant, results of stool PCR and blood tests listed above from specimens collected within 14 days of initiating study product will be used as Day 0 blood and stool results. Out of range laboratory values detected by Day 0 blood test results will be documented on a CRF, and potential subjects will be excluded if an out of range laboratory value for tests monitored as potential adverse events is found, as described in Toxicity Table (Protocol Appendix E, based on Day 0 blood test results. However, subjects will only be excluded based on serum hemoglobin if they meet exclusion criterion 8.

4) Participants with no laboratory exclusion criteria (from *L reuteri* stool PCR, HIV, leucocyte count, severe anemia, AST, ALT, T. bili, BUN, creatinine) will have a standardized history and physical examination performed by a physician. Results of serum hemoglobin (exclusion criterion #8) and HIV serum antibody (exclusion criterion #7) will be reported as promptly as possible, usually within 48 hours, to determine if participants meet these exclusion criteria. If the stool specimen is positive for *L. reuteri,* the child will be excluded from the study (exclusion criterion #10). Subjects with negative stool PCR results for *L. reuteri,* negative HIV serology and adequate hemoglobin levels (per inclusion/exclusion criteria) will progress to the initial medical examination. The screening will take place in a nationally certified (INS- certified) clinical trial center in the community of Santa Clara. Subjects with positive results on HIV serology or inadequate hemoglobin levels will receive appropriate counseling and referral to medical services available in the community for patients with these conditions. HIV seropositive children will be referred to the only HIV referral (Hospital Regional de Loreto) directly by the study MD.

5) Subjects not excluded on the basis of laboratory tests will receive a baseline medical exam. A baseline assessment for presence of symptoms monitored for as AEs

will be done on Day 1 prior to randomization and receipt of initial dosing of study product. Subjects who have developed an exclusionary clinical adverse event since onset of the screening process (e.g. diarrhea, bronchospasm) will not be allowed to be randomized, but may be re-evaluated for inclusion at a later date by restarting the screening process for a period of thirty days following the onset of the AE.

7.4 Product Administration period

On days 1-5 participants will attend the study clinic to receive study product. The health worker will administer LR or PL and this treatment will be continued once daily for a total of five days. Participants will be monitored for 20 minutes. Any dose vomited within 20 minutes of administration will be repeated. Vital signs are monitored and symptoms are recorded.

Study participants will have daily visit at the community-based study clinic on study days 1-5 to detect symptoms, adverse events, and to administer the test article (LR or PL) under directly observed therapy. Parents of subjects will be instructed to in the use of a diary card, provided by the study personnel, to record any symptoms the infant experienced during the treatment phase and the first 28 day follow-up period. Parents of subjects will be instructed to record both solicited AEs (clinical symptoms listed as monitored AEs in Protocol Appendices C and E below) and unsolicited AEs on the diary cards. Field workers will verify information recorded, determine and document the severity of each AE recorded on the form, and assist with completing the card as needed. In the event that the AEs in question are assessed to be of a more severe intensity than what is recorded by the subject's parents, the diary card may be revised to reflect this change. However, field workers will not "downgrade" any solicited or unsolicited adverse events recorded by the parents. Parents of subjects will be instructed to contact the field worker or study physician immediately if the enrolled child develops fever or becomes ill. Subjects with AEs will be referred to the study physician, who evaluate the subject, generate an AE report, and make an initial determination of association with study product or not. AEs not listed in Appendices C, D, and E will be reported but not graded for severity.

On day 5, when the final dose is administered, study personnel will collect a 3 ml. blood sample for CBC, AST, ALT, total bilirubin, creatinine, and BUN. Out of range laboratory values will be documented on the appropriate CRFs for laboratory values.

Temperature will be documented daily while receiving study intervention, and participants with new onset of Grade 3 or 4 fever following initiation of intervention therapy (defined as oral, axillary, or temporal temperature $\geq$ 39.0 degrees C) and participants who appear especially ill or toxic to the field worker will have a physician evaluation. In these cases, blood will be examined in for malaria parasites by thick smear and a blood culture will be performed. In the event of positive blood cultures, presumptive antibiotic treatment and hospitalization (if deemed necessary by the attending physician) will be facilitated by project personnel. Antibiotic therapy will be the standard pediatric doses of Ceftriaxone and Gentamicin (for common causes of bacteremia from urinary and pulmonary sources)

with the addition of clindamycin if the study product is the suspected origin. Further details on procedures for evaluating and empirically treating subjects with fever are found in the section on Adverse Events, Bacteremia. Serious Adverse Events (SAEs) are defined in Section 9.2.2, and any participant who develops an SAE, regardless of cause, will discontinue receiving study product if this is still in progress, but will continue with study visits, data collection, and safety monitoring for the 3 month follow-up period of the study.

In order to compensate for the time and inconvenience of multiple visits, upon completing enrollment screening we will give participants an improved water storage container and a small food supply basket (containing milk, rice, oil, and tuna) and a small toy for your child. Following the 5th dose of study product we will offer another toy for their child. On study day 28 we will give a second food basket and a third toy that their child can choose for successful completion of the study.

7.5 Follow-up

A. First 28 Days. The first 28 day follow-up period includes intense monitoring for adverse effects, starting on the day when the first dose of probiotic or placebo is administered (day 0), and continuing past the end of the treatment phase on day 5 and ending on day 36, or 36 days after the first dose was administered. Symptom data for AE monitoring will be completed daily by the participant's parent or guardian for 6 weeks and verified by project staff during home visits on days 8, 12, 15, 18, 24, and 28. Information on symptoms and medication use will be recorded by the parents of participant on diary cards provided by the study. Temperature will be recorded on these visits by project staff. Diary cards used will be similar to those used in the Phase One study for children ages 2-5 years (NCT020124122). On day 28, field workers will collect diary cards and document data on clinical symptoms, vital signs, and repeat blood tests (as on day 5).

Subjects with fever will be referred to the study physician for a full physical exam and collection of specimens for fever evaluation (malaria smear, blood culture). Subjects with serum hemoglobin less than 11 gm/dL at baseline or at day 5 (or Hb less than 9.5 gm/dl at baseline or at day 5 for infants 5 months of age or younger) will be treated with iron supplementation in the form of iron sulfate drops or chewable iron tablets, according to American Academy of Pediatrics guidelines [Clinical Report—Diagnosis and Prevention of Iron Deficiency and Iron-Deficiency Anemia in Infants and Young Children (0–3 Years of Age) Baker RD, Greer FR, The AAP committee on Nutrition; Pediatr 126 (5): 1-11, 2010] (PMID:20923825). When indicated, iron supplementation will be provided starting at least 5 days after the last dose of study product to prevent confounding the assessment of study related adverse events with the common side effects of supplemental iron therapy (gastrointestinal upset, etc), an administration of iron supplementation will be controlled for in analysis of adverse event data (clinical and laboratory test outcomes by study group at days 5 and 28). Treatment will consist of an oral liquid formulation dosed at 3-6 mg elemental iron/kg/24 hours. Iron supplementation will be continued for 2 months after initiation, and serum hemoglobin will be measured using blood collected by fingerstick tested in a HemoCue device at the end of the 2 months treatment period. Children with serum hemoglobin less than 11 gm/dL at this 2 month point (or less than 9.5 gm/dl at baseline or at day 5 for infants 5 months of age or younger) will be referred to a local pediatrician for further evaluation and individualized treatment if needed. Individuals with helminth infection will be treated with albendazole therapy based on cumulative testing results on study day 36. For ascaris and hookworm this consists of one dose of albendazole, whereas doses will be given for 3 days for trichuris and strongyloides infections.

Stool samples will be obtained on day 3, 5, 12, 15, 18, 24, 28, and 36 for the presence of *Lactobacillus reuteri* by PCR.

B. Day 28 to 3 month endpoint. After day 28, parents of the subjects will be instructed to report all episodes of febrile illness occurring during the 3 month follow-up period, and to present to the study clinic for fever evaluation whenever the parents of the subject

suspects they are febrile or a new febrile episode is documented. Febrile episodes detected by participant presentation during the follow-up period will be handled in the same way as febrile episodes detected by active surveillance during the treatment phase. Study personnel will contact parents of participants 3 months after starting study treatment to document new onset of AEs and SAEs not previously recorded, onset of new medical conditions, hospitalizations, and use of new medications and herbal or homeopathic treatments not previously documented.

In all, 13 study contacts are expected with study participants (See Appendix A). The study is expected to be conducted in a 7 month period.

7.6 Final Study Visit

On the final visit, the CRF for study completion or non-continuation form is filled out to allow for CRF and laboratory specimen form missingness to be tracked.

7.7 Early Termination Visit

All subjects who do not complete the full 3 months of study participation for any reason will have a termination visit that documents the reason and date of non-continuation with all study procedures. If the participant agrees to continue with study visits, data collection, and partial study tests or procedures (ie no blood draws) this is documented only on protocol deviation forms for the procedures that the participants parent specifically decline.

7.8 Unscheduled Visit

Unscheduled visits to the clinic will be documented on an AE form. This would both document encounters initiated by the patient coming to the clinic, or the laboratory seeking out the patient because of an unexpected laboratory finding (positive malaria smear, for example).

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

Clinical evaluations include a standardized medical history and physical examconducted by a physician in an interview as part of the screening process. No personal health records will be sought and medical history will be based on parental interview. A standardized physical examination will also be done that will include length, weight, temperature, heart rate, respiratory rate, ENT exam, cardiac, pulmonary, and abdominal exams at the time of screening only. Targeted physical examinations will be done with all AE investigations, and vital signs (temperature, pulse, respiratory rate) reported in each instance.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

- **Hematology:** hemoglobin, white blood cells (WBC) with differential count, platelet count done during screening and on D5 and D28.
- **Biochemistry:** creatinine, blood urea nitrogen, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) done on screening and D5 and D28.
- **Immunology**: HIV test to be done as part of screening process.

8.2.2 Special Assays or Procedures

Quantification of viable count of probiotic will be done per existing laboratory SOP. Detection of *Lactobacillus reuteri* will be done following DNA extraction with a soil MoBio kit with bead-beating per existing laboratory protocol. Specimen reception, barcoding and handling will be done as per laboratory SOP. Sample preparation for international shipping will be done by IATA certified staff and shipped under relevant DHHS CDC permits and in compliance with national and international laws.

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9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

1. Evidence of invasive infection resulting from the administration of *L. reuteri* DSM 17938. Baseline and daily temperature measurements will be made during the study period. Complete hemogram with differential count obtained at baseline, on day 5, and on day 28. Vital signs will be monitored daily during the administration of the treatment (days 1-5). The evaluation of all febrile episodes will be done and include a physician exam and the evaluation of blood smears for malaria and blood cultures for the evaluation of possible bacteremia.

2. Clinical tolerance. The inclusion of a placebo group will allow for comparisons of daily symptom measurements to be compared between the treatment and placebo group. The symptoms will be monitored for at household visits, and will be graded as adverse events according to defined criteria.

Symptoms monitored as AEs include fever, vomiting, diarrhea, irritability, rash, pruritis, and wheezing or brochospasm. Diagnostic microbiology will be performed on all stool specimens from subjects who develop diarrhea during the 90 days following initiation of therapy.

3. Evidence of toxicity resulting from the L. reuteri DSM 17938 preparation. Participants will have baseline, day 5, and day 28 assessment of hematologic status and renal and hepatic function. Assessment of renal function will include blood urea nitrogen (BUN) and creatinine (Cr). Assessment of hepatic toxicity will include transaminases (AST, ALT) and total bilirubin. Hematologic status will be determined by CBC to measure hemoglobin and absolute leucocyte count.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

Adverse Event: ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of study product including active or placebo.

According to the U.S. DHHS Common Rule an AE **does** include any:

- exacerbation of a pre-existing illness, such as asthma.
- increase in frequency or intensity of a pre-existing episodic event or condition.
- condition detected or diagnosed after study product administration even though it may have been present prior to the start of the study.
- continuous persistent disease or symptoms present at baseline that worsen following the start of the study.
- symptoms associated with disease not previously reported by the subject will be recorded as an AE.

According to the U.S. DHHS Common Rule (http://www.hhs.gov/ohrp/policy/advevntguid.html) an AE **does not** include a/an:

- medical or surgical procedure (eg, surgery, circumcision, transfusion); the condition that leads to the procedure is an AE.
- pre-existing diseases or conditions present or detected at the start of the study that do not worsen.
- situations where an untoward medical occurrence has not occurred (eg, hospitalization for elective surgery, social and/or convenience admissions).

All AEs including local and systemic reactions not meeting the criteria for "serious adverse events" (See Section 9.2.2) will be captured on the ADVERSE EVENT CRF. Information to be collected includes event description, time of onset, clinician's (MD) assessment of severity, relationship to study product and time of resolution/stabilization of the event. All AEs occurring while on study product or within 90 days of follow-up must be documented appropriately regardless of relationship to the study product. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it will be recorded as an AE.

Specific AEs that will be monitored in this project:

During probiotic therapy, study field workers will monitor for other potential adverse effects during their home visits, based on parental interview and information recorded on a study log. **Expected AEs** that are common in this study population will include fever, nausea/upset stomach, vomiting, diarrhea, irritability, rash, pruritis, and wheezing or bronchospasm. Diarrhea episodes will be characterized as watery or dysenteric. Space will also be provided for the open end documentation of any other symptom which could

potentially be related to probiotic use. Appendix B and D details expected AEs and how they will be graded by severity**.** Grading of AEs will be confirmed by the project physician. AEs not listed as Expected AEs in the Toxicity Table will be reported but not graded for severity. Responses to AEs encountered will be at the discretion of the project physician and study monitor, based on type of AE encountered and its severity.

Severity of Event: Expected AEs will be assessed by the clinician using a protocol defined grading system. For events not included in the protocol defined grading system, the following guidelines will be used to quantify intensity.

Level 1 = Mild (awareness of a symptom but the symptom is easily tolerated)

Level 2 = Moderate (discomfort enough to cause interference with usual activity)

Level 3 = Severe (incapacitating; unable to perform usual activities; requires change in intensity of caregiver activity or bed rest)

Level 4 = Life threatening

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Method, Frequency, and Time Period for Detecting AEs

At each visit, after the parent of the subject has had an opportunity to spontaneously mention any problems, the investigator will inquire about AEs by asking the following standard questions of the parent:

"Has your child had any (other) medical problems since your last visit/assessment?"

"Has your child taken any new medicines, other than those given to you in this study, since your last visit/assessment?"

Relationship to Study Products: AEs will be assessed for relationship to study product. The clinician's assessment of an AE's relationship to test article (study drug) is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event will be reported. All AEs must have their relationship to study product assessed using the terms: associated or not associated.

In a clinical trial, the study product must always be suspect. To help assess, the following guidelines will be used.

- **Associated** – The event is temporally related to the administration of the study

product and no other etiology explains the event.

- **Not Associated** – The event is temporally independent of study product and/or the event appears to be explained by another etiology.

9.2.2 Serious Adverse Events (SAE)

Definition of a Serious Adverse Experience (SAE):

Serious adverse event (SAE): A Serious Adverse Event is defined as an AE meeting one of the following conditions

- Death during the 3 month period of protocol defined surveillance
- Life Threatening Event (defined as a subject at immediate risk of death at the time of the event)
- An event requiring inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance
- Results in persistent or significant disability/incapacity
- Any episode of bacteremia due to *Lactobacillus reuteri*, the study probiotic
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject 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 convulsions that do not result in inpatient hospitalization.
- Any monitored lab value meeting the Grade 3 or Grade 4 toxicity criteria

Bacteremia: Any episode of bacteremia due to *Lactobacillus reuteri*, the study probiotic, will be considered a Serious Adverse Event. Subjects started on probiotic/placebo therapy will be monitored for adverse events during the 5 days of probiotic therapy and at 2-5 day intervals (when symptoms are recorded and stools are collected) following discontinuation of therapy. Temperature will be documented daily while receiving study intervention and at follow-up visits which include AE monitoring, and participants with new onset of fever following initiation of intervention therapy and participants who appear especially ill or toxic to the field worker will have a physician evaluation. Subjects will also be instructed to present to study personnel for evaluation whenever they suspect they are febrile or a new febrile episode is documented. In cases where fever is grade 3 or 4, or when the subject appears particularly ill or toxic in the estimation of the field worker or

physician, blood will be examined in for malaria parasites by thick smear (a standard daily diagnostic procedure in our laboratory) within 8 hours of collection, and antimalarial treatment provided by the Ministry of Health will be administered to subjects with fever and parasitemia. Subjects evaluated in this manner will have a blood culture specimen taken and close monitoring. Presumptive medical therapy will be started at the discretion of the study physician. In the event of positive blood cultures, presumptive antibiotic treatment and hospitalization (if deemed necessary by the attending physician) will be facilitated by project personnel (including procurement). Antibiotic therapy will be the standard pediatric doses of Ceftriaxone and gentamicin for presumed or confirmed common causes of sepsis in this population (*E. coli, S. pneumonia*) and clindamycin will be used if study product associated bacteremia is suspected (no source of lower respiratory infection, urinary tract infection, or diarrheal illness noted) or supported by microbiologic data (gram positive rods on initial gram stain).

Documenting SAEs

Any AE and SAE occurring during the study must be documented in the SAE CRF and on form FDA 3500 MedWatch. A separate MedWatch form should be used for each SAE. However, if at the time of initial reporting, multiple SAEs are present, they may be reported on the same "SAE" page. If a subject dies during participation in the study or in the 85 days following the administration, NIDDK will be provided with a copy of any post-mortem findings, including histopathology.

Post-study AEs and SAEs

Investigators will not actively seek AEs or SAEs in study participants beyond day 28. However, if the investigator learns of any AE at any time after active surveillance ends on day 28 or a subject has completed the study at 3 months, and such event(s) is (are) reasonably related to the study participation, the investigator will promptly notify NIDDK and document the event in as much detail as can be ascertained.

9.2.3 Unanticipated Problems

The Office for Human Research Protections (OHRP) Common Rule 45 CFR §46.103 considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (in the guidance document, possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

An incident, experience, or outcome that meets any of the three criteria above may warrant consideration of substantive changes in the trial in order to protect the safety, welfare, or rights of subjects or others. Examples of corrective actions or substantive changes that might need to be considered in response to an unanticipated problem include:

- changes to the research protocol initiated by the investigator prior to obtaining IRB approval to eliminate apparent immediate hazards to subjects
- modification of inclusion or exclusion criteria to mitigate the newly identified risks
- implementation of additional procedures for monitoring subjects
- suspension of enrollment of new subjects
- suspension of research procedures in currently enrolled subjects
- modification of informed consent documents to include a description of newly recognized risks
- provision of additional information about newly recognized risks to previously enrolled subjects.

Some incidents, experiences, and outcomes that occur during the conduct of human subjects research represent unanticipated problems but are not considered adverse events. These may involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased risk of harm, but no harm occurs. Unanticipated problems must be reported, even if they do not involve adverse events. However, adverse events that do not meet the criteria to be considered unanticipated problems are not subject to these reporting requirements.

Incidents or events that meet the OHRP criteria for unanticipated problems involving risks to subjects or others require the completion of an AE report form.

Additional reporting requirements. Abuse or illegal activities will be reported to Peruvian authorities in a manner consistent with local laws. A written note, documenting the concern, and witnessed of reported behavior giving rise to the concern, is issued to the Peruvian community designated “Defensor de Pueblo” the local legal authority that investigates cases of child abuse and exploitation.

9.2.4 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

Baseline lab tests will be reviewed by the study physician as part of the screening process. In order to be eligible to participate in the study, all monitored baseline laboratory values must be within the normal range for the reference laboratory.

Laboratory values provided by the clinical laboratory that are not among the group of monitored laboratory tests do not have to be within the normal range in order for a consented subject to become a participant, but these reportedly abnormal values will be noted on the FORM FOR EVALUTION OF LABORATORY VALUES. These non-monitored laboratory values provided as part of the complete blood count (CBC) include platelet count and percentage of various leukocyte categories in the differential. Monitored laboratory values are:

- Hemoglobin
- Total leukocyte count
- Total serum bilirubin
- Blood urea nitrogen (BUN)
- Creatinine
- Alanine transaminase (ALT)
- Aspartate aminotransferase (AST)

Monitored laboratory values will be reviewed by the study physician within 48 hours of receiving the report from the reference laboratory, and values will be recorded on the appropriate CRF (FORM FOR EVALUATION OF LABORATORY VALUES). Abnormal values meeting toxicity level 1 or 2 which do not warrant further clinical investigation will be noted as such on the laboratory values CRF along with an assessment of relationship to the study product. These abnormal values will be reported to the oversight agencies as AEs according to procedures described below. Abnormal values meeting toxicity level 1 or 2 which may, in the judgment of the study physician, warrant further investigation will result in a clinic visit for full evaluation recorded on the ADVERSE EVENT CRF and will be followed to completion and resolution as with other AEs. Abnormal values meeting toxicity levels 3 or 4 will be considered SAEs and will be evaluated, monitored, and reported according to procedures described for SAEs.

Documenting AEs

Any AE occurring during the study must be documented in the subject's medical records and on the appropriate AE CRF, i.e. the AE Report Form. Information to be collected on the AE Report Form includes event description, time of onset, investigator assessment of severity, and relationship to the study product, time of resolution of the event, seriousness, and outcome. All adverse events occurring during the study must be documented appropriately regardless to the relationship of the event with study participation.

The investigator or study physician should attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. A diagnosis, if known, or clinical signs and symptoms if diagnosis is unknown, rather than the clinically significant abnormal laboratory finding, should be completed on AE CRF form. If no diagnosis is known and clinical signs and symptoms are not present, then the abnormal finding should be recorded. The laboratory data should either be recorded on the AE forms with the reference range and baseline value(s) or copies of the laboratory reports and reference ranges should be sent with the CRF pages.

9.3 Reporting Procedures

AE Reporting:

AEs. This study will report AEs to the DSMB and NIDDK quarterly. Any medical condition that is present at the time that the patient is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study it should be recorded as an AE.

AEs will be reported annually to the following institutional representatives at the fax and email addresses provided:

1.	Tulane University SPHTM.	Ms. Roxanne Johnson Director, Tulane University Biomedical IRB rjohnso@tulane.edu Fax 504-988-4766 Tel. 504-988-2665
2.	Johns Hopkins Bloomberg School of Public Health	Ms. Joan Petit, IRB Office Director irboffice@jhu.edu Fax: 410-502-0584 Tel. 410-502-1999
3.	Asociación Benéfica PRISMA	Salomon Zavala Sarrio, IRB Chairman szavalas@gmail.com Fax 011-511-6165501 Tel. 011-511-6165500 Ext 246
4.	Instituto Nacional de Salud (Lima, Peru)	Contact information to be provided
5.	NIDDK	Jose Serrano, MD PhD Program Official for PRIDEC-Peru Division of Digestive Diseases and Nutrition National Institute of Diabetes and Digestive and Kidney Diseases National Institutes of Health serranoj@mail.nih.gov Tel. 301-594-8871 Fax: 301-480-8300
7.	BioGaia AB	Karin B Diderot

BioGaia AB
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8. DSMB

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6707 Democracy Blvd, Rm 644
Bethesda, MD 20892-5450
Phone: 301 594 7024
Fax: 301 480 8300
Email: torrancer@niddk.nih.gov

Ms. Torrance will be the NIDDK designate for communications with the DSMB.

9.3.1 Serious Adverse Events

Any AE considered serious by the study physician or which meets the aforementioned criteria must be submitted on a SAE form to the appropriate IRBs, INS, DSMB designate and the NIDDK. The SAE form will be sent as an email attachment or fax to each relevant institutional representative within the time frame below:

* All deaths, whether related or unrelated, will be recorded on the Serious Event Form and on MedWATCH FDA 3500 (2/13) and sent by fax and email within 24 hours of the site awareness of the death.

* Serious adverse events other than death, regardless of relationship, will be reported via fax and email by the site within 24 hours of becoming aware of the event.

Other supporting documentation of the event may be provided upon request as soon as possible.

All serious adverse events will be:

- recorded on the appropriate serious event case report form
- followed through resolution by a study physician
- reviewed by a study physician

All SAEs will be followed until satisfactory resolution of the event or until the study physician deems the event to be chronic or the patient to be stable.

The investigator, or responsible person according to local requirements, must comply with the applicable local regulatory requirements related to the reporting of SAEs to regulatory authorities and the IRB/EC.

SAEs will be reported to the institutional representatives at the fax and email addresses shown in the section entitled Reporting of Adverse Events.

9.3.2 Regulatory Reporting for Studies Not Conducted Under NIDDK-Sponsored IND

Procedures for reporting AEs and SAEs are described in the corresponding sections on AE and SAE reporting.

9.4 Type and Duration of Follow-up of Subjects after Adverse Events

Follow-up of AEs
All AEs will be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or the subject is lost to follow-up. The ADVERSE EVENT CRF includes space for up to two follow-up visits for the same AE. The investigators will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate as completely as practical the nature and/or causality of the AE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. New or updated information should be recorded on the originally completed AE CRF with all changes signed and dated by the investigator.

9.5 Halting Rules

The study will be halted if two or more individuals develop similar SAEs for which a relationship to the study product cannot be excluded upon study physician and medical monitor review. Participants who develop an SAE, regardless of cause, will discontinue the study treatment if treatment is still ongoing.

Subsequent review of serious, unexpected, and related AEs by the Medical Monitor, IRB, or NIDDK may also result in suspension of further administration of study product. NIDDK, typically in conjunction with DSMB recommendations, retains the authority to suspend additional enrollment and study interventions/administration of study product for the entire study, as applicable.

Examples of findings that might trigger a safety review are the number of SAEs overall

and by treatment group during the closed DSMB sessions, the number of occurrences of a particular type of SAE, severe AEs/reactions, or increased frequency of events.

9.6 Safety Oversight

Safety oversight for this Phase 1 study Safety of *Lactobacillus reuteri* in healthy children aged 2-24 months is outlined in the attached Data and Safety Monitoring Plan. A medical monitor has been contracted to evaluate AE reporting and they and NIDDK staff and study staff will review open reports that do not specify treatment group when adverse events and out of range values are summarized on a quarterly basis. Additionally, an NIDDK appointed DSMB will monitor safety data by treatment group for this Phase 1 study by examining a closed report in which out of range laboratory values and adverse events are reported by treatment group on a quarterly basis. This closed report will be prepared based on the final open report. Our project biostatistician (unmasked) will communicate with the project biostatistician who is not involved in any data entry or cleaning of the data while the trial is ongoing and transfer the data and treatment assignment by PID to the biostatistician to elaborate the same tables as those present in the DSMP, but with treatment assignment. This will be reviewed by unblinded members of the NIDDK as well as the DSMB.

10 CLINICAL MONITORING

Clinical monitoring will be carried out in two ways:

1. The investigators have outlined a detailed Data and Safety Monitoring Plan, described in a separate DSMP document. The clinical and statistical monitors performing this function are listed in section 9.6 Safety Oversight. Procedures for and frequency of this clinical monitoring are described in the DSMP.

2. NIDDK will provide regulatory and compliance monitoring through an NIDDK-appointed site monitor, contracted through a certified research monitoring agency such as Westat. Frequency and scope of this oversight will be determined by NIDDK.

10.1 Site Monitoring Plan

Site monitoring is conducted to ensure that the human subject protection, study procedures, laboratory, study intervention administration, and data collection processes are of high quality and meet the sponsor, ICH E6 and, when appropriate, regulatory guidelines.

11 STATISTICAL CONSIDERATIONS

Data will be collected, checked, and entered into and a data management system in a Microsoft ACCESS database with identity and time stamping. All patients will be assigned a unique identifier (Personal Identification or PID) which will be the link for all databases and appear on all derived biospecimens. We will use filters for the invalid entries and the identification of missing and out-of-range values and a double data entry system. All inconsistencies between entries must be resolved with differences able to be reviewed by supervisors and auditors. Access to the database will be restricted combined physical access restriction and password protection. Analyses will be conducted in STATA and SAS.

11.1 Study Hypotheses and Analysis

A. **The primary hypotheses** are that *L. reuteri* therapy at the specified dose is safe and well-tolerated in children aged 2-24 months. There are three areas of safety and tolerability that will be assessed in this Phase 1 trial.

1. **Evidence of bacteremia or invasive infection resulting from the administration of L. reuteri 17938**. Baseline and daily temperature measurements will be made during the study period during the product administration phase and then on all scheduled home visits. Complete blood counts with differential counts will be obtained at baseline, on day 5, and on day 28. The evaluation of all documented febrile episodes will include an examination by a physician, and thick smear for malaria, and blood culture for the evaluation of bacteremia is the temperature is greater than 39ºC using temporal artery probe measures.

   Specific outcome parameters in this category include:

   a. Mean daily temperature
   b. Incidence of fever on study days 1-5
   c. Frequency of temperature readings categorized as fever (adjusted for number of subjects per group)
   d. Number of episodes of *L. reuteri* bacteremia

2. **Evidence of toxicity resulting from the L. reuteri DSM 17938 preparation.** Participants will have baseline, day 5, and day 28 assessment of hematologic status and

renal and hepatic function. Assessment of renal function will include blood urea nitrogen (BUN) and creatinine (Cr). Assessment of hepatotoxicity will include transaminases (AST, ALT) and total bilirubin. Hematologic status will be determined by CBC to measure hemoglobin count and absolute neutrophil count, as mentioned above.

Specific outcome parameters in this category include:

a. Mean values for laboratory tests monitored (adjusted for number of subjects per group). These are listed in Appendix D.
b. Frequency of out of range values for laboratory tests monitored (adjusted for number of subjects per group). These are listed in Appendix D.

2. **Clinical tolerance**. The inclusion of a placebo group will allow for comparisons of the prevalence of daily symptom measurements between treatment groups. The symptoms monitored on the daily diary card (fever, anorexia/decreased oral intake, vomiting, diarrhea, irritability, rash, itching, wheezing) are graded by the parent(s) of the participant as mild, moderate, severe, or very severe. Parents report of severity will be analyzed, except in the case that the physician or study team deems them to be more severe than reported (the study team may upgrade, but NOT downgrade the severity of the symptom).

   Specific outcome parameters in this category include:

   a. Frequency and severity of symptoms monitored as AEs (adjusted for number of subjects per group)
   b. Frequency of all AEs (adjusted for number of subjects per group)
   c. Frequency of SAEs (adjusted for number of subjects per group)

All continuous variables will be summarized using descriptive statistics including number (N), mean, standard deviation (SD), median, maximum, and minimum. All categorical variables will be summarized using frequency counts and percentages.

Changes from baseline temperature and fever graded by defined toxicity categories will be summarized using descriptive statistics by treatment group.

Concomitant medications, including those taken prior to study drug administration, will be listed by subject. All concomitant medications taken after initiation of the study treatment will be tabulated overall and by formulation. The number and percentage of subjects who take at least 1 concomitant medication and the number and percent of subjects who take each specific concomitant medication will be presented.

Treatment-emergent AEs will be defined as any event with a start date occurring on or after treatment Day 1 or, if pre-existing, worsening after treatment Day 1. If a subject reports the same AE more than once, then that subject will only be counted once for the

summary of that AE, using the most severe intensity.

Treatment-emergent AEs will be summarized as follows to the DSMB during closed sessions:

- All treatment-emergent AEs;
- All treatment-emergent AEs by intensity;
- SAEs;
- All treatment-emergent SAEs by relationship to study drug; and
- AEs that led to premature discontinuation of study drug.

All SAEs and AEs leading to premature withdrawal from the study will be listed.

Hematology and blood chemistry parameters will be summarized at baseline and at the end of the study. Laboratory values and changes from baseline in laboratory values will be summarized descriptively for all subjects and by treatment group. A summary of shifts from baseline to final evaluation will be given for each parameter for all subjects and by treatment group. The normal range for each parameter will be used to create categories of low, normal, or high. Any result higher than the upper limit normal (ULN) will be categorized as high, any result lower than the lower limit of normal (LLN) will be categorized as low, and any result within the LLN and ULN will be categorized as normal. The number and percentage of subjects in each shift category from baseline to final evaluation will be shown for each parameter. Only the hematology and blood chemistry tests with a numeric normal range and at least one follow-up value will be analyzed for shifts from baseline. The number and percentage of subjects with clinically significant values will be presented.

B. The secondary outcome measurements are

1. To determine the duration of shedding of *L. reuteri* DSM 17938 following controlled administration.

Since the longitudinal nature of the repeated PCR assays results in correlated outcomes, generalized estimating equations (GEE) will be used to model the binary response whether subjects exhibited a positive PCR result during the various period of study. The duration of shedding of *L. reuteri* will be assessed using Kaplan Meier Product limit estimates.

11.2 Sample Size Considerations

The sample size for this Phase 1 safety trial in healthy children aged 2-24 months is determined by historical precedent for safety in human use.

Enrollment for the prior Phase 1 studies in adults (Safety of LR in Healthy volunteers #NCT00774163) and children (Safety of LR in healthy children 2 to 5 years of age in Peru #NCT02124122) have been able to recruit and retain >95 subject (Phase 1 child study in progress). We have high and similar rates of retention in our other studies with more complexity and participant burden, even among our birth cohorts.[37]

Missing data will not be imputed. Data will be analyzed per treatment group assignment after the administration of a single dose of product for all safety and tolerability analyses. Data regarding the duration of carriage will be restricted to those individuals who complete 5 days of therapy.

The amount of subjects in this study will likely be underpowered to detect differences in most secondary outcomes. This is in part due to the lack of information on key parameters needed for sample size calculations. For example, in this age and context, to calculate the duration of *L. reuteri* carriage following administration it is not known how many subjects will become positive for *L. reuteri* by PCR following administration. Similarly, it is not known how frequent intermittent passage of *L reuteri* positive stool is in individuals in this community, although about 10% of individuals in this age range test positive. This data collected as part of this Phase 1 study will provide important information of use in subsequent phase 2 trial [Efficacy of *Lactobacillus reuteri* in healthy children aged 2-24 months in the treatment of environmental enteropathy] .

11.3 Planned Interim Analyses (if applicable)

No formal interim analyses are planned; however, accumulated phase 1 trial data will be reviewed by treatment group every 3 months by the NIDDK and DSMB.

11.3.1 Safety Review

Subject accrual, status adherence data on visits and intervention, adverse events and rates of adverse events will be reported to the DSMB every three months. SAEs will be reported within 24 hours of occurrence. Details of the interval reports are specified in the DSMP.

The study would be halted if two or more subjects develop a similar SAE for which the relationship to the study product cannot be excluded. This would include, but not be limited to bacteremia with the product strain.

Individuals with a SAE during the period of probiotic administration will have the intervention stopped regardless of the determined relationship to the study product, but follow-up monitoring visits and testing would be completed to the greatest extent possible, and the SAE would be managed until resolved.

INS requests SAE reporting, annual reports, and study termination report. Annual reporting to IRB is planned for Tulane, Johns Hopkins University and AB PRISMA.

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12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

All participants will have a chart composed of a copy of the signed consent document, all CRFs, clinical laboratory values (CBC, AST, ALT, BUN, Creatinine, and total bilirubin and screening day 0 *L. reuteri* PCR status, but not subsequent Lr PCR tests), diagnostic testing results performed as part of adverse event evaluations, and copies of hospital records in the case of a SAE. These charts will be available for review by study personnel, the Medical Monitor, the INS, the regional directorate of Health of Loreto, authorized representatives of NIDDK, OHRP, and other Department of Health and Human Services agencies, as applicable; the Ethics committee of AB PRISMA, and the Institutional Review Boards of Johns Hopkins University and Tulane University. Study source documents are stored in locked cabinets within locked rooms of private research space when not being reviewed. Databases are present in restricted areas of these buildings and are password protected.

The study product control, including the review of temperature and shipping logs, viability tests, and product tracking logs are available for review by auditors from the agencies mentioned above.

Tests related to PCR carriage are generated in experimental files. These results are then matched to SID and transferred to the database (in the case of ELISA results) or transferred via a access file in which the technician manually reads the photo file and inputs the result by SID which is then added to the database. All experimental files are available for review.

All source documents, with the exception of microbiome data, will have primary storage at the laboratory and field sites in Iquitos Peru. Microbiome data will be stored in in the absence of identifiers in commercial laboratories in the United States and on servers located at Johns Hopkins University. The Laboratory and offices at AB PRISMA are used for long term storage of physical documents and the secure storage of electronic versions of the data on a server. The building has 24hr staff presence and is a private facility with limited access.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Quality Assurance

To ensure the quality and integrity of laboratory and clinical data, detailed SOPs will be written to clarify correct procedures and handing of data and specimens that extends from field activities to data management. Staff will be trained in CITI and the SOP portions that correspond to their responsibilities in the study. Study responsibilities will be specified in the Site personnel and signature log. Training will be documented and documentation will consist of a training duration, specific areas covered in training, and register of participants. This training, which will occur prior to the study and reinforced during the study, will be documented in the study file for review by study monitors.

Laboratory protocols

Laboratory protocols for the collection, registration and barcoding of all biologic samples will be detailed.

Product Assurance

The Certification of Assurance of the study product, obtained by the product supplier, will contain the following information.

Identity

Identity of the test product will be confirmed by standard carbohydrate utilization tests (API 50CH test (BioMerieux) pattern and the plasmid profile of the strain will be documented.

Purity

Information on the content of Salmonella, *E. coli*, yeast and mold, and total aerobic count per USP <61> .[43]Evaluation for contamination with Shigella will be by FDA protocol BAM Chapter 6.). The release limit of Salmonella, Shigella and *E.coli* is absent in 10g of product, yeast and mold < 100 CFU/g and total aerobic count < 1000 CFU/g.

Viable counts of probiotic product strain

Existing laboratory protocols will be employed, with modifications as necessary, for quantification of the viable count of product strain (name protocol). Viable counts will be performed prior to study enrollment and every month following study initiation until the last dose of product is administered. Tolerance for variability will be $\pm$ 1 log CFU count. If the results are not within this range, the product will be re-analyzed from two vials from the same stock. If again not within range, this stock will be discarded and replaced with new study product provided by BioGaia, the product manufacturer.

All study personnel engaged in study activities must have training documented in the form

of signatures on training logs. For laboratory scientists, this includes practicums demonstrating test proficiency in assay performance and the correct storage and naming of experimental files. Upon completion of testing, they sign the SOP to acknowledge proficiency certification and compliance with the protocol SOP.

Quality Control

CRFs and source documents will be reviewed for completeness by a field or data entry personnel daily and forms will be reviewed on site by the Study Coordinator, the study physician, and /or overseeing investigators. Upon completion, forms are further evaluated for missing or incomplete data by data management personnel prior to data entry.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The investigators will ensure that this study is planned and conducted in full conformity with the principles set forth in the Declaration of Helsinki, CIOMS, International Ethical Guidelines for Biomedical Research Involving Human Subjects (2002), and in accordance to laws governing the conduct of clinical trials in Peru (XV article 28 of law 26842).

14.2 Institutional Review Board

The relevant IRB and oversight committees are the following, which must approve of the protocol, parental permission documents, and any amendments to the protocols. They also must be made aware of protocol deviations affecting the safety of participants, product concerns, or any new information that may affect the balance of risk and benefit to participants.

1.	Johns Hopkins School of Hygiene & Public Health	Joan Pettit, IRB Director jhsph.irboffice@jhu.edu Fax: 410-502-0584 Tel. 410-502-1999
2.	Tulane University SPHTM	Ms. Roxanne Johnson Director, Tulane University Biomedical IRB rjohnso@tulane.edu Fax 504-988-4766 Tel. 504-988-2665
3.	Asociación Benéfica PRISMA	Salomon Zavala Sarrio, IRB Chairman szavalas@gmail.com Fax 011-511-6165501 Tel. 011-511-6165500 Ext 246
4.	Instituto Nacional de Salud (INS; Lima, Peru)	Contact information to be provided

14.3 Informed Consent Process

Consent will be obtained by CITI certified employees of PRISMA with experience in biomedical research and community based research. Both parents must sign the consent form for children of this age to participate in clinical trials in Peru. The discussion will begin in a public forum where interested parties are invited by public loudspeaker to a community center. Individuals who retain interest in having their children participate after this forum will be invited to the study center where the process will be repeated in a private setting.

The study involves young children. To ensure the parental permission to have the child participate is valid, the study will be explained in detail to ensure that parents understand the risks and benefits for their particular child. The study team has extensive experience working with young children and their parents and the stress of study participation to the child participants will be minimized in part by clinical rapport gained from this experience.

A standard signed consent document will be used. Children of guardians lacking signatory capacity will not be allowed to participate. Study participants will be given a copy of the consent document to ensure their understanding (by giving them opportunities to present and discuss with 3rd parties, and to provide necessary contact information with study personnel or local or international IRBs overseeing the study.

14.3.1 Informed Consent/Assent Process (in Case of a Minor)

All subjects are under the age of 2 years and will participate with parental permission.

14.4 Exclusion of Women, Minorities, and Children (Special Populations)

Not relevant.

14.5 Subject Confidentiality

The confidentiality of research subjects is strictly maintained by the study team. Although the study monitors and agencies may access study information in the oversight of the clinical trial to ensure quality measures are being met or that human subjects are being treated appropriately. Data regarding subjects will not be released to other third parties.

The prolonged storage of source documents with identifiers is done at a secure space with limited access and rigorous security. Digital records of the study and nearly all source documents are identified by PID only to diminish risk of the dissemination of information regarding the identity or characteristics of subjects.

Databases lack identifiers and are further protected by password. All study personnel, including data entry personnel, receive specific training on their obligations regarding the privacy of participants and their families.

14.6 Study Discontinuation

In the case that the study is discontinued based on safety concerns, follow-up will be maintained to document all relevant safety outcomes.

14.7 Future Use of Stored Specimens

Data collected will be stored in servers at AB PRISMA and Johns Hopkins University Bloomberg School of Public Health. Specimens will be stored at the laboratories of AB PRIMSA in Iquitos Peru, with the option of secure storage at Johns Hopkins University. In the parental permission form, there is a provision for the use of residual sample for long term use for research related to the discovery and evaluation of new tests for enteric infections, microbiome research, testing for biomarkers of undernutrition, and gastrointestinal function. Subjects can withdraw their consent to have their samples in this biorepository and still participate in the study. Subjects may withdraw their consent to have their samples stored at a date no later than 2 years after the completion of the study. De- identified samples will be shared with third parties only under a materials and transfer agreement assuring compliance with restriction (No human DNA analysis, not for profit, not to be used to derive patents) are upheld.

15 DATA HANDLING AND RECORD KEEPING

Data collected in paper format will be transferred to a data unit at the research center and entered into a data management system. Records will be checked by a study physician or coordinator prior to being transferred for data entry.

Personal identifiers (such as names, birthdates, addresses) will be collected and held on a small number of forms that will not be entered into the electronic database.

All data from the study will be held in secure space locally to permit validation of the electronic database by auditors (including the Medical monitors, study PIs, INS staff, or other trial auditors from or designated by NIDDK).

15.1 Data Management Responsibilities

All study data must be held in secure areas with limited access. Transfer of physical files and electronic data will be kept to a minimum. Transfer of files and backup files will all be done on password protected files. Transfer of study files will always be done by a study team member. The site data manager has responsibility for the security and integrity of the physical files, the existence and safety of back up files, and the protection of digitally stored information. Data is held on a server in Iquitos Peru with a mirror copy at Johns Hopkins in Baltimore Maryland.

All records and laboratory reports must be reviewed by the study physician, PI, study coordinator or designee before being transferred to the data unit. All corrections made on CRFs will be made by striking thru the original text with a single line and by writing the correct data above, beside or below the original entry. The correction must be accompanied by the initials of the individual making the change and the data of the change. The original entry must not be obscured.

If the form is stamped as being already entered in the electronic database, it is the responsibility of the individual initialing the change on the paper form to contact the data center to ensure entry of the change in the study database.

The data management team in Iquitos will ensure records from participants are adequate and complete. They will discuss active issues regarding incomplete or data that appears to have unexpected values with the Study Coordinator to minimize recurrence.

15.2 Data Capture Methods

Data captured on case report forms will be documented on paper forms. Clinical laboratory data will be entered directly from the source document. These forms will be entered into the study base on an ongoing basis. Once forms are entered, the forms are stamped to communicate this state to the study team. Details of data management are included in a separate data management SOP.

Data will be entered in a MS ACCESS with filters for invalid entries and missing values by project personnel at the data unit and time and date stamping. Complete double data entry with verification will be performed. Validation of the DDE system will be performed using STATA or a similar program. All inconsistencies will be resolved by the data manager in communication with the Study Coordinator or project PI. These files will be saved and available for review as part of the audit trail to document data integrity.

15.3 Timing/Reports

The report content and timing is specified in the DSMP.

15.4 Study Records Retention

Study documents will be retained for a minimum of 7 years after the data is accrued in accordance with regulations regarding the conduct of clinical trials in Peru. They will be secured in locked furniture in the AB PRISMA offices in Iquitos.

15.5 Protocol Deviations

A protocol deviation is the con-compliance with the specified clinical trial protocol, GCP, MOP, or laboratory SOP procedures. The non-compliance can be the result of logistics, the investigator, study site staff, or the subject. It is however, always the responsibility of the site PI to identify protocol deviations, characterize the nature, extent, and reason for the deviation, and report them as appropriate to the INS, the IRBs of AB PRISMA, Tulane, Johns Hopkins University, and the sponsor in a timely manner.

Protocol deviations are recorded on a form and become part of the study file reported to the IRB annually if the deviation is characterized as one warranting reporting. All protocol deviations should be included in the reports to the DSMB as well.

Study deviations that are recorded that are purely the result of study compliance (such as missing stool specimens, or specimens collected 3 days late) are reviewed by the study staff but not reported to IRBs.

16 STUDY ADMINISTRATION

16.1 STUDY LEADERSHIP AND GOVERNANCE

16.1.1 Steering Committee

The Steering Committee will govern the conduct of the study. The Steering Committee will be composed of the study PI and co-PI and representatives of the sponsoring NIH Institute (NIDDK), the study coordinator (AB PRIMSA), the local PI (Cesar Ramal Asayag) and the PI of the laboratory of AB Prisma in Iquitos. The Steering Committee will oversee protocol and MOP development and meet by phone once prior to study initiation for final review of study materials. The steering committee members will receive the reports of the medical monitor and meet by teleconference on an ad hoc basis.

16.1.2 Executive Committee

An executive committee is not required to coordinate and oversee a small single site study that already has DSMB oversight
.

16.1.3 Conflict of Interest Policy

The study product is supplied free of charge by BioGaia, (or with a minimal charge to cover product labelling and shipping). BioGaia does not participate in study design, data analysis, or sponsor this trial with additional funds. No member of the investigative staff owns stock in or receives any compensation from BioGaia, nor have they in the past. BioGaia makes no determinations regarding the study outcomes, nor do they have any capacity to influence data analysis or the dissemination of results.

16.2 SUBCOMMITTEES

No subcommittees are planned for this small single site study. Prior to enrollment the trial will be registered at clinicaltrials.gov. All resulting publications will be submitted to the digital archive at Pubmed Central as per NIH public access regulations (https://grants.nih.gov/grants/NIH-Public_Access-Plan.pdf) and preferentially submitted to journals that have open access options.

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APPENDIX A: STUDY PROCEDURES
Version 18 MAR 2015
Appendix A. Study schedule for participants 0 to 2 years of age. Blood collection at 5 and 28 days can have a 48 hour window to allow for difficulty in scheduling visits. Stool collection has an inherent challenge in collecting samples on time. A 48 hr window will be allowed, after which the sample will be counted as missing. The scheduling of visits at 36 and 90 days will have a one week window, after which a protocol deviation form must accompany the safety information collected at these time points (data will be sought and recorded even beyond the scheduled window).

Procedure	PRE-RX DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 8	DAY 12	DAY 15	DAY 18	DAY 24	DAY 28	DAY 36	DAY 90
Informed Consent	X													
Medical History	X													
Physical Examination	X													
Record Temperature	X	X	X	X	X	X	X	X	X	X	X	X		
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X		
Stool Sample	X			X		X		X	X	X	X	X	X	
Blood Chemistry	X					X						X		
Hematology	X					X						X		
Assign Treatment Number	X													
Dispense Diary Cards		X												
Receive Study Product		X	X	X	X	X								
Maintain Diary		X	X	X	X	X	X	X	X	X	X	X		
Collect Adverse Events		X	X	X	X	X	X	X	X	X	X	X		x
Collect Diary Cards												X		

APPENDIX A: STUDY PROCEDURES

APPENDIX A.1: Planned laboratory tests

Hematology – Hemoglobin, WBC and differential count, platelet count. **Biochemistry** – BUN, creatinine, total bilirubin, AST, ALT.
Blood samples have a target volume of 3ml, a minimum of 1.5 ml.

Immunology – HIV rapid test done as part of screening
Probiotic carriage- PCR is done on stool samples to detect *L. reuteri* per laboratory SOP.

Appendix B. Definitions for Adverse Events Monitoring

Safety of *Lactobacillus reuteri* in healthy children ages 2-24 months in Peru

Estimating Clinical Severity:

Grade 1 (mild): Symptoms causing no or minimal interference with usual activities

Grade 2 (moderate): Symptoms causing greater than minimal interference with usual activities

Grade 3 (severe): Symptoms causing inability to perform usual activities

Grade 4 (potentially life-threatening): Symptoms causing inability to perform basic self-care functions, or if medical intervention is needed to prevent permanent impairment or death

APPENDIX C: Clinical adverse events to be monitored : Symptoms*

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
1. Altered feeding/Anorexia	Mild	Moderate—decreased oral intake	Severe—little oral intake	Unable to ingest food or fluid for more than 24 hours
2. Vomiting	1 episode/24 hours	2 -3episodes/24 hours	4-6 episodes/24 hours	> 6 episodes/24 hours or hypotensive shock
2. Diarrhea	3 liquid stools per 24 hours	4 liquid stools per 24 hours	Liquid stools, 5-8 per 24 hours	Liquid stools, > 8 per 24 hours, or requires hospitalization
4. Irritability	Slightly irritable, no interference with activity	Moderately irritable, some interference with activity	Significant; prevents daily activity or seeks medical attention	Incapacitating or requires hospitalization
5. Rash	Localized macular rash	Diffuse macular, maculopapular, or morbilliform rash	Grade 2 rash with vesicles, bullae, ulcerations, target lesions, or angioedema	Severe generalized rashes (e.g. Stevens Johnson syndrome, toxic epidermal necrolysis
6. Pruritis	Localized itching, relieved spontaneously or in > 48 hours	Localized itching lasting 48 hours or longer	Generalized itching	Generalized, severe itching or generalized itching lasting > 48 hours
7. Bronchospasm or wheezing	Mild wheezing, or minimal increase in respiratory rate	Wheezing with nasal flaring, intercostal retractions, or pulse oximetry 90-95%	Wheezing with dyspnea at rest, inability to perform normal activities, or pulse oximetry <90%	Respiratory failure or anaphylaxis

* Based on NIAID Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, December 2004 (http://www.niaid.nih.gov/labsandresources/resources/daidsclinrsrch/documents/daidsaegradingtable.pdf)

APPENDIX D: LABORATORY VALUES*

Serum Chemistries (normal range for 0-2 year old children at our reference lab)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
8. Bilirubin, mg/dL (≤ 1.0)	1.1-1.9	2.0-2.9	3.0-7.5	>7.5
9. Blood Urea Nitrogen BUN mg/dL (≤ 23)	24-60	61-80	≥81	Requires dialysis
10. Creatinine, mg/dL (≤ 0.4)	0.5 – 1.0	1.1 – 1.6	1.7 – 2.0	> 2.0 or requires dialysis
11. Liver Function Tests –ALT (≤ 52) units per liter (U/L)	53-200	201-400	401-600	> 600
12. Liver Function Tests –AST (≤ 39) units per liter (U/L)	40-200	201-400	401-600	> 600

Hematology	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
13. Children 6-24m : Hemoglobin gm/dL (≥ 11)	10.0 – 10.9	7.0 – 9.9	< 7.0	Cardiac insufficiency due to anemia
13a.Children 2-5 m: Hemoglobin gm/dL (≥9.5)	9.4-8.5	6.5-8.4	<6.5	Cardiac insufficiency due to anemia
14. Total WBC Count - cell/mm^3 (6,000-17,500)	17,501 – 20,000	20,001 – 25,000	25,001 – 28, 000	> 28,000

***** Based on NIAID Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, December 2004 (http://www.niaid.nih.gov/labsandresources/resources/daidsclinrsrch/documents/daidsaegradingtable.pdf), adapted for normal reference values for age for our reference laboratory (Asociacion Civil Selva Amazonica: Iquitos, Peru; www.selvaamazonica.org)

APPENDIX E. FEVER SEVERITY GRADING

Body Temperature	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
15. Fever (°C)	38.0 – 38.4	38.5 – 38.9	39.0 – 40	> 40

APPENDIX F. PRODUCT INFORMATION

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Lactobacillus reuteri

Lactobacillus reuteri is one of the most well researched probiotic species, especially in the very young.

To date 137 clinical studies using BioGaia's human strains of *Lactobacillus reuteri* have been performed in more than 11 700 individuals of all ages. Results are published in 108 articles in scientific journals (February 2015).

In this section you will learn about the different aspects of *Lactobacillus reuteri*, such as the history of this probiotic, its natural occurrence and about Reuterin, the antimicrobial substance that *Lactobacillus reuteri* produces.

- See more at: http://www.biogaia.com/lactobacillus-reuteri#sthash.N1KjzKol.dpuf

History of Lactobacillus reuteri

Lactobacillus reuteri is a Gram-positive bacterium whose natural habitat is the digestive tract of mammals and birds.

The species ***Lactobacillus reuteri* was recognized and recorded in scientific classifications of lactic acid bacteria, as early as the turn of the 20th century**. At this time it was mistakenly grouped as a member of *Lactobacillus fermentum*. In the 1960s, further work by German microbiologist Gerhard Reuter could distinguish *Lactobacillus reuteri* from *Lactobacillus fermentum*. Reuter reclassified the species as "*Lactobacillus fermentum* biotype II".

***Lactobacillus reuteri* was eventually identified as a distinct species in 1980 by Kandler et al. They chose the species name "reuteri", after discoverer Gerhard Reuter**, and *Lactobacillus reuteri* has since been recognized as a separate species within the Lactobacillus genus.

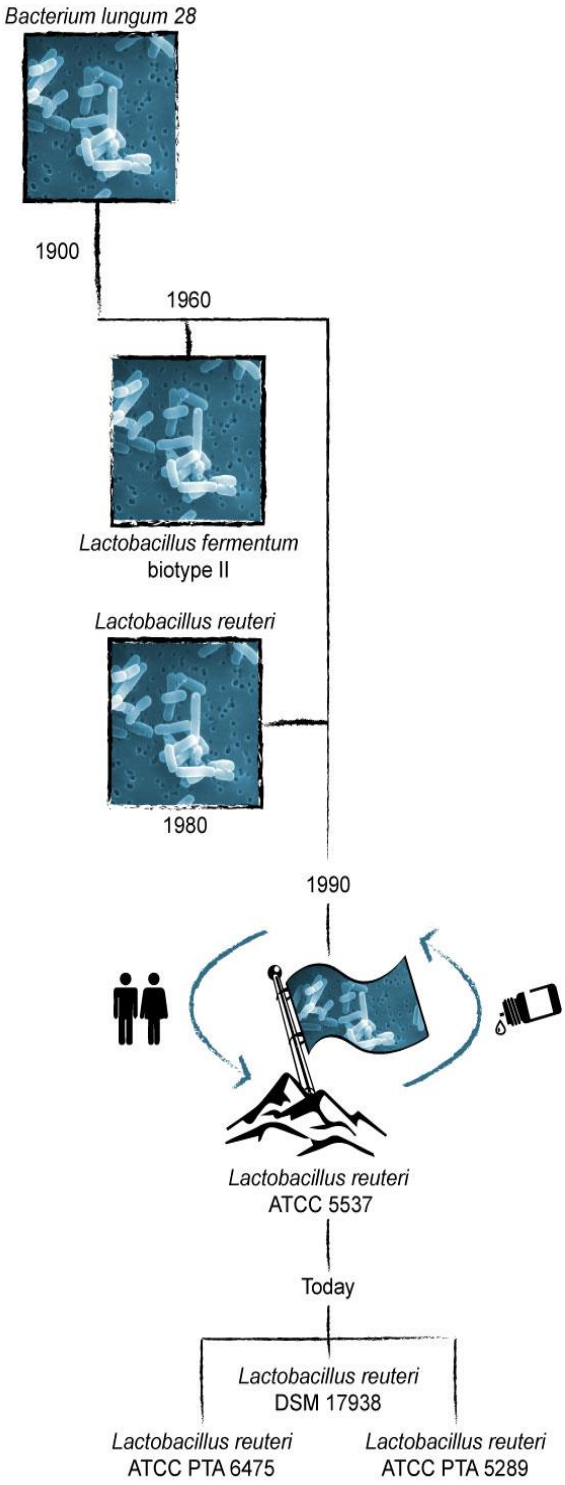
The first strain of *Lactobacillus reuteri* for human use was isolated in 1990 from the breast milk of a Peruvian mother living in the Andes. This strain was deposited at the American Type Culture Collection (ATCC) as *Lactobacillus reuteri* SD 2112 (SD = safety deposit), and was later given the number ATCC 55730.

In 2007 *Lactobacillus reuteri* ATCC 55730 was replaced by the "daughter strain" *Lactobacillus reuteri* DSM 17938. The only difference between the strains is the loss of two plasmids of ATCC 55730 that carried resistance to tetracycline and lincomycin, respectively.

The new strain *Lactobacillus reuteri* DSM 17938 can be considered comparable to *Lactobacillus reuteri* ATCC 55730 in all aspects of probiotic function: genetic sequence information has shown the irrelevance of the genes of the plasmids to the functions of the strain, and also confirmed that all tested probiotic-related functions of *Lactobacillus reuteri* DSM 17938 are retained from those of *Lactobacillus reuteri* ATCC 55730.

The commercial name of *Lactobacillus reuteri* DSM 17938 is *Lactobacillus reuteri* Protectis. Other human strains that are used commercially are *Lactobacillus reuteri* ATCC PTA 5289 and ATCC PTA 6475.

- See more at: http://www.biogaia.com/history-lactobacillus-reuteri#sthash.zJI8di1z.dpuf



Natural occurrence

Lactobacillus reuteri is one of very few probiotic species whose natural habitat is the digestive tract and it is therefore especially well adapted to thrive in this kind of environment. It is among the first bacterial species to become naturally established in the normal microbiota of the newborn. Most other probiotic strains are only temporary or transient inhabitants of the gastrointestinal tract, derived from the intake of food.

In humans *Lactobacillus reuteri* has been isolated from:

- Breast milk
- The mouth
- The stomach
- The small intestine (duodenum and ileum)
- The colon (large intestine)
- Faeces
- The vagina

- See more at: http://www.biogaia.com/natural-occurrence#sthash.d26hjspa.dpuf