**Trial protocol**

# Efficacy of probiotic supplementation in preterm and small for gestational age infants. A multi-centre, placebo- controlled, individually-randomised trial

**(Probiotics in preterm and small for gestational infants, PROPS trial)**

**Version 1.4**

**Date 8October2025**

## Executive summary

**Background:** Preterm and small for gestational age (SGA) infants have a 2- to 10-fold higher risk of mortality than infants born at term and with normal birth weight, and are particularly vulnerable to infection, malabsorption, necrotizing enterocolitis, difficulty feeding, and growth failure. Numerous trials conducted in the last ten years have reported that probiotics can improve short- and long-term mortality, necrotizing enterocolitis and sepsis rates, and growth and neurodevelopment in preterm and low birth weight infants. However, most trials have been small and with high to moderate risk of bias. There have been no trials in SGA infants. The World Health Organization (WHO) guideline development group (GDG) and other organizations have recommended that further large high-quality trials are implemented to provide evidence of sufficient quality and applicability to inform policy and practice.

**Sponsor, funder and coordination:** The trial is sponsored by the WHO Newborn and Child Health and Development Unit (NBC) and funded by the Bill & Melinda Gates Foundation (BMGF). It is coordinated by a coordinating centre (CC) at Johns Hopkins University. There is a dedicated trial steering committee (TSC) which includes the WHO, CC, and the lead principal investigators from each site.

**Aims and objectives:** The overall aim of the trial is to assess the effect of probiotic supplementation on mortality, morbidity, and growth in preterm or term SGA infants in the first 6 months of life in South Asia and Sub-Saharan Africa.

For preterm infants, the primary objective is to assess the effectiveness of probiotic supplementation on mortality from enrolment to 6 months of age. For term SGA infants the primary objective is to assess the effectiveness of probiotic supplementation on underweight-free survival from enrolment to 6 months of age.

Secondary objectives are: to assess effects on safety outcomes (serious adverse events [SAEs] and probiotic sepsis from enrolment to 6 months of age; to assess effects on morbidities (sepsis, necrotising enterocolitis, severe diarrhoea and growth [wasting and underweight]) from enrolment to 6 months of age; to assess effects on abundance of *B. Infantis* and *L. rhamnosus* and other bacteria in infant faecal specimens at enrolment, 7 days, 28 days and 6 months of age; and in maternal faecal specimens at enrolment; and (iv) to assess effects on the primary outcome in specific subgroups (gestational age strata, birth weight strata, vaccination status, feeding type, early initiation of enteral feeding, micronutrient supplement intake, receipt of antibiotics).

**Timeline:** The trial will be conducted over 28 months. Infants will be enrolled over a period of 18 months and followed up until they reach 6 months of age. There will be 4 months for additional follow-up and data cleaning and analysis. It is anticipated that the first infant will be enrolled in November 2025.

**Study setting and recruitment:** Study recruitment will be at hospitals in Bangladesh, Ethiopia, Kenya, Nigeria and Pakistan, which were selected due to the high rates of preterm birth and malnutrition among infants in those countries. Infants will be enrolled at < 48 hours after birth (< day 2 after birth) and supplementation will commence at that time. A total of 14,000 infants will be enrolled across the 5 participating countries.

**Randomisation:** Randomisation of the recruited infants will be done at the time of enrolment and will be done by site and separately for preterm and term SGA infants.

**Intervention and placebo administration:** The intervention will be a high-quality, pharmaceutical grade product with known safety containing the strains *Bifidobacterium longum subsp. Infantis* DSM33361 (*B. Infantis*) and *Lactobacillus rhamnosus GG* DSM33156 (*L. rhamnosus GG*). The doses will be 0.35 billion colony forming units (CFU) *B. infantis* and 1 billion CFU *L. rhamnosus GG* once daily for 28 days as these are the doses and duration previously used in clinical trials and are in routine use in hospitals. The strains will be lyophilized (freeze dried) mixed with maltodextrin powder to a total of 0.5 grams and provided in individual sachets (i.e. one sachet for *B.infantis* and one sachet for *L. rhamnosus GG)*.

The placebo will be maltodextrin powder alone without the probiotic organisms and will be identical in appearance, taste, consistency and packaging to the intervention.

The supplements will be given daily for 28 days. Each infant will receive 2 sachets. The intervention group will receive one sachet containing *B.infantis* mixed with maltodextrin powder and one sachet containing *L.rhamnosus* mixed with maltodextrin powder. The placebo group will receive two sachets containing maltodextrin powder alone. Research staff will visit families and mix the supplements in 1-2 mls of sterile water at the point of care and provide the supplements as directly observed therapy (DOT) (i.e. the mother will give the infant the supplement directly observed and supervised by research staff). The visits will occur either in a health facility or at home if the infant has been discharged.

**Safety assessments:** No serious adverse events or drug reactions are expected. However, infants will be assessed daily for 7 days after each supplement dose.

**Outcome assessments.** After supplementation is completed, infants will be followed up every 4 weeks at home for primary and secondary outcome endpoint assessment by an independent outcome assessment team until the infant reaches 6 months of age. Endpoint data will also be collected on all infants admitted to study hospitals.

**Ethical and regulatory issues**: This trial is registered in the UK's Clinical Trial Registry (ISRCTN12028895). Approval will be sought from the WHO Ethics Review Committee (ERC) and the ERCs and regulators in each country. Informed written consent will be sought from the mother of the infants before infants will be enrolled in the trial. Mothers have the right to withdraw their infant from the trial at any time without giving a reason for withdrawal. The trial will be monitored by an independent external monitor, Data safety and monitoring board (DSMB) and external advisory group (EAG) as well as the ERCs at WHO and in each country.

**Impact of the trial and post-trial access if study supplementation is effective:** There is an imperative to clarify the effect of probiotics in preterm and term SGA infants using a programmatically feasible formulation and approach that is effective and safe in high burden LMIC settings, especially Asia and Africa. Currently, due to lack of data, WHO has only made a conditional recommendation on the use of probiotics in preterm infants, and there are no current WHO guidelines on the use of probiotics in term SGA infants. In the event the study demonstrates that probiotic supplementation provides significant public health benefits for preterm and SGA infants, the findings from this trial will be used to update WHO recommendations and develop new recommendations. The findings will also be used to develop LMIC national and hospital level clinical practice guidelines. If the probiotic supplement is proven to be efficacious and safe, then WHO/NBC will work with the WHO prequalification department and UNICEF supply division to increase availability and access to the probiotic product in the study countries and in all LMICs. Results from this trial will help inform policy and practice for probiotic supplementation in

preterm and SGA infants, improve the certainty of evidence on critical outcomes and strengthen the understanding of optimal strains, dosing and duration of probiotic supplementation and related impact in high burden LMICs.

**Summary of key elements**

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|---|---|
| **Study title** | Efficacy of probiotic supplementation in preterm and small for gestational age infants. A multi-centre, placebo-controlled, individually-randomised trial (Probiotics in preterm and small for gestational infants, PROPS trial) |
| **Study design** | Double-blind, individually-randomized, placebo-controlled, parallel-group clinical trial |
| **Study sites** | Infants will be recruited in hospitals in Bangladesh, Ethiopia, Kenya, Nigeria, and Pakistan. Follow up will be through visits in the hospital and at home after discharge. |
| **Trial period** | 28 months: this includes 18 months of enrollment, 6 months follow-up, 4 months for final follow-up, site data cleaning, and data analysis |
| **Primary objectives** | |
| *Preterm infants* | *Objectives*<br>Assess the effect of probiotic supplementation on all-cause mortality from enrolment to 6 months of age<br>*Endpoints*<br>Denominator: number of preterm infants enrolled<br>Numerator: number of deaths from enrolment to 6 months of age<br>*Number to be enrolled*<br>9,453 preterm infants across all sites (50% intervention, 50% placebo)<br>This provides 90% power to detect a 20% reduction in mortality in the intervention group, assuming 10% mortality in the placebo group |
| *Term small for gestational age infants* | *Objectives*<br>Assess the effect of probiotic supplementation on underweight-free survival at 6 months of age<br>*Endpoints*<br>Denominator: number of small for gestational age births enrolled<br>Numerator: number of enrolled infants who have not died and who are not underweight (weight-for-age z-scores less than -2 standard deviations) at 6 months of age<br>*Number to be enrolled*<br>4,465 term small for gestational age infants across all sites (50% intervention, 50% placebo)<br>This provides 90% power to detect a 20% greater underweight free survival in the intervention group, assuming 23% underweight free survival in the placebo group |
| **Safety objectives** | The following safety objectives will be assessed in both preterm and term SGA infants from enrolment to 6 months of age:<br>- episodes of serious adverse events (SAEs)<br>- episodes of sepsis due to the probiotic strains *B. Infantis* or *L. rhamnosus* |

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| **Secondary objectives** | The following secondary objectives will be assessed in both preterm and term SGA infants from enrolment to 6 months of age:<br>1. To assess the effect of probiotic supplementation on the following outcomes at hospital discharge, 1 month and 6 months of age:<br>- all cause mortality (for the 6 month endpoint, this will be evaluated as a secondary outcome for term SGA infants, and as a primary outcome for preterm infants) and cause specific mortality due to necrotising enterocolitis, sepsis, severe diarrhoea<br>- episodes of necrotising enterocolitis, sepsis, severe diarrhoea<br>- episodes of pathogen specific sepsis (including *Streptococcus, Staphylococcus, Klebsiella, Escherichia coli , Acinetobacter spp*) including their serotypes and genotypic sequences<br>- episodes of hospitalisation<br><br>2. To assess the effect of probiotic supplementation on the prevalence of underweight, wasting, and stunting and mean weight for age (WAZ), weight for length (WLZ), and length for age (LAZ) z scores at 6 months of age<br><br>3. To assess the effect of probiotic supplementation on the abundance of *B. Infantis* and *L. rhamnosus* and other bacteria in infant faecal specimens at enrolment, 7 days, 28 days and 6 months of age; and in maternal faecal specimens at enrolment, including their serotypes and genotypic sequences.<br><br>4. To assess the effects of probiotic supplementation on the primary outcome in the following subgroups:<br>- prematurity (<32weeks, 32-<37weeks)<br>- birth weight (< 1.5kg, 1.5 to < 2.5kg, 2.5+ kg )<br>- preterm and SGA status (preterm SGA, preterm AGA, term SGA)<br>- vaccination (fully, partially, not vaccinated) from enrolment to 6 months of age<br>- feeding type (exclusive, predominant, partial) at 1 month, 3 months, 6 months of age<br>- early initiation of enteral feeding (including mother's milk, donor milk, infant formula) (within < 1hour, 2-< 24hour, 24+ hours after birth)<br>- micronutrient supplement intake (multiple micronutrients, iron, vitamin A, vitamin D, zinc) from enrolment to 6 months of age<br>- receipt of antibiotics from enrolment to 6 months of age |

## List of acronyms and abbreviations

| | |
|---|---|
| AGA | Appropriate for gestational age |
| ATCC | American Type Culture Collection |
| ADR | Adverse drug reaction |
| AE | Adverse event |
| AESI | Adverse event of special interest |
| *B. Infantis* | *Bifidobacterium longum subsp. infantis* |
| CC | Coordinating centre |
| CFU | Colony forming unit |
| CRF | Case record form |
| CI | Confidence interval |
| DSM | Deutsche Sammlung von Mikroorganismen Culture Collection |
| DSMB | Data safety and monitoring board |
| EAG | External advisory group |
| ERC | Ethical review committee |
| HIC | High-income country |
| ICHGCP | International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Harmonised Guideline for Good clinical practice E6 |
| ICHGCP R3 | International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Harmonised Guideline for Good clinical practice E6(R3) |
| JHU | Johns Hopkins University |
| LBW | Low birth weight |
| LMIC | Low- or middle- income country |
| *L. rhamnosus GG* | *Lacticaseibacillus rhamnosus GG* |
| MCA | Department of Maternal, Newborn, Child and Adolescent Health and Ageing, WHO |
| MD | Mean difference |
| NBC | Newborn and Child Health and Development Unit, MCA, WHO |
| OR | Odds ratio |
| PD | Protocol deviation |
| PI | Principal investigator |
| RCT | Randomized controlled trial |
| RR | Relative risk |
| SAE | Serious adverse event |
| SGA | Small for gestational age |
| spp. | Several species |
| TSC | Trial steering committee |
| WHO | World Health Organization |
| UNICEF | United Nations Children's Fund |

## List of tables and figures



## List of annexes

**Version log**

| Version | Version date | Action | Log of changes | Date of WHO ERC approval |
|---|---|---|---|---|
| 1.0 | 12Dec2024 | Submitted to WHO ERC for prereview | | |
| 1.1 | 27Dec2024 | Revised post WHO ERC prereview | Clarified timing of screening<br>Removed process evaluation, this will be submitted separately<br>Change of guardian to mother | |
| 1.2. | 6March2025 | Revised post WHO ERC review | Clarified mandatory reporting<br>Clarified anonymisation of data<br>Clarified timing of adverse events<br>Clarified stratification and loss to follow up | |
| 1.3. | 30March2025 | Revised post SAP review | Corrected sample size<br>Removed vaccine antigen subgroup analysis<br>Removed CFUs<br>Other minor changes | 18April2025 |
| 1.4. | 8Oct2025 | Amendment 1 | Added JHU as coordinating centre and coordinator of data management<br>Updated start date of trial<br>Updated WHO responsible officer names<br>Added registration number of trial<br>Updated ICHGCPR2 to ICHGCPR3<br>Addition of stunting (length for age z score) as secondary outcome<br>Clarified WHO's role in safety and protocol deviation monitoring<br>Removed typo about transport media for faeces collection<br>Updated definition of SAE to align with ICHGCPR3<br>Clarified sites will retain ownership of their own data<br>Clarified blood culture processes<br>Clarified care seeking at study facility would be encouraged.<br>Clarified study endpoint definitions<br>Minor wording, formatting corrections | |

## Administrative information

| Sponsor | | |
|---|---|---|
| Sponsor responsible officers | Dr Ameena Goga<br>Dr Sachiyo Yoshida | World Health Organization, Newborn and Child Health and Development Unit (WHO/NBC) |

| Coordinating Center | | |
|---|---|---|
| Coordinating centre responsible officer | Dr Kayur Mehta | Johns Hopkins University |

| Principal investigators | | |
|---|---|---|
| Bangladesh | Prof Mohammod Shahidullah | Projahnmo Research Foundation, Dhaka, Bangladesh |
| Ethiopia | Prof Mahlet Abayneh Gizaw, Dr Adamu Bayissa | St Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia<br>Armauer Hansen Research Institute, Addis Ababa, Ethiopia |
| Kenya | Prof Benson Singa | Kenya Medical Research Institute, Nairobi, Kenya |
| Nigeria | Prof Adejumoke Idowu Ayede | College of Medicine, University of Ibadan and University College Hospital, Ibadan, Nigeria |
| Pakistan | Prof Fyezah Jehan | Aga Khan University, Karachi, Pakistan |

| Clinical trial registration (CTR) | | |
|---|---|---|
| **Registry name** | **Clinical trial registration (CTR) registration number** | **Date registered** |
| ISRCTN | ISRCTN12028895 | 8 April 2025 |

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## 1. Background and rationale

### 1.1. Importance

Global estimates of prematurity, small for gestational age (SGA) and low birth weight (LBW) range from 10% to 40% with the highest prevalence in Asia and sub-Saharan Africa.[1-4] Preterm infants have a gestational age below 37 weeks at birth, SGA infants have birthweight below the tenth percentile for age and sex,[5] and LBW infants have a birth weight below 2.5 kg. [1-3] Preterm and SGA infants have a 2- to 10-fold higher risk of mortality than infants born at term and with normal birth weight, and are particularly vulnerable to infection, malabsorption, necrotising enterocolitis, difficulty feeding, and growth failure. [4,6-8] Preterm and SGA infants also have a higher risk of impaired neurodevelopment including cerebral palsy, attention deficits and poor educational outcomes. [9,10] These problems are largely due to early life complications arising from intrauterine stresses, immaturity of organ and immune systems, and malnutrition in early postnatal life.[4] Improvements in outcomes in preterm and SGA infants have been slow in all countries, especially low- and middle- income countries (LMICs), due to the complexities of preventing complications and providing medical care for small and vulnerable infants.[4,11-15]

### 1.2. Probiotic definition and mechanism of action

The World Health Organization (WHO) and the Food and Agricultural Organization of the United Nations (FAO) define probiotics as live microorganisms that when administered in adequate amounts confer a health benefit on the host.[16] Only products containing microorganisms that have been proven to improve health outcomes can be called probiotic.

Preterm infants have immature gastrointestinal microbiota which are different from term infants.[17] It has been hypothesized that providing probiotic bacteria from the healthy term infant gastrointestinal tract may result in health benefits to preterm infants by 'restoring' a normal gastrointestinal microbiome so it becomes similar to a term healthy newborn.

Preclinical studies have shown that probiotics may work by: outcompeting gastrointestinal pathogens for nutrients, limiting pathogen growth, producing inhibitory organic acids, producing antimicrobial compounds, stimulating differentiation and proliferation of enterocytes, enhancing expression of intestinal digestive enzymes, and improving intestinal mucosal barrier integrity.[18-20] These effects result in maturation of the immune system, providing protection from infections, particularly gastrointestinal, blood stream and respiratory infections

### 1.3. Clinical trials of the effectiveness of probiotics in preterm and SGA infants

#### 1.3.1 Probiotic clinical trials

In the last ten years trials of proposed probiotic products have reported improvements in: short- and long-term mortality, rates of necrotising enterocolitis and sepsis, and longer term growth and neurodevelopment in preterm and LBW infants.[21-24] A recent Cochrane review of randomised controlled trials (RCTs) in preterm infants[21]reported: moderate-certainty evidence from 51 trials of 10,170 participants of a decrease in all-cause mortality by the time of hospital discharge (relative risk [RR] 0.76, 95% confidence interval [CI] 0.65 to 0.89); low-certainty evidence from 54 trials of 10,604 participants of a decrease in necrotising enterocolitis by the time of hospital discharge (RR 0.54, 95% CI 0.45 to 0.65);

moderate-certainty evidence from 47 trials of 9,762 participants of a decrease in invasive infection by the time of hospital discharge (RR 0.89, 95% CI 0.82 to 0.97); and low-certainty evidence from five trials of 1,518 participants of little or no effect on neurodevelopment between 18 months and 3 years (RR 1.03, 95% CI 0.84 to 1.26).

Three systematic reviews have reported small clinically important effects of probiotic products on growth outcomes in 'all infants' ( i.e. preterm, term, AGA, SGA infants combined).[25-27] The highest quality of these systematic reviews[25] reported low to moderate certainty evidence of the effects of probiotics on growth (improvement in weight (standardised mean difference [SMD] 0.26, 95% CI 0.11 to 0.42) and height (SMD 0.16, 95% CI 0.06 to 0.25; at follow up to 6 years). There appears to be no systematic reviews of impacts on growth in preterm or SGA infants separately.

To our knowledge there have been no RCTs of the effect of probiotic products in SGA infants. There appears to have been only one retrospective cohort study of 1,464 infants conducted in Australia which showed similar effects on necrotising enterocolitis in term SGA as in appropriate for gestational age (AGA) infants.[28,29]

### 1.3.2 Genera specific clinical trials of *Lactobacillus* and *Bifidobacterium*

Bacterial genera (or classes) proposed as probiotics for preterm and SGA infants include gram positive anaerobes present in term healthy newborns including: *Bifidobacterium, Lactobacillus, Sacchromyces, Bacillus, Streptococcus,* and *Enterococcus*.[21]

*Bifidobacterium* and *Lactobacillus* have consistently been reported to produce statistically significant health benefits in preterm infants in recent systematic reviews.[22-24] The largest network meta-analysis to date of 63 trials involving 15,712 preterm infants,[22] reported that the combination of *Bifidobacterium* and *Lactobacillus* improved all-cause mortality (odds ratio [OR] 0.56 95% CI 0.39 to 0.80) necrotising enterocolitis (OR 0.35 95% CI 0.20 to 0.59), culture proven sepsis (OR 0.87, 95%CI 0.60 to 1.27), full enteral feeding OR -2.15 95%CI -3.78 to -0.51, and days of hospitalisation (-2.84 days, 95%CI -6.21 to 0.54).

### 1.3.3 Strain specific clinical trials of *Lacticaseibacillus rhamnosus GG* and *Bifidobacterium longum subsp. infantis*

A bacterial strain is the genotypic and phenotypic characterization of a microorganism.[30] WHO/FAO recommends testing of all bacterial strains using defined methods and registration (or 'deposition') of genotypic sequences in international culture collections such as the American Type Culture Collection (ATCC) and Deutsche Sammlung von Mikroorganismen (DSM).[16] As technologies advance (such as 16S ribosomal DNA and whole genome sequencing [WGS]) and more strains are registered and used trials, probiotic effects have increasingly been shown to be 'strain specific'.[30,31] i.e. the effects of one genotypic strain being unique and not attributable to other probiotics of the same genus or species.[30,31] Some strains in a genera have been shown to have no effect on clinical outcomes and some strains marked effects.

The effects of *Bifidobacterium* and *Lactobacillus* are also considered to be ''strain'' specific in preterm infants.[22,23,32,33] *Lacticaseibacillus rhamnosus GG* and *Bifidobacterium longum subsp. Infantis* are considered by many researchers and guideline developers to be the most important strains for use in preterm infants [28,29] [34]

#### *-Lacticaseibacillus rhamnosus* GG (*L. rhamnosus GG*)

*Lacticaseibacillus rhamnosus* GG (*L. rhamnosus GG*) (DSM33156) is the most studied *Lactobacillus* probiotic strain in preterm infants. There have been 24 trials of *L. rhamnosus GG* (DSM33156) of 2387

infants,[23,32,35] and these studies show effects on: all-cause mortality (3 RCTs, 207 participants, RR 0.99, 95% CI 0.42 to 2.33), necrotising enterocolitis (5 RCTs, 851 participants, RR 0.50, 95% CI 0.26 to 0.93), late onset sepsis (7 RCTs, 1037 participants, RR 1.08, 95% CI 0.84 to 1.39), time to reach full enteral feeds (2 RCTs, 19 participants, SMD 0.11 days, 95% CI -0.22 to 0.45), and duration of hospital stay (3 RCTs, 293 participants, SMD -0.14 days, 95% CI -0.37 to 0.09).

Preclinical trials indicate that the *L. rhamnosus GG* (DSM33156) mechanism of action includes: avidity for human intestinal mucosal cells, adhesion to intestinal epithelial cells inhibiting the growth of pathogens, adherence to the intestinal mucus,[36,37] formation of biofilms enhancing ability to protect and strengthen cytoskeletal integrity, and inhibition of colonisation by pathogens.[38,39]

***-Bifidobacterium longum subsp. infantis (B. infantis)***

*Bifidobacterium longum subsp. infantis* (*B. infantis*) (DSM33361) is the most studied *Bifidobacterium* probiotic strain in preterm infants. There have been 3 trials of *B. infantis* DSM 33361 of 2788 infants.[40-42] A meta-analysis of these trials show similar effects[23] to *L. rhamnosus GG* on: necrotising enterocolitis (2 trials of 1244 participants, RR 0.24, 95% CI 0.064 to 0.670); late onset sepsis (2 trials of 1244 participants, RR 0.80, 95%CI 0.47 to 1.30); all-cause mortality (2 trials of 1244 participants, RR 0.89, 95% CI 0.32 to 2.30). In addition, 1 trial of 115[40] infants has also shown effects on multi drug resistant organism (MDRO) colonization (RR 0.99, 95%CI 0.54 to 1.81) and eubiosis scores (achieving microbiota similar to term infants) (I trial of 179 infants, OR 1.28, 95%CI 1.19 to 1.38).

There have been 12 small trials of other *B. infantis* strains trials enrolling a total of 838 infants.[33] 11 of these trials did not report the strain, the remaining trial,[43] used the DSM24687 strain in 93 infants. Evidence was varied and inconclusive for all trials.

Preclinical trials indicate that the *B. infantis* mechanism of action includes: utilization of human milk oligosaccharides (HMOs) which selectively encourage the growth of a healthy gut microbiota while suppressing the proliferation of enteropathogens;[44,45] production of tryptophan metabolites and indole-3-lactic acids which possess anti-inflammatory properties and aid in the maturation of the immune system; colonisation of the infant gastrointestinal tract establishing a protective barrier against harmful bacteria and enhancing nutrient absorption and digestion; out-competition of pathogens reducing their ability to colonize and disrupt the gut ecosystem; and promotion of maturation of the innate immune response.[46,47]

### 1.3.4 Effects of *Lacticaseibacillus rhamnosus GG* and *Bifidobacterium longum subsp. Infantis* combined

*L rhamnosus GG* and *B. Infantis* may have additive and synergistic effects.[21,22,34] A meta-analysis of 8 trials of 1266 infants[34] used *L. rhamnosus GG and B. infantis* in combination in preterm infants (2 alone, 6 with other strains) and showed effects on: all-cause mortality (8 trials of 1226 participants, RR 0.43, 95% CI 0.14 to 1.31); necrotising enterocolitis (8 trials of 1201 participants, RR 0.37, 95% CI 0.09 to 1.49); late onset sepsis (4 trials of 812 participants, RR 0.63, 95% CI 0.60 to 1.14); reduction in days to reach full enteral feeds (6 trials of 1111 participants, MD -1.19, 95% CI -1.85 to 0.47); reduction in days of hospitalisation (6 trials of 1101 participants, MD -2.50 95%CI -4.55 to -2.45). However, sample sizes were small for these trials, confidence intervals were wide, estimates imprecise and evidence was very low certainty.

## 1.4. Safety

### 1.4.1 Manufacturing

Probiotic products for preterm and SGA infants must be manufactured according to Good Manufacturing Practice (GMP)[48] and WHO/FAO guidelines.[16] In addition, manufacturers should provide certificates of compliance and analysis and to be able to address: dose, potency, formulation, and testing for purity, quality, contamination, species and strain identity, storage conditions, stability and viability at end of shelf life, packaging and labelling.[16]

### 1.4.2 Dose and potency

Dose ranging studies in adults, children and preterm infants have shown little variation in dose response to probiotics.[49-51] A small trial of 149 preterm infants showed no clear differences in colonization between dosing either 1 billion or 10 billion CFU of the same strain daily.[51] Another trial also in preterm infants, reported that administration of a daily dose of 1 billion CFU of 2 probiotic strains resulted in increased colonisation compared to a weekly or biweekly dose of the same strains.[49] A systematic review of probiotic dose ranging studies in different clinical settings and patient groups showed that antibiotic-associated diarrhoea varied by dose but there was no variation in effect on necrotising enterocolitis.[50]

In a recent meta-analysis of 28 trials of 6729 infants receiving *L. rhamnosus* GG or *B. infantis*,[34] the *L. rhamnosus GG* DSM33156 doses ranged from 1 to 6 billion CFUs. The *B. infantis* doses ranged from 0.18 to 0.6 billion colony forming units (CFU). In the three *B. Infantis* DSM 33361 trials all infants received 0.35 billion CFUs per day. In the subgroup analysis there was no evidence of effect modification by dose.

Based on the available clinical trials, the European Society for Paediatric Gastroenterology Hepatology and Nutrition Committee on Nutrition (ESPGHAN) has recommended *L. rhamnosus GG* DSM33156 doses of 1 to 6 billion CFU and *B. infantis* DSM33361 doses of 0.3 to 0.35 billion CFU.[35]

### 1.4.3 Formulation

Probiotics can be provided for preterm infants in a lyophilized (freeze dried) formulation in sachets, 'oil in droppers', capsules or in readymade infant formula.[52] In the meta-analysis of 28 clinical trials of 6729 infants[34] receiving *L. rhamnosus GG* or *B. infantis*, 20 trials provided probiotics in lyophilized ('freeze dried' ) form in sachets and then reconstituted in 1 to 10 mls of water or milk and provided once or twice daily orally or via a gastric feeding tube.[21-24] Three trials provided probiotics in a smaller volume 'oil in dropper' formulation and the remaining 3 did not describe the formulation.[21-24] In the subgroup analysis there was no evidence of effect modification by formulation type.

### 1.4.4 Testing for purity, quality, contamination, species and strain identify

Standard testing for bacteria during manufacture includes certification that the products are free from: *Bacillus spp* including *B. cereus*; *Cronobacter spp*. including *C. sakazakii* (previously known as *E. sakazakii*); *Salmonella spp* including *S. typhii*; *Staphylococcus spp* including *S. aureus; Clostridium spp.* including *C. difficile*; *Listeria spp* including *L. monocytogenes; Enterobacter spp*. ; and all yeasts and moulds.[53,54]

A number of reports have shown that product labels on commercial or medical probiotic products do not match actual contents e.g. species identity and bacterial count, or that they are contaminated with nonprobiotic bacteria.[54,55] All manufacturers must provide a certificate of analysis that shows correct genotypic product identity at the strain level performed on inoculation material.[16]

### 1.4.5. Storage and stability

*L. rhamnosus* GG and *B. infantis* stability is well known to be influenced by temperature.[49,56,57] Bacterial counts in lyophilized preparations drop faster in warm and humid environments than when they are stored at 2 to 8 deg Celsius. A stability analysis at end of shelf life must be provided by all manufacturers. After reconstitution viability drops significantly and it is recommended to provide *L. rhamnosus* GG or *B. infantis* immediately after reconstitution i.e. reconstitute at or close to the point of care.[35] None of the 28 trials of 6729 infants receiving *L. rhamnosus* GG or *B. infantis*,[34] reported timing of reconstitution or storage conditions.

### 1.4.6 Preparation and mixing

There are many reports of environmental and cross infant contamination from probiotics when used in the hospital ward and at home.[40,58,59] Cross colonisation may not necessarily be an adverse effect (in case of a safe product). However, it is important for untested products and in individually randomised clinical trials. Precautions to avoid spillage is needed when preparing probiotic supplements.

To reconstitute, probiotics can be mixed with sterile water, breastmilk or infant formula.[60,61] Preparation must also avoid other bacteria being introduced. WHO Codex Alimentarius guidelines must be followed.[62]

### 1.4.7 Clinical and serious adverse events

No serious adverse events (SAEs) (e.g. deaths, hospitalisations, sepsis, gastrointestinal complications, severe vomiting, severe diarrhoea, anaphylaxis) were reported to be related to the intervention in the 28 clinical trials of 6729 infants[34] receiving *L. rhamnosus GG* or *B. infantis*.[35] No episodes of probiotic sepsis (defined as sepsis due to an administered probiotic strain) were also were reported. A recent systematic review[63] reported 32 cases of probiotic sepsis when strains were used in observational studies and routinely in hospital (7 *L. rhamnosus GG* and 4 *B. infantis*). All cases were reported within 7 days after supplementation. All infants recovered with no sequelae and there were no deaths. All cases were in infants under 28 weeks gestation (mean gestational age 25 weeks) or were in infants with significant gastrointestinal comorbidities (short gut syndrome , congenital intestinal atresia, volvulus, gastrostomy , gastroschisis, jejunostomy, necrotising enterocolitis). In one case the only risk factor was that the infant was a twin. An additional case of probiotic sepsis has recently been reported of an infant under routine care in a hospital in the USA who died.[64,65] This infant was under 28 weeks gestation and 1000 grams and received the *Bifidobacterium longum subsp. Infantis Evivo* MCT oil strain EVC001.[64,65] It is not known whether this death was related to the probiotic sepsis or incidental.

### 1.4.8 Drug and vaccine interactions

No interactions with medicines or antibiotics have been reported in the 28 trials of 6729 infants[34] receiving *L. rhamnosus GG* and *B. infantis*. No interactions with vaccinations have also been reported. The potential for probiotics to transmit antibiotic resistance genes has been described and probiotic manufacturers should include these analyses prior to commercial or clinical trial use.[66,67]

## 1.5. Other implementation considerations

### 1.5.1 Timing of initiation

The optimal timing of initiation of probiotics is unclear. In the early days after birth ‘natural’ (breast milk driven) bacterial colonisation is just beginning, the infant immune system is underdeveloped, and the

preterm gastrointestinal barrier function is impaired by inadequate tight junctions and reduced mucus layer. In the meta-analysis of 28 trials of 6729 infants receiving *L. rhamnosus* GG or *B. infantis*,[34] all trials initiated probiotics as soon as infants were assessed as being stable and able to take enteral feeds within the first week after birth. Probiotics may have their maximum effect if given in the early days after birth as sepsis risks are highest on day 1 and 2 after birth and decrease thereafter. [1-3,67] In contrast, rates of necrotising enterocolitis are higher after infants reach day 3-4 of age, [1-3] however, probiotic supplementation may also impact during earlier preclinical phases of necrotising enterocolitis. Feasibility considerations are also important as many preterm infants are discharged from hospital within 48 hours after birth in LMICs and may these infants miss the opportunity to commence probiotics in hospital unless they are started early.[4]

### 1.5.2 Duration of supplementation

There is also no clear evidence as to when probiotic supplementation to preterm infants should be ceased. In the meta-analysis of 28 trials of 6729 infants receiving *L. rhamnosus* GG or *B. infantis*,[34] infants were dosed until 28 days or discharge except in four trials which dosed the infants to hospital discharge. There was no modification of effect by duration of dosing reported in the meta-analysis.

### 1.5.3 Infant feeding

Gastro-intestinal microbiota are different in human milk and formula fed infants.[68] Human milk contains human milk oligosaccharides (HMOs) which are an important substrate for *Bifidobacteria* spp and *B. infantis* (150). [69,70] In vitro studies have shown that the growth of some Bifidobacterium spp is enhanced in the presence of human milk oligosaccharides (HMOs).[44,71]

It is hypothesized that probiotics might be more effective in infants fed human milk than preterm formula.[23,70] In a large recent meta-analysis of 49 trials and 5044 infants, the effect of any probiotics on necrotising enterocolitis was only significant in human milk fed infants who were either exclusively breastfed (RR 0.75, 95% CI 0.65 to 0.86) or mixed fed (RR 0.85, 95% CI 0.69 to 0.99). In the meta-analysis of 28 trials of 6729 infants receiving *L. rhamnosus* GG or *B. infantis*,[34] all infants had received human milk. The subgroup analysis by amount of human milk feeding showed no difference.

It is unknown if probiotics are more effective in infants fed mother's own milk or donor human milk. Donor milk still contains HMOs, however many beneficial bacteria present in fresh human milk are destroyed in the process of pasteurization.[72]

## 1.6. Global policy implications

The care of preterm, SGA and LBW infants are a global priority in the United Nations Every Woman Every Child (EWEC) Global Strategy for Women's, Children's and Adolescents' Health 2016–2030,[73] the United Nations Children's Fund (UNICEF) Every Child Alive campaign,[74] the World Health Organization (WHO) 2025 Global Nutrition Targets,[75] and the joint WHO–UNICEF Every Newborn Action Plan (ENAP) to end preventable deaths.[76,77]

In 2022, a World Health Organization (WHO) guideline development group (GDG) reviewed the evidence for the impact of probiotics in preterm and term SGA infants and their final guidelines included a conditional recommendation for the use of probiotics for human milk fed infants < 32 weeks gestation or < 1.5kg at birth.[78] However, the evidence of impact on all critical outcomes (mortality, necrotising enterocolitis, sepsis and growth) was graded as low quality due to heterogeneity, risk of bias, indirectness and imprecision. In addition the GDG was not able to make a recommendation on type (i.e. genera,

species or strain), formulation (e.g. powder or drops), dose, timing or duration of probiotic administration as there was insufficient evidence.

The WHO and other organisations[35,78,79] have recommended that further large high-quality trials are implemented to provide evidence of sufficient quality and applicability to inform policy and practice. The WHO GDG recommended that rigorous new research was needed to improve certainty of evidence on critical outcomes and also to understand optimal strains, dosing and duration of probiotic supplementation and related impact in LMICs.[78]

### 1.7. Summary

There is an imperative to clarify the effect of probiotics in preterm and term SGA infants using a programmatically feasible formulation and approach that is effective and safe in high burden LMIC settings, especially Asia and Africa.

WHO aims to coordinate a large definitive multi-country multi-centre individually-randomised double-blind parallel-group placebo-controlled trial of the impact of probiotic supplementation on mortality, morbidity and growth to six months in human milk fed preterm and term SGA infants.

Five high quality sites (two in Asia and three in Africa) have been chosen for the trial following an open call for expressions of interest. WHO plans to take a pragmatic approach to the choice of probiotic, dose and duration for use in these LMICs. Based on the available evidence, we propose using a multi strain high quality pharmaceutical grade product with an established safety profile that has been used in vulnerable preterm and term SGA infants in multiple countries.

Based on our evidence review we have chosen to use the strains of *B. Infantis* DSM33361 and *L. rhamnosus GG* DSM33156 in combination in our trial as they have been specifically developed for preterm infants and used extensively in preterm infants without safety concerns. These strains have proven individual effects and may have additive and synergistic effects without evidence for antagonism or harm.

We have chosen a study population of healthy preterm infants. We will exclude infants from participation if they have confirmed sepsis or cannot feed enterally for example if they have major congenital are mechanically ventilated, post surgery or too unwell to be fed.

The strains will be produced at high quality using standards specifically developed for preterm infants (called ‘’pre-inf’’ standards). The strains will be provided with documentation of compliance and analysis necessary for administration to preterm and SGA infants including certificates of: formulation, preparation, administration, testing for quality and purity, testing for contamination with bacteria, testing for species and strain identity and purity, and testing of ‘stability’ and viability at end of shelf life.

Infants will be enrolled within 48 hours after birth (day 2) and dosing will commence at that time. Infants will be given doses of 0.35 billion CFU *B. infantis* and 1 billion CFU *L. rhamnosus GG* once daily for 28 days as these are the doses and duration in most common use and recommended by guideline developers. The formulation we will use is sterile, lyophilized and provided in sachets that can be mixed in sterile water in a volume of no more than 1-2 mls.

The trial will be powered for mortality effect and also for assessment of necrotising enterocolitis, sepsis and growth. All enrolled infants will be monitored closely for both efficacy and safety including probiotic sepsis (with anaerobic culture capability) and followed to 6 months of age.

## 2. Trial setting

The trial will be implemented in five countries (Bangladesh, Ethiopia, Kenya, Nigeria and Pakistan) which were selected following an open call for expressions of interest. Table 1 summarizes the key characteristics of the trial sites. All sites have high rates of preterm and SGA birth, mortality risk and malnutrition among infants in the first 6 months of life. All site hospitals are generalist public hospitals with a defined population catchment area. The hospitals admit women for all normal and higher risk deliveries from the nearby areas. All investigator teams have experience in conducting large hospital and community-based intervention trials.

**Table 1 Characteristics of trial sites**

| **Country** | **Bangladesh** | **Ethiopia** | **Kenya** | **Nigeria** | **Pakistan** |
|---|---|---|---|---|---|
| **Institution** | Projahnmo Research Foundation (PRF) | St Paul's Millennium Medical College [SPHMMC] and Armauer Hansen Research Institute (AHRI) | Kenya Medical Research Institute (KEMRI) | University of Ibadan (UI) | Aga Khan University (AKU) |
| | | | | | |
| **Planned recruitment over 18 months** | | | | | |
| **-preterm < 37 weeks** | 2,200 | 2,000 | 2,000 | 2,000 | 1,300 |
| **-term small for gestational age (SGA)** | 900 | 900 | 900 | 900 | 900 |
| **-total recruitment** | 3,100 | 2,900 | 2,900 | 2,900 | 2,200 |
| **Geographic location (region, district [s])** | Sylhet district, North-East region | Addis Ababa city, Oromia district | Kisumu, Migori, Kisii, Homa Bay, Western Kenya | Ibadan city, Lagos city | Karachi city, Sindh province |
| **Urban/rural** | Periurban | Urban and periurban | Semirural | Urban and periurban | Urban and periurban |
| **Socio economic status** | | | | | |
| **-unimproved source of drinking water (tanker/ truck /spring/river/lake) as main drinking water source** | 4.2% | 6.4% | 20.0% | 34.2% | 5.7% |
| **-use of flush toilet or improved sanitation facility** | 58.8%, | 11.8% | 10.0% | 53.4% | 78.8% |

| | | | | | |
|---|---|---|---|---|---|
| **-access to electricity** | 91.8% | 95.3% | 36.0% | 59.4% | 94.5% |
| **-women with no formal education** | 22.5% | 14.2% | 20.0% | 36.0% | 36.5% |

All data are unpublished data from trial sites, 2023

## 3. Aims and definitions

### 3.1. Aims, objectives, outcomes

The **overall aim** of the trial is to assess the effect of probiotic supplementation on mortality, morbidity, and growth in preterm or term SGA infants in the first 6 months of life in South Asia and Sub-Saharan Africa.

**Primary objectives**
In preterm infants: to assess the effect of probiotic supplementation on all-cause mortality from enrolment to 6 months.
In term SGA infants: to assess the effect of probiotic supplementation on 'underweight-free survival' at 6 months.

**Safety objectives**
The following safety outcomes will be assessed in both preterm and term SGA infants from enrolment to 6 months of age:
- episodes of serious adverse events (SAEs) (as defined in table 2 below)
- episodes of sepsis due to the probiotic strains *B. Infantis* or *L. rhamnosus GG*

**Secondary objectives**
1. To assess the effect of probiotic supplementation on the following outcomes at hospital discharge, 1 month and 6 months of age:
- all cause mortality (for the 6 month endpoint, this will be evaluated as a secondary outcome for term SGA infants, and as a primary outcome for preterm infants) and cause specific mortality due to necrotising enterocolitis, sepsis, severe diarrhoea
- episodes of necrotising enterocolitis, sepsis, severe diarrhoea
- episodes of pathogen specific sepsis (including *Streptococcus, Staphylococcus, Klebsiella, Escherichia coli , Acinetobacter spp) including their* serotypes and genotypic sequences
- episodes of hospitalisation

2. To assess the effect of probiotic supplementation on the prevalence of underweight, wasting, and stunting and mean weight for age (WAZ), weight for length (WLZ), and length for age z scores at 6 months of age

3. To assess the effect of probiotic supplementation on the abundance of *B. Infantis* and *L. rhamnosus* and other bacteria in infant faecal specimens at enrolment, 7 days, 28 days and 6 months of age; and in maternal faecal specimens at enrolment, including their serotypes and genotypic sequences.

4. To assess the effect of probiotic supplementation on the primary outcome in the following subgroups:
- prematurity (<32weeks, 32-<37weeks)
- birth weight (< 1.5kg, 1.5 to < 2.5kg, 2.5+ kg )
- preterm and SGA status (preterm SGA, preterm AGA, term SGA)
- vaccination (fully, partially, not vaccinated,) from enrolment to 6 months of age
- feeding type (exclusive, predominant, partial) at 1 month, 3 months, 6 months of age
- early initiation of enteral feeding (including mother's milk, donor milk, infant formula) (within < 1hour, 2-< 24hour, 24+ hours after birth)
- micronutrient supplement intake (multiple micronutrients, iron, vitamin A, vitamin D, zinc) from enrolment to 6 months of age
- receipt of antibiotics (by AWaRe classification[80] [Access, Watch, Reserve]) from enrolment to 6 months of age

## 3.2. Study definitions

Key study definitions are provided in tables 2a-2c below.

**Table 2a. Primary and secondary outcome definitions**

| |
|---|
| All-cause mortality = proportion of infants who die from any cause from enrolment to 6 months of age |
| Underweight free survival = proportion of infants with weight for age z score (WAZ) > = -2 standard deviations (SD) at 6 months of age who have not died |
| Necrotising enterocolitis = incidence of suspected necrotising enterocolitis (modified Bells grade 2 or greater criteria) from enrolment to 6 months of age |
| Sepsis =<br>-Suspected sepsis = incidence of suspected sepsis from enrolment to 6 months of age<br>-Confirmed sepsis = incidence of suspected sepsis with bacterial pathogens isolated from blood cultures from enrolment to 6 months of age |
| Severe diarrhoea = incidence of severe diarrhoea from enrolment to 6 months of age |
| Hospitalisation = incidence of hospitalisation for any cause from enrolment to 6 months of age |
| Underweight = proportion of infants with weight for age z score (WAZ) < -2 standard deviations (SD) at 6 months of age |
| Wasting = proportion of infants with weight for length z score (WLZ) < -2 SD at 6 months of age |
| Stunting = proportion of infants with length for age z score (WLZ) < -2 SD at 6 months of age |

See Annex 1 for case definitions

**Table 2b. Safety definitions**

| |
|---|
| Serious adverse event (SAE) = any AE as defined below that is considered serious at any dose if it: results in (i) death, (ii) requires inpatient hospitalisation, (iii) is life-threatening, (iv) results in persistent or significant disability/ incapacity, and (v) requires prolongation of the existing hospitalisation, or (vi) ) any other important medical event that may not be immediately life-threatening or result in death or hospitalisation, that may jeopardise the participant or that may require intervention to prevent serious outcomes [81] |
| Adverse event of special interest (AESI) = Events (serious or non-serious) of scientific and medical concern specific to the supplement whether or not considered related to the supplement that are: (i) cases of suspected sepsis and (ii) cases of suspected severe diarrhoea as defined according to the trial clinical case definitions (see Annex 1) that occur within 7 days of receiving the supplement.[81] |

| |
|---|
| Adverse event (AE) = Any untoward medical occurrence which does not necessarily have a causal relationship with the supplement. It is any unfavourable and unintended sign, symptom, or disease temporally associated with the use of the supplement, whether or not related to the supplement. This includes any unintended effects of trial interventions or trial conduct.[81] |
| Probiotic sepsis = an infant with suspected sepsis who has a study probiotic strain specific pathogen isolated in blood cultures[35] |

**Table 2c. Other study definitions**

| |
|---|
| Preterm infants are defined as infants less than 37 weeks gestation.[2,3] |
| Term infants are defined as infants 37 weeks gestation or greater[2,3] |
| SGA infants are defined as infants with birthweight below the 10$^{th}$ percentile for age and sex[5] |
| AGA infants are defined as infants with birthweight between the 10$^{th}$ and 90$^{th}$ percentile for age and sex[5] |
| LBW infants are defined as infants who weigh < 2.5kg at birth [2,3] |
| |
| Exclusive breastfeeding is defined as no other food or drink, not even water, except breast milk (infants are allowed to receive oral rehydration salts [ORS], drops and syrups [vitamins, minerals and medicines]) [82] |
| Predominant breastfeeding is defined as breast milk plus water based fluids (e.g. water, juice or tea) but no solids, milks or gruels; [82] |
| Partial breastfeeding is defined as breast milk plus solids, milks or gruels and can include water-based fluids.[82] |

See Annex 1 for case definitions

## 4. Design, study phases and timeline

This will be a multi-country multi-centre individually-randomised double-blind parallel-group placebo-controlled superiority trial implemented in five countries: two in Asia (Bangladesh, Pakistan) and three in Africa (Ethiopia, Nigeria, Kenya).

The trial will be conducted in compliance with the protocol, all country regulatory requirements, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Harmonised Guideline for Good Clinical Practice E6(R3) (ICHGCPR3) and CONSORT guidelines.[81,83]

As shown in Figure 1 below, preterm and term SGA infants will be screened and assessed for eligibility, enrolled within 48 hours after birth (by day 2), dosed daily for 28 days, and followed up 4-weekly in person for outcome ascertainment until they reach 6 months of age.

Separate teams will implement: screening and enrolment, intervention delivery, clinical, outcome assessment, and data management procedures.

**Figure 1. Trial flow**

**Figure 1a. Preterm infants trial flow**

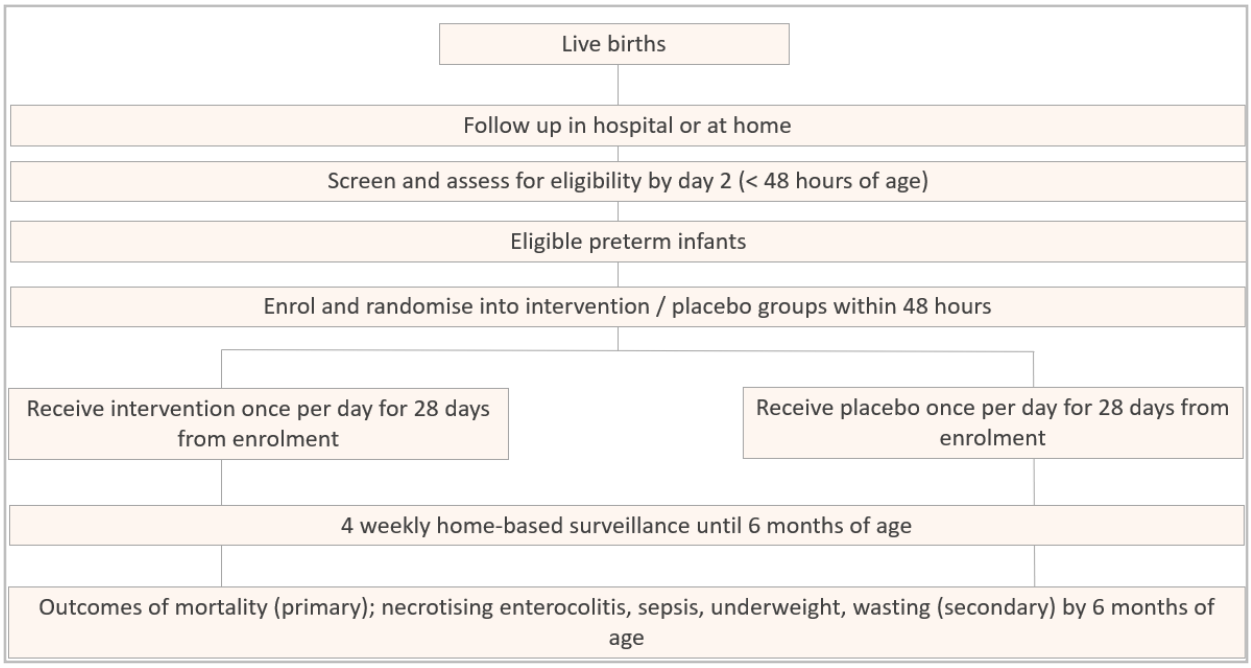
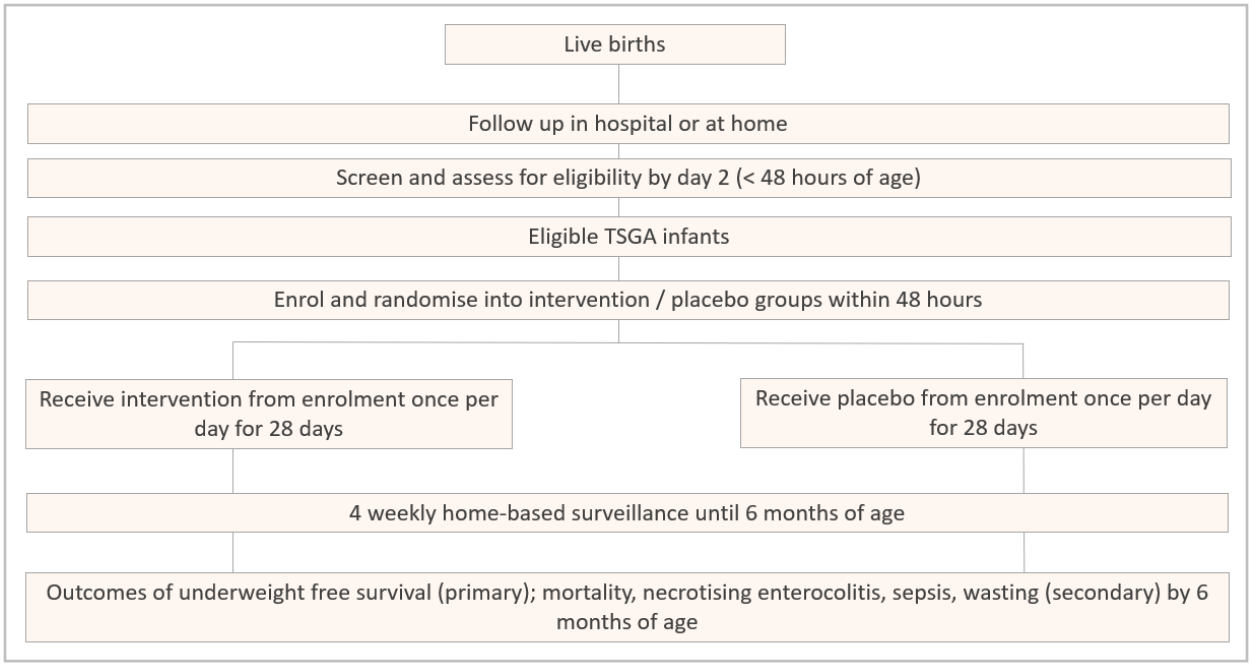



**Figure 1b. TSGA infants trial flow**




The trial will be conducted over 28 months. Infants will be enrolled over 18 months and followed up until they reach 6 months of age. There will be 4 months for final follow up, data cleaning and analysis.

Study procedures and tools will be piloted over an initial 4 month run in period. Further details can be found in Annex 2.

## 5. Trial organisation

### 5.1. Site teams

The implementation of the trial will be done by the site teams in the 5 countries (Bangladesh, Ethiopia, Kenya, Nigeria, Pakistan) headed by the trial PIs as listed on page 9. There will be dedicated teams in each of the 5 sites for all trial activities including: screening and enrolment, intervention delivery, clinical procedures, outcome assessment, laboratory procedures, data management, and oversight. Each team will have supervisors to train, support, monitor and provide ongoing quality assurance. Support and staffing is provided for these teams so there will be no disruption to normal hospital services.

### 5.2. Sponsor and Coordinating Centre

WHO/MCA will be the sponsor of the trial. There will be a dedicated coordinating centre (CC) based at John Hopkins University who will coordinate the trial. The CC roles will include the preparation of standard operating procedures (SOPs), daily and monthly reviews of data, and regular site visits. Consultants will be employed to assist the CC. This will include: clinical experts in regulatory trials, infectious disease, data management and statistics.

### 5.3. Trial steering committee (TSC)

There will be a trial steering committee (TSC) which will include all principal investigators (PI), the sponsor, and the CC. The TSC will review progress on a regular basis with the timing to be decided.

### 5.4. Data Safety and Monitoring Board (DSMB)

A Data Safety Monitoring Board (DSMB) will be constituted by WHO to review data, monitor the progress of the trial and assess safety of the intervention. Assessments of progress, safety and unblinded interim analyses will be conducted at timings decided by the DSMB. The DSMB will also advise the study team on trial continuation, modification or termination based on pre-decided stopping rules. Further details are in section 19 below.

### 5.5. Expert advisory group (EAG)

An expert advisory group (EAG) will be established consisting of senior neonatologists, probiotics, infectious disease and epidemiology experts. The EAG will provide ongoing expert advice at all stages of the trial. The EAG will be regularly updated on progress (timing to be decided) and be available for other ad hoc meetings if the need arises.

## 6. Sample size

The following assumptions have been used for sample size calculations for each population (preterm and term SGA): 90% power, 5% significance and 20% effect (i.e. effect of public health significance).

Disease rates in the comparator group were based on unpublished data from the sites and are presented in table 3 below.

For effect sizes, the recent Cochrane systematic review and metanalysis described above (Sharif et al 2021[21]) provided effect estimates for probiotics in preterm infants for: mortality (RR 0.76, 95% CI 0.65 to 0.89); necrotising enterocolitis (RR 0.54, 95% CI 0.45 to 0.65); and invasive infection / sepsis (RR 0.89, 95% CI 0.82 to 0.97). Also, as described above, one large high quality study has also shown effects of *B. infantis* on necrotising enterocolitis (RR 0.46, 95% CI 0.23- 0.93) and sepsis (RR 0.81, 95% CI 0.61 to 1.08). One retrospective cohort study showed similar effects on necrotizing enterocolitis in term SGA as in appropriate for gestational age (AGA) infants.[28,29] An additional systematic review has provided evidence of effect estimates on growth (improvement in weight (SMD 0.26, 95% CI 0.11 to 0.42) and height (SMD 0.16, 95% CI 0.06 to 0.25; at follow up to 6 years).[25] These effect sizes for preterm and SGA infants are all higher than the effect of ''public health significance (20%)'' that we have chosen to drive the sample size calculations, i.e. our effect size of public health significance can be considered to be conservative. Other baseline assumptions are derived from site, country and regional estimates.[4,84-89]

Based on these data and assumptions within the sites we calculate that the sample sizes needed for the primary endpoints, across 5 sites, are 8,602 preterm and 3,750 term SGA infants. These calculations were performed using Stata version 16.0.

Birth rates are shown in table 1 above and Annex 3. Overall we consider that in 18 months in each site the following numbers of infants can be enrolled: Bangladesh 3,100, Ethiopia 2,900, Kenya 2,900, Nigeria 2,900, Pakistan 2,200 (14,000 total infants, of whom 9,500 are preterm and 4,500 are term SGA). These numbers will allow for 9% loss to follow up in determining the primary endpoint among preterm infants (9,453) and 16% loss to follow up in determining the primary endpoint among term SGA infants (4,465) for a total of 13,918 infants to be enrolled.

**Table 3. Sample size calculations**

| | **Preterm study** | **Term SGA study** |
|---|---|---|
| **Primary outcome** | **Mortality at 6 months** | **Underweight free survival at 6 months** |
| Intervention effect | 20% reduction | 20% improvement |
| % in comparator | 10% | 20% |
| % in intervention | 8% | 24% |
| Power, significance level | 90%, 5% | 90%, 5% |
| Total needed at 6 months | 8,602 | 3,750 |
| Total infants needed to recruit at birth assuming loss to follow up rates* | 9,453 preterm | 4,465 term SGA |

*9% in preterm, 16% in term SGA

We consider that these sample sizes will be also sufficient to ascertain impact on the secondary outcomes. Specifically for the confirmed sepsis outcome, for preterm infants, assuming 90% power, 5% significance, 10% loss to follow up and baseline rate of culture confirmed sepsis in the comparator group of 7.5% at one month, we estimate that the sample size of 9500 preterm infants will be sufficient to detect a 20% effect at 80% and a 24% effect at 90% power. This will also provide sufficient sample size for assessing effects at six months of age at 80% and 90% power assuming that rates may rise to 10% in preterm infants at six months. For term SGA infants, assuming 90% power, 5% significance, 10% loss to follow up and baseline rate of culture confirmed sepsis in the comparator group of 5.0 % at one month, we estimate that the sample size of 4200 term SGA infants will be sufficient to detect a 30% effect at 80% and a 35% effect at 90% power. This will also provide sufficient sample size for assessing effects at six months of age at 80% and 90% power assuming that rates may rise to 7% in term SGA infants at six months.

## 7. Screening, assessment of eligibility, enrolment

### 7.1. Screening

Hospital staff (i.e. the doctors and nurses in the hospital who are routinely caring for mothers during labour and babies after birth) are part of the study team and have approval from the hospital administration units to notify live births to the site research team. Notification will occur as soon as possible after birth and within 48 hours.

The hospital staff will ask all mothers of all live born babies for authorisation to be approached by the research team as soon as feasible after birth and within 48 hours. The mothers will be given a copy of the screening information sheet by the hospital staff or the hospital staff will read the information sheet to the mother. If the mother gives authorisation, the research team will then visit the family while the baby is still in hospital, inform the family about the study and ask the mother of the infant for their informed written consent to conduct the screening procedures (as listed in the table below).

Mothers will be given as much time as possible to reflect on participation. The research team will explain that consent is needed before the baby is 48 hours old because probiotics may have maximum benefit in very young babies under 2 days of age. The research team will explain that if the mother is unable to decide if will not influence any care she receives from the study hospital.

### 7.2. Consent for screening

The mother of the infant will be asked for informed written consent for the infant to be screened. Sometimes the mother will need to discuss with the father of the baby before consent is given. Only mothers above the legal age of consent can give consent for screening. The consent will be done by senior experienced research staff who are not hospital staff. A standardised informed consent form will be used and the procedures in section 7.4 below will be followed.

### 7.3. Inclusion and exclusion criteria

Inclusion criteria
- < 48 hours of age at the time of enrolment
- Preterm (AGA or SGA) or term SGA infants
- Likely to be in the study area for the next 6 months

Exclusion criteria
- < 28 weeks gestation
- Not taking enteral feeds (e.g. due to a major congenital abnormality, mechanically ventilated, post surgery or too unwell to be fed)
- Confirmed sepsis at the time of enrolment
- Indwelling central lines (including umbilical catheter/central venous line) (infants with peripheral intravenous lines will not be excluded)
- Multiple births (e.g. twins, triplets)

### 7.4. Assessment of eligibility

| The following procedures will be followed. The assessments will occur in the health facility or at home if the infant has already been discharged. Inclusion criteria | Method of assessment |
|---|---|
| < 48 hours of age at the time of enrolment | The research team will check the hospital record and ask the family and hospital staff and make a final decision on the date and time of birth which will be entered into a pre-programmed tablet based electronic case record form (eCRF). The tablet will use an inbuilt algorithm to calculate the age of the baby in hours. |
| Preterm (AGA or SGA) or term SGA infants | The research team will assess the gestational age and measure the infant weight as described below and enter the data into the eCRF. The eCRF will use inbuilt algorithms to calculate the infants' grouping (preterm SGA, preterm AGA, term SGA, term AGA) based on the data entered. |
| Likely to be in the study area for the next 6 months | The research team will ask the mother of the infant |
| | |
| Exclusion criteria | Method of assessment |
| < 28 weeks gestation | The research team will assess the gestational age as described below |
| Not taking enteral feeds (e.g. due to a major congenital abnormality, mechanically ventilated, post surgery or too unwell to be fed) | The research team will review the hospital record and also ask the mother if the baby is currently (i.e. right now) able to take enteral feeds. This includes by breastfeeding, cup, gastric tube or other enteral means such as bottles. If possible the mother will be asked to demonstrate that the infant is taking enteral feeding, but if direct observation of enteral feeding is not possible this will not be an exclusion criterion. |

| | |
|---|---|
| Confirmed sepsis at the time of enrolment | The screening and enrolment team will review the hospital record to ascertain if the infant has blood culture results showing that the infant has any bacteria isolated from blood cultures |
| Indwelling central lines | The research team will review the hospital record and gently examine the infant to ascertain if the infant has any indwelling central lines such as umbilical catheters or central venous lines |
| Multiple births (e.g. twins, triplets) | The research team will review the hospital record and also ask the mother |

There may also be other reasons for not enrolling the infant. Reasons may include refusal from the mother, the baby is too unwell, the mother may have mental health issues or communication issues, the baby may be enrolled in another trial or there may be another reason. The reasons will be recorded in the study forms.

### 7.5. Assessment of gestational age

The infant's gestational age is needed to assign the infants into the correct study population (preterm or term SGA). A 'best obstetric estimate' (BOE) method will be used to allocate the infant's gestational age.[90] These algorithms are widely used in clinical practice[90] and use a hierarchical sequence of early trimester ultrasound, any obstetric ultrasound, and maternal recall of the date of the onset of the last menstrual period (LMP), to calculate the estimated date of delivery (EDD) and the infant's gestational age at birth. The screening team will review data from the mother's 'handheld' personal health records and will also ask for permission to obtain facility records to obtain information on the date of the mother's last menstrual period, estimated date of delivery, and ultrasound measurements wherever they are available. This information will be captured into the study data management system ( electronic case record forms [eCRFs] on hand-held tablet devices) which will have an inbuilt computerised algorithm that calculates the gestational age. The sites will work to ensure that all pregnant women in the participating hospitals have access to antenatal ultrasound as early as possible in their pregnancy (ideally before 20 weeks) to minimise the reliance on late trimester ultrasound and LMP in these algorithms. Neonatal examinations such as Dubowitz and Ballard will not be performed. If an ultrasound result is not available, all attempts will be made to determine the mother's LMP date. However, if the LMP date cannot be obtained, then this data point will be scored as not known and the infant will be excluded from the study. The assessment will occur in the health facility or at home if the infant has already been discharged.

### 7.6. Assessment of weight

The screening team will measure the infant's weight at < 48 hours after birth (day 2) using standardised electronic scales purchased especially for the study. These will be digital weighing scales with a graduation of 10 grams or less. This weight will be entered into eCRFs on hand-held tablet devices. The assessments will occur in the health facility or at home if the infant has already been discharged.

### 7.7. Categorisation of preterm and term SGA status

The screening team will assess the gestational age and measure the infant weight within 48 hours of birth as described above and will enter the data into a preprogrammed eCRF. Inbuilt algorithms will first calculate the infant's prematurity status. If the infant is preterm then the algorithm will use the Intergrowth 21 growth reference standards[91] to calculate preterm SGA (PSGA) or preterm AGA (PAGA) status. If the infant is term then the algorithm will use the WHO growth reference standards for term infants[92] to calculate if the infant is term SGA (TSGA) or term AGA (TAGA).

### 7.8. Consent and enrolment for the main trial

The mother of the infant will be asked for informed written consent for the infant to be randomised and included in the trial. This consent will be done by senior experienced research staff who are not hospital staff. At the time of screening the mother will be asked if she would like to hear about the main trial as well or if she would like the team to return at a more convenient time. A standardised informed consent form will be used and the procedures in section 7.4 below will be followed. The mother of the infant is required to give consent in this trial because biological specimens will be taken from the mother and her health care record will be accessed. Sometimes the mother will need to discuss with the father of the baby before consent is given. Only mothers above the legal age of consent can give consent in this trial.

### 7.9. Baseline data collection

At the time of enrolment baseline data will be collected by the enrolment team. These visits can occur at home or a health facility depending on site level procedures.

The mother will be asked for permission to review hospital records, handheld personal health records and discharge summaries and for the information to be recorded on the study CRFs. The data collection will be:

**Labour and delivery**. Site of delivery (e.g. inborn, outborn, name of hospital), type of delivery (including normal vaginal birth, Cesarean section, instrumental delivery), complications during labour.

**Mother's health care history**. Ethnicity, education level, routine antenatal and postnatal care, including vaccination status, morbidities during the antenatal and postnatal period and treatments given.

**Infant's health care history**. Morbidities after screening (such as congenital abnormality, birth asphyxia, sepsis suspected or confirmed, septic shock, respiratory distress syndrome, gastric surgery) and treatments given, invasive procedures (including central venous line insertion, umbilical catheter insertion, surgery, mechanical ventilation), routine health care including vaccination status and micronutrient supplementation received.

**Feeding practices.** Timing of initiation of enteral feeding of baby, what and how the baby has been fed since birth.

**Anthropometry.** Infant's length and head circumference ( measured by the research team using standardised equipment and procedures). Growth measurements including WAZ and WLZ and LAZ will be calculated by inbuilt algorithms that use the WHO growth standards for term infants,[92] and the Intergrowth-21 standards for preterm infants.[91]

**Faecal specimen from infants.** A fresh faecal specimen will be collected from a randomly selected 30% of infants. This specimen will be taken prior to the first dose of the supplement if possible. If not a specimen will be collected as soon as possible after supplementation begins. These will be fresh specimens collected directly from the infant diaper. Samples will be placed into specimen containers and transported to the hospital laboratory as soon as possible.

**Faecal specimen from mothers.** A fresh faecal specimen will be collected from the biological mothers of the 30% of infants who are randomly selected for faecal sample collection. This specimen will also be taken prior to the first dose of the supplement if possible. If not a specimen will be collected as soon as possible after supplementation begins.. This will usually be collected by the mother herself, However if the mother wishes it the hospital or research staff may collect it with the mother's permission. It will be collected into standard faecal specimen containers and processed as described above for the infant specimens.

### 7.10. Co-enrolment guidelines

Infants who are enrolled in other intervention studies will not be included in the PROPS trial.

## 8. Assignment of interventions

### 8.1. Allocation and sequence generation

Each site will have their own randomisation lists and randomisation will be done separately for the preterm and term SGA infants. i.e. There will be two randomisation coding 'lists': one for preterm infants and one for term SGA infants. The randomisation coding lists will be prepared and held by an external statistician who is not part of the study. Allocation to intervention and control groups will be done through a computerised server-based system with computer generated random numbers. A back up paper-based randomisation system will also be available if internet connectivity is disrupted. . Random permuted blocks of variable sizes (6, 8, 10 or 12) will be used during randomisation. This 'blocking' will also be prepared by the external statistician and will be accessible only to that statistician.

### 8.2. Allocation concealment and blinding

The participants (i.e. participants, care providers), hospital staff and all investigators (i.e. the site teams including the intervention, data collection, data management and data analysis teams, the WHO, and CC (including the project managers and internal statistics team) will be blinded to the group allocation, i.e. they will not know if the infant receives intervention or placebo sachets.

The intervention powder will be identical in appearance, consistency, taste and smell to the placebo powder. The intervention and placebo will be placed in foil sachets that are also identical in appearance and packaging. The sachets will be labelled, assembled into boxes and packs by an experienced packing team. The packing team will ensure that all labelling does not distinguish between placebo and intervention groups.

Each infant who is randomised will be allocated a randomisation number which will become their participant ID number. The randomisation sequence will be generated by the external statistician who is independent to the PROPS trial. A designated staff member will ensure that this code is sent to designated teams only and not to any other staff.

### 8.3. Emergency unblinding procedures

Clinical circumstances that will necessitate unblinding are not anticipated. The external statistician will hold the randomisation code that contains each participant's treatment allocation. However, the treatment allocation for each infant is also needed in each local site. This will be provided in the form of a sealed encrypted ''code break'' electronic file individual for each infant that contains each participant's treatment allocation. The file will be designed such that a participant's treatment may be disclosed, without affecting the blinding of the whole trial if it is in the direct interests of the participant.

Breaking of the blind for individual participants in emergency situations is only permitted in case of an important adverse event where the knowledge of the product is required for therapeutic decisions for the management of the participant. The principal investigator will be the one who will take the decision to break the trial blind. If time permits, the principal investigator will discuss this decision with the WHO project managers and in case of disagreement, it will be the Investigator's opinion that prevails. It is the wellbeing of the participant affected that overrides any other consideration.

The person who opens the file must record the reason and the date of opening, and then sign and date a print out of the process. It will be recorded in the study forms that the code is broken, why, when and by whom. The investigator will record the event of unblinding in the participant's medical record, including the reason for unblinding, but not the treatment allocation if this can be avoided.

It may be necessary to unblind an individual participant's treatment for the purposes of expedited reporting of any unexpected serious adverse event to relevant bodies. In this situation, every effort will be made to maintain the blinding of the personnel involved in data analysis. Information on whether the blinding has been broken for any participant will be collected before the database is declared clean and is released to the statistician at the end of the project. After completion of the trial, all emergency code break files will be returned to WHO.

## 9. Intervention and placebo supplementation

### 9.1. Intervention and placebo formulation, sachets, dose and duration

The intervention will be a high-quality, pharmaceutical grade product with known safety containing

*Lactobacillus rhamnosus GG DSM33156* and *Bifidobacterium longum subsp. Infantis DSM33361.* The doses will be 1 billion CFU of *L rhamnosus GG* and 0.35 billion CFU *B. Infantis* at 'end of shelf' i.e. on administration to the infant. Each strain will be mixed with 0.5 grams of maltodextrin lyophilized powder and provided separately in its own foil sachet.

The placebo will contain identical maltodextrin lyophilized powder but will be provided alone (i.e. without the probiotic strains) and will be packaged in its own identical foil sachets. It will be identical in appearance, taste, consistency and packaging to the intervention.

Each infant will receive two sachets. In the intervention group the infant will receive one *L rhamnosus GG* sachet and one *B. Infantis* sachet. In the comparator group the infant will receive two placebo sachets.

We have allowed 50% overage to account for accidental spillage, and vomiting or spitting of the dose by the infant. That is, we have allocated 84 sachets for each infant, two for each day for 28 days (56 sachets) and 28 back up doses.

The intervention and placebo supplements will be provided daily for 28 days from enrolment.

### 9.2. Manufacturing

A reputable manufacturer with a demonstrated record of high-quality production of these strains for preterm infants has been engaged to produce the trial intervention and placebo. The manufacturer will produce the supplements according to their high quality specifications and standards for preterm infants and will pack them into the identical intervention and placebo foil sachets. The sachets will be maintained at 2 to 8 deg C throughout the manufacturing process. After production of the sachets, the manufacturer will 'handover' to an specialised packing team who will organise the labelling, packing and shipping to the sites. The manufacturer will provide documentation about which batches are intervention and placebo only to designated teams.

The manufacturer will also provide certificates of compliance and analysis to be able to address: dose, potency, formulation, and testing for purity, quality, contamination, species and strain identity, storage conditions, stability and viability at end of shelf life.

### 9.3. Labelling, packing and shipping to sites

The packing team will carefully label all sachets and boxes, ensuring that the intervention and placebo sachets are kept separate. The team will assemble the sachets into packs, cartons and pallets for each site, label the cartons and pallets with the secondary and tertiary labelling and packaging according to country regulations, ship the cartons to each study site, provide the necessary regulatory and customs clearance documents, ensure adequate storage conditions (refrigeration at 2 to 8 deg C) are in place on arrival in country, and ensure a smooth transition to customs officials and the onsite investigators in country. The study teams will ensure the cold chain of 2 to 8 deg C throughout.

### 9.4. In country transport and storage

The sachets will be transported to each site and stored maintaining the cold chain with temperature between 2 to 8 deg C in each study site (via refrigerators or cold boxes). All sachets will be logged on

arrival in the site and site logs, registers, and store rooms will be maintained by designated site staff according to ICHGCP procedures.

### 9.5. Administration and back up doses

The intervention delivery team will log out 4 sachets (2 for the primary dose and 2 as the backup dose) for each enrolled infant each day and place the sachets in dedicated cold boxes (similar in appearance to vaccine carriers) to maintain the temperature of the sachets at 2-8 deg C throughout the day until return to the office.

To ensure the supplements are mixed 'fresh' i.e. just prior to feeding to the infant, the sachet contents will be reformulated by the dedicated intervention delivery team in designated areas in the respective site offices near the hospital wards or in the individual homes of participating infants. According to the manufacturer's instructions, to maintain cell count, the reformulated supplement must be used within 30 minutes. The manufacturer's instructions will be strictly followed to ensure hygiene and to avoid environmental contamination. The research staff will use dedicated gloves throughout the process of preparation and administration and will dispose of the gloves using the standardised study SOPs. In the hospital wards, the contents of each sachet will be mixed in 1-2 ml of sterile water in the study office and placed in a syringe labelled with the infant's study details. The prefilled syringes will then be placed in separate containers within cold boxes at 2 to 8 degrees Celsius for transport to the hospital ward. At the home visits, the sachets will be transported in their packaging in the cold boxes at 2 to 8 degrees Celsius. The intervention team will bring with them a small table. They will use this table to reformulate the sachet into study syringes using exactly the same method that is used for the hospital ward sachets.

The supplements will be administered enterally (i.e. orally or by gastric tube) to the infant using syringes in health facilities or home. The sachet contents will ideally be given to the infant by the mother who will be directly observed by the intervention delivery team (directly observed therapy [DOT]). It may take some time to administer the supplement to young preterm and SGA infants. Mothers will be advised to take as much time as is needed to administer the supplement.

If the intervention delivery team becomes aware that an infant has vomited soon after the study sachet ingestion (within approximately 15 minutes), they will give the backup dose to the infant. The dosing will be repeated once only. If the supplement is spilled then the back up dose can be given. There are no other reasons for giving the back up dose.

The amount of supplement administered to the baby will be recorded on the study eCRF.

If the infant refuses any of the primary dose, the back up dose should not be given.

Spare intervention and placebo sachets labelled with spare randomisation codes will be kept onsite. If it appears that all 84 allocated doses will be used (i.e. their primary dose and their back up dose) we will be able to allocate these spare doses to the infant whilst remaining blinded.

An intervention delivery CRF will be completed by the intervention delivery team which will include details of the sachets provided.

If the family have been told not to enterally feed the infant i.e. the infant has been made 'nil by mouth' or 'nil orally' by the medical team, or is unable to take enteral feeds, then the supplementation will be ceased until the infant is able to take feeds again.

### 9.6. Unused supplements

If infants are not able to be supplemented (e.g. the family were not at home), the unused sachets will be brought back to the study office. If cold chain has been maintained and the sachets are not damaged then the sachets can be logged back into the study store and reused for revisits as per the study SOPs.

At the end of the study all unused sachets will be destroyed according to national guidelines and the destruction process will be documented as per national guidelines.

### 9.7. Quality assurance of the intervention and placebo

Quality assurance processes will be implemented for all steps of the intervention process. For the manufacturer, quality assurance will include: predelivery testing for purity, quality, contamination, species identity and purity, and testing of 'stability' and viability at end of shelf life.

### 9.8. Concomitant care and interventions that are permitted or prohibited during the trial

Infants will be able to receive all other health care from the hospitals and health facilities that they require. There are no restrictions. We will record receipt of other probiotics, antibiotics and other medicines during the monthly data collection visits.

## 10. Safety monitoring

### 10.1. Definitions

**Adverse event (AE).** AEs are defined in this trial according to ICHGCPR3 as any untoward medical occurrence which does not necessarily have a causal relationship with the supplement. They are any unfavourable and unintended sign, symptom, or disease temporally associated with the use of the supplement, whether or not related to the supplement. This includes any unintended effects of trial interventions or trial conduct.

**Adverse event of special interest (AESI).** AESIs are defined in this trial according to ICHGCP as events (serious or non-serious) of scientific and medical concern specific to the supplement whether or not considered related to the supplement: (i) cases of suspected sepsis (including probiotic sepsis); and (ii) cases of suspected severe diarrhoea, both as defined according to the trial clinical case definitions (see Annex 1) and that occur within 7 days of receiving the supplement.

**Serious adverse event (SAE).** SAEs are defined in this trial according to ICHGCP as any AE as defined above that is considered serious at any dose if it: results in (i) death, (ii) requires inpatient hospitalisation, (iii) is life-threatening, (iv) results in persistent or significant disability/ incapacity, (v) requires prolongation of the existing hospitalisation, or (vi) any other important medical event that may not be immediately life-threatening or result in death or hospitalisation, that may jeopardise the participant or that may require intervention to prevent serious outcomes.

### 10.2. Safety assessments and reporting

There will be three types of safety assessment.

**Immediate.** All infants will be observed by research staff for 15 minutes after supplementation to determine if there are any concerns or if the infant will spit or vomit the supplement.

**Active surveillance (solicited).** The research staff will review all infants daily in person or by telephone for 7 days post supplementation and ask about all possible AEs, AESIs and SAEs as defined above using a dedicated CRF.

**Passive surveillance (spontaneously reported).** All research staff, monitoring staff and health facility staff will be asked to report all AESIs and SAEs throughout the trial using the same dedicated CRF.

All events that are suspected to be due to the product, and all AESIs and SAEs. occurring within 7 days of supplementation will be reported to the sponsor (WHO) within 24 hours of identification using standardised CRFs.

The clinical management of the infants is described in section 14 below. All these events will also be reviewed by the PIs to make the final diagnoses and standardised CRFs will be completed.

### 10.3. Adverse events of special interest (AESI)

All AESIs will also be reported on standardised eCRFs and sent to the sponsor within 24 hours of identification in the site. If the infant is admitted to a study hospital a hospital CRF will also be completed. All AESIs will be adjudicated by the dedicated study adjudication committee (as per section 16) and reported to WHO within 24 hours of the final adjudication being completed.

### 10.4. Serious adverse events (SAE)

All SAEs occurring within 7 days of supplementation will be reported to the sponsor within 24 hours of identification. They will be recorded on a dedicated 'initial SAE' CRF and monitored until resolution. At resolution a 'SAE close out' CRF will be completed by the PI which will include the final diagnosis and the grading of certainty and expectedness according to ICHGCP.

All SAEs will be communicated to the responsible agencies (e.g. ERCs, regulators) according to WHO and site specific procedures.

## 11. Outcome assessment

### 11.1. Four weekly outcome assessment visits

Each infant will be visited in person every 4 weeks by the outcome team. These visits can occur at home or a health facility depending on site level procedures. The mother will be asked for permission to review hospital records, handheld personal health records and discharge summaries and for the information to be recorded on the study CRFs. All study CRFs will be developed for the trial and will be standardised and pretested before use. The data collection will be:

**Vital status.** If the infant has died, and if so date of death, if the infant is hospitalised and if the infant is present at the visit.

**Recall of illnesses.** Recall about illnesses including infections, health careseeking including hospitalisations and treatments given.

**Health care use.** Any contact with health care providers for preventive care including vaccination status micronutrient supplements received, medicines received and their dates.

**Feeding practices.** Feeding practices (what and how the baby has been fed) since the last visit and in the last 24 hours.

### 11.2. Six month outcome assessment visit

This visit will occur at home or a health facility depending on site level procedures. At these visits data collection will include the same data collection as described for the outcome visits that occur every 4 weeks:

**Vital status.** If the infant has died, and if so date of death, if the infant is hospitalised and if the infant is present at the visit.

**Recall of illnesses.** Recall about illnesses including infections, health careseeking including hospitalisations and treatments given.

**Health care use.** Any contact with health care providers for preventive care including vaccination status micronutrient supplements received, medicines received and their dates.

**Feeding practices.** Feeding practices (what and how the baby has been fed) since the last visit and in the last 24 hours.

In addition the following will be collected:

**Anthropometry.** Weight, length, and head circumference (measured by the outcome team using standardised equipment and procedures). Growth measurements including WAZ and WLZ will be calculated by inbuilt algorithms that use the WHO growth standards for term infants,[92] and the Intergrowth-21 standards for preterm infants.[91]

### 11.3. Faecal specimen collection visits

Faecal specimens will be collected by the outcome team from a 30% random subsample of infants at all sites at day 7, week 4 and month 6 of infant age. Wherever possible the week 4 and month 6 faecal

specimen visit will be done at the same time as the outcome assessment visits. The faecal specimens will be fresh specimens collected directly from the infant diaper or via a faecal swab. Samples will be placed into specimen containers and transported to the hospital laboratory as soon as possible.

## 12. Hospital assessments

### 12.1. Clinical procedures

In the study hospitals there will be a designated 'clinical team' comprising hospital staff members and study research staff . All members of the clinical team will be nationally accredited as doctors, nurses, medical assistants, clinical officers or the equivalent accreditation in the site's country.

The clinical team will be trained in the standard study clinical case definitions for suspected sepsis, necrotising enterocolitis and severe diarrhoea (as per Annex 1). The clinical team will be also trained in ICHGCPR3.

The clinical team will review each enrolled hospitalised infant daily using a dedicated 'daily hospital review' CRF.

If an enrolled hospitalised infant aged under 6 months meets the study case definition for these conditions the clinical team will complete two study CRFs, one at presentation with the illness and the other at discharge.

If the infant meets the clinical case definition for suspected sepsis a blood culture specimen (2mls of blood) will be taken by the clinical team and inoculated into a paediatric aerobic blood culture bottle. In addition, for safety reasons, we will also monitor probiotic sepsis from anaerobic bacteria (i.e. *L. rhamnosus GG* and *B.infantis* are anaerobes). We will use standard procedures for this which is to perform anaerobic culturing on all specimens that flag positive from the aerobic bottles.

We will also perform molecular tests using study product specific PCR primers on the broth from blood culture bottles that flag positive to detect study-specific probiotic strains. However, these PCR results will not be able to be used for clinical care of the infants as they are not able to be done in real time. The results will allow us to understand the incidence of probiotic blood stream infection.

In line with WHO recommendations, all infants with suspected sepsis will receive broad spectrum antibiotics (e.g. empirical ampicillin and gentamicin)[93]. These antibiotics are highly effective against *L. rhamnosus GG* and *B. infantis*. Thus, all infants will be covered for possible probiotic sepsis regardless of the results of the tests.

If infants have suspected necrotising enterocolitis, in line with standard normal routine care, they will also receive additional tests for necrotising enterocolitis which may include a full blood count and abdominal X-Ray (AXR).

Any test that is needed for the study that is not normally free of charge to the family in the hospital, including blood cultures, will be paid for by the study.

We will develop study level training and SOPs for these activities in collaboration with the participating hospitals. The specimens will be processed as per the procedures in section 13 below.

## 13. Laboratory procedures

There will be a designated laboratory team comprising hospital staff members, laboratory staff and study research staff. All hospital staff members will be nationally accredited as laboratory technicians. The laboratory team will be also trained in ICHGCP. A 'hub and spoke' model will be used for the laboratory procedures; meaning that the hospital laboratories will process the specimens and transport all specimens to a centralised well equipped laboratory. All laboratories will be nationally accredited as laboratories that can manage specimen processing for hospitals.

**Laboratory procedures for blood cultures.** All blood culture specimens will be transported from the point of care to the hospital laboratory at room temperature within 1 hour. They will be logged in the hospital laboratory and then transported at room temperature to the central laboratory within 24 hours. The blood culture specimens will be processed in the central laboratory using automated blood culture equipment and standard methods and the isolates will be frozen at minus 60 to 80 degrees Celsius in case further testing is needed. The results of all blood cultures will be immediately communicated to the clinical team.

**Laboratory procedures for faecal specimens.** All faecal specimens will be transported from the point of care to the hospital laboratory as soon as possible. The faecal specimens will be processed in the central laboratory using standard methods and will be frozen at minus 20 to minus 80 degrees Celsius until analysis.

We will develop study level training and SOPs for these activities in collaboration with the participating hospitals.

## 14. Health care management in the enrolled infants

At all times the research staff will follow study SOPs for assessment and management of morbidities.

If an infant is considered to be unwell at any time the research staff will immediately assist the family in seeking health care from an accredited health care provider according to the SOPs. Families will be encouraged to seek care from a study hospital. However, the family are free to seek care from any health care provider of their choosing.

All participants in the study hospitals will be managed according to local clinical practice guidelines including antibiotic therapy. Site investigators are responsible for ensuring that the local clinical guidelines adhere to relevant WHO guidelines, or if a local guideline does not exist, that the relevant WHO guidelines are used.

If the baby becomes unwell we may need to share the baby's study information with the doctors or nurses caring for the baby. If we need to do this we will inform the mother first.

## 15. Retention

All randomised infants will have contact details (address, phone number, relatives) taken at enrolment, in order to facilitate community follow up to 6 months of age.

Infants will then have regular inperson visits from the study team: daily for 28 days for supplement administration, and daily for 7 days after the last supplement dose has been received. There will also be

scheduled follow up outcome assessment visits every four weeks after enrolment. Thus it is anticipated that loss to follow up from time of discharge to 6 months will be low. In some sites, infants and their mother will be invited back to the clinic for follow up visits. In others, the follow up visit will be done at home. For the home visits, if the family is not home at the time of the visit, two additional attempts to reach the family will be made before the visit is classified as a 'missed visit'.

## 16. Endpoint management

### 16.1. Central adjudication committee

All reports of deaths, sepsis, necrotising enterocolitis and severe diarrhoea (suspected and confirmed) will be reviewed by a central blinded adjudication committee and a standardised adjudication CRF will be completed. The committee will use the case definitions in Annex 1 including those for Bell stage necrotising enterocolitis. The committee will review each event ''case by case'' and decide on the final ''confirmed'' diagnosis and whether each individual event would be counted as a study endpoint. If a death occurs in the community we will administer a standard WHO verbal autopsy form and this will also be reviewed by the adjudication committee and a final cause of death assigned. We will develop study level training and SOPs for these activities in collaboration with the participating hospitals.

### 16.2. Withdrawing infants from the study and censorship

A participant will be discontinued from the study by the PI if:
- there are events considered by the family or the PI to be intolerable.
- the infant has a SAE that is likely to be related to the trial intervention
- there is early termination of the study by the sponsor

All mothers of the infants can also decide to discontinue study participation or decline provision of study supplements to their infant at any point. The mother does not have to provide a reason for withdrawal. However, if it is known, the reason for withdrawal will be recorded in the infant case report forms (CRF). If the infant's mother decides to withdraw the infant from the study then we will ask for permission for the infant's data to be retained in the databases. If the mother refuses then all data will be deleted.

All other withdrawal, discontinuation or censorship reasons will be documented including.
- completed study (i.e. primary and secondary outcome information complete)
- infant died from enrolment to end of follow up
- family left the study area permanently

Withdrawn or discontinuing participants will not be replaced by others, i.e. possible dropouts will not influence the sample size once the study has started.

### 16.3. Treatments to trial participants if they have been withdrawn

Infants who are withdrawn from the trial will receive the same standard of preventive and curative health services, as recommended and provided by the national health system, as the infants who remain in the trial.

## 17. Data management

### 17.1. Responsibilities

A web-based, GCP-compliant system will be used for data collection and management. A data management team will be contracted to develop and implement the data management system. A separate data management SOP will be developed. The process is shown in figure 2 below.

The CC has the overall responsibility for the implementation and conduct of data management. Data will be managed locally by each site and centrally by the CC. The WHO and CC will have real-time access to the data from each site to monitor study progress and safety events. The PI has the responsibility for the accuracy of data transmitted from the respective site. The study statistician will be responsible for overseeing data management, as well as development of the statistical analysis plan and execution of the pre-planned data analysis (and any subsequent secondary analyses). The first version of the statistical analysis plan including ''shell''(or ''dummy'') tables will be finalised before recruitment begins.

### 17.2. Data collection

Data will be collected electronically using tablets or hand-held devices. Range and logic checks will be built in to ensure data quality. Real time data will be transferred to local and web-based servers. Logic errors and checks across different forms will be run regularly by the sites and WHO; queries will be given to field teams for confirmation and corrections incorporated. The data collection system will also have offline capability and back-up paper forms will be used in some sites. Each site will have a data manager responsible for local query management and ensuring CRFs are correctly filled out. Any discrepancies will be immediately addressed.

### 17.3. Data quality assurance

Key measures to ensure study data quality are listed below:

**Overall study management:**
- All study investigators will undergo ICHGCP training and will have up to date certification
- The study protocol will be registered with an international clinical trial registry (CTR) platform and all changes to the trial protocol will be updated in the CTR as soon as they occur.

**Study sites:**
- Data collectors will be trained to correctly introduce the study to potential participants, administer the consent forms and correctly complete all CRFs and SOPs, as per the study SOPs
- Each site will pilot all trial procedures, including pilot testing the forms, before recruitment formally commences. Periodic refresher courses will also be conducted as necessary.

**Data management system:**
- A validation system will be built into the data entry and management system to ensure consistency, accuracy and completeness of the data collected (including range checks, consistency checks and skips).
- A SOP for data management will be developed and implemented

- Monthly data management calls with each site, the CC and the database manager will occur. Monthly reports will be generated on key variables for each site, so that all involved (i.e. the site database team, the CC and the overall trial database managers) review this together. Data queries will be discussed, and those pending will be resolved.

**Monitoring**
- As described in the monitoring section, all study procedures will be carefully monitored and corrective actions implemented where needed.

### 17.4. Confidentiality

This trial will generate an anonymised research dataset, with de-identified information of characteristics and outcomes from all infants participating in the trial.

The following measures will be taken to ensure participant confidentiality:
• Trial data for each participant will be identified by a unique anonymous ID number
• The local trial register linking personal information and trial ID numbers, and all personal information of participants, will be kept separate from the CRFs
• Trial documents will be kept securely under lock and key in the research offices and will not be accessible, other than to the researchers
• Data will be entered by trial ID number in the password-protected data management system to which only trial staff will have access
• The trial report will not contain the names of any participants
• After completion of the trial, the trial's documents will be archived in accordance with institutional and national rules for clinical research archiving.

### 17.5. Data access

WHO will be the data controller and custodian for the combined all-site data. The sites will be the custodian and retain ownership of their own respective site data. Each site will have access to its own site data during the trial to facilitate self-monitoring by the site team. i.e. Each site will hold identifiable data to ensure they can follow up each mother and infant, especially if there are any adverse events, but also to ensure outcome data can be collected and data queries addressed. Thus each site will hold a key that links personal data with participant IDs. Data with unique IDs but without names or identifiers (i.e. coded data) will be transferred to the CC and to WHO to enable WHO to report to the DSMB and the CC to monitor data quality and progress and coordinate the final data analyses. Identifiable data will be retained at the site level until the files are clean, analysis completed, and results published. At the end of the trial after the analyses are complete and published the key that links the personal data with the participant held by the site will be destroyed. At that stage the data will become anonymous. This anonymized individual-level patient data will be stored at WHO in perpetuity. After the trial is finished WHO will retain all these electronic data on WHO servers in electronic files permanently. Only WHO staff will have access to these electronic data after the trial is finished. WHO will bear costs and responsibilities for hosting these data.

In line with WHO's open access policy, trial findings will be made available in an open-access publication. Once the trial is completed and primary results published, the trial dataset will be made available for study partners to use for further analyses. For the purposes of re-analysis, sub-analysis or secondary

analysis of individual-level patient data, WHO will indicate that interested institutions or groups are able to submit requests for additional analyses of the dataset, which will be reviewed by WHO staff. Priority will be given to submissions from research collaborators who participated in the trial.

## 18. Data analysis

### 18.1. Responsibilities

The study statistician will be responsible for development of the detailed statistical analysis plan and execution of the pre-planned data analysis and any subsequent additional analyses. The first version of the statistical analysis plan will be finalised and approved by all PIs before recruitment begins.

### 18.2. Design

The primary analysis will be done by intention to treat (ITT) using individual level study site data combined together from all five sites. Analyses will be done separately for the preterm and term SGA infants. Analyses will be based on all enrolled infants with outcome data available. Any participants with protocol deviations, including infants who no longer wish to receive their allocated intervention but are prepared to have data collected, will be analyzed in the arm to which they were allocated in adherence to the ITT strategy. Data from participants whose mother withdraws their consent for their data to be used will be excluded from the analysis. However, if withdrawal of consent is only for continuing to participate in the study, but not for using the data, then data available from these participants will be included in the analysis.

### 18.3. Baseline characteristics and comparability between the two groups

Baseline characteristics of infants and their mothers and families will be compared in the intervention and control arms to check if randomisation achieved adequate balance in these characteristics between the groups. Summary values (means, proportions) for maternal, infant and sociodemographic characteristics among the groups will be presented in the baseline table. We will not perform any test of significance. The large sample size is likely to yield a balance between the groups. However, we will carefully examine the size of any baseline differences. Imbalanced characteristics that may influence the primary outcomes will be adjusted accordingly. Numbers and baseline characteristics of infants lost to follow-up will also be compared between study arms to detect any imbalances.

### 18.4. Flow of participants

The flow and number of participants through assessment of eligibility, randomisation, follow-up, and analysis will be presented along with reasons for exclusions and withdrawals for all time points.

### 18.5. Main effects

**For binary outcomes** – the number of participants, number of missing values and percentages by arm will be reported. The intervention group will be compared against the placebo group for the primary

outcome using risk ratios with 95% confidence intervals . A binomial model including study group (i.e. intervention or placebo) and country as covariates will be used with a log link to estimate risk ratios.

**For continuous outcomes** – the number of participants, the number of missing values, minimum, maximum, means and standard deviations by arm will be reported. The intervention group will be compared against the placebo group using mean differences and their 95% confidence intervals estimated by general linear models including country in the model as a covariate.

The primary analysis will be a complete case analysis (including only infants with complete outcome data) if there is less than 5% missing data on the primary outcome, or if we are unable to identify predictors for the missingness. If a complete case analysis is not appropriate, we will account for the missing data using multiple imputation, with the missing data assumed to be missing at random.

### 18.6. Safety outcomes

The following safety outcomes will be assessed in both preterm and term SGA infants from enrolment to 6 months of age:
- episodes of serious adverse events (SAEs)
- episodes of sepsis due to *B. Infantis* or *L. rhamnosus GG*

Results will be presented as simple counts, means, standard deviations in the intervention and comparator group. Standard methods for analysing continuous outcomes will be used as described above.

### 18.7. Faecal specimens

We will assess the effect of probiotic supplementation on the abundance of *B. Infantis* and *L. rhamnosus* and other bacteria in infant faecal specimens at enrolment, 7 days, 28 days and 6 months of age; and in maternal faecal specimens at enrolment, including their serotypes and genotypic sequences.

Results will be presented as simple counts, means, standard deviations in the intervention and comparator group. Standard methods for analysing continuous outcomes will be used as described above.

### 18.8. Subgroup analyses

The following subgroup analyses will be conducted for the primary endpoint:

Prerandomisation
- prematurity (<32weeks, 32-<37weeks)
- birth weight (< 1.5kg, 1.5 to < 2.5kg, 2.5+ kg )
- preterm and SGA status (Preterm SGA, Preterm AGA, Term SGA)

Post randomisation
- vaccination (fully, partially, not vaccinated,)
- feeding type (exclusive vs non exclusive) (formula vs no formula)

- early initiation of enteral feeding (including mother's milk, donor milk, buccal colostrum, infant formula) (within < 1hour, 2-< 24hour, 24+ hours after birth) (and mode of feeding e.g. direct breastfeeding, cup, gastric tube)
- micronutrient (MMN) supplement intake (any iron containing MMN, any vitamin A containing MMN, vs none)
- receipt of antibiotics (by AWaRe classification[80] [Access, Watch, Reserve]) from enrolment to 6 months of age

Post-randomisation subgroup analyses are at risk of bias and are advised against by some authors. However, in the current trial we believe there are good scientific reasons to investigate the post-randomisation subgroups defined a-priori above as there are plausible reasons why the effects of treatment could be different in the different subgroups. Before conducting these post-randomisation subgroup analyses, we will first examine whether the intervention has an effect on the stratifying variable. Statistical tests for effect modification by the factors mentioned above will be performed. These tests will have lower power but will help to avoid overinterpretation of the results of the subgroup analyses. All of the above subgroup analyses will be treated as hypothesis generating rather than hypothesis testing. The subgroup analyses are all are pre-defined and not data driven.

### 18.9 Other analyses

We will also describe rates of all outcomes by study site and hospital.

Final analyses will also compare the baseline characteristics of discontinuing and withdrawn infants to those who do not discontinue, in order to assess the possibility of follow-up bias.

We will also perform a per-protocol analysis (i.e. the full 28 days of supplementation) as well as the ITT analysis. We will also do analyses for infants who have received shorter courses of supplement for 1 week, 2 weeks, 3 weeks.

### 18.10. Additional methods for protecting against bias

Randomisation into the trial, group allocation, and distribution of study supplements will be done by study teams who are not part of the outcome evaluation. As described in the sections above, the interventions will be double-masked, i.e., the study supplements will look and taste identical and will be coded with randomisation codes. To ensure adherence, all study supplements will be given under direct observation.

## 19. Data safety and monitoring board

### 19.1 Roles and responsibilities

An independent Data Safety Monitoring Board (DSMB) will be appointed prior to commencement of the study. The roles and responsibilities of the DSMB in this trial include:

- To safeguard the interests of the trial's participants, potential participants, investigators and sponsors

- To assess the safety and efficacy of the trial's intervention according to adverse event data available while the trial is ongoing, and all other data available at a predefined schedule. This schedule will be fixed in advance by the DSMB.

- To monitor the trial's overall conduct and quality, and protect its validity and credibility

- To make recommendations to the trial steering committee concerning continuation or termination of study or any other modification necessary based on the observed effects of the intervention

The DSMB will meet before the trial onset and at prespecified intervals to review progress and safety throughout the trial. Additionally, any member of the DSMB may request an additional meeting at any point.

### 19.2 Composition

The DSMB will be independent and composed of qualified professionals who are completely uninvolved in the running of the trial and who cannot be unfairly influenced by those involved in the trial. The DSMB charter will be developed by WHO and approved by the DSMB members. It is planned that the DSMB will be composed of 4-5 members, including an independent chair, a statistician, and 2-3 technical experts familiar with the intervention, infant health care, and clinical trial methodology.

### 19.3 Interim analysis

The interim analysis will be conducted by an external statistician uninvolved in the running of the trial. At least one interim analysis will be conducted. The first will tentatively be conducted when the primary outcome is available for 40-50% of the target sample size. The DSMB will decide on the need for additional interim analyses at their first meeting.

The data will be sent to the DSMB by trial arm (A or B), but will not be unblinded (that is, the DSMB will not know whether A or B are the intervention or placebo arms). The data will be discussed at a closed session DSMB meeting that only the DSMB attends. The DSMB may request unblinding (i.e. to know if A and B are intervention or placebo) if the DSMB considers that it is necessary. Only the DSMB and the external statistician will receive the data and attend the meeting.

After the DMSB meeting, the chair of the DSMB will send a report to WHO with their recommendations. The DSMB might consider discussing the results with WHO in a joint meeting if needed. WHO will decide on the continuation of the trial and will report to the WHO ERC.

### 19.4 Stopping rules

The DSMB will make the final decision to terminate the trial. The DSMB will convene before the onset of the trial to agree on specific stopping rules for the trial. Tentatively, DSMB may recommend termination, dropping the placebo arm, or other modification of the study under the following conditions:

1. In an interim analysis, there is strong evidence of a mortality benefit (reduced mortality) from the study intervention. The DSMB will agree on the exact definition of “strong evidence”, but according to the Peto-rule, in an interim analysis a mortality difference should be considered statistically significant only if the 2-sided p-value is lower than approximately 0.001.[94] Simulation studies have shown that the Peto’s rule is not overly extreme and may result in little inflation of type 1 error in the final analysis.[95]

2. In an interim analysis, there is strong suspicion of harm (increased mortality or incidence of other SAEs) from the study intervention. For harm assessment, no fixed statistical rules will be applied, but the DSMB will holistically consider point estimates and confidence intervals for mortality / SAE incidence differences, p-values from appropriate statistical tests and other relevant factors, when determining its recommendation about study continuation or discontinuation.

3. In an interim analysis, there is strong evidence of futility (mathematical expectation predicting from the accrued data that the major study hypotheses cannot be supported with statistical significance with the originally planned sample size). While stopping rules will be utilized to help the DSMB to assess the justification of trial continuation, the DSMB will consider the balance of risks and benefits as well as consistency with external evidence. Thus, the DSMB recommendation to continue or discontinue will not be based on any single issue or value in any of the analyses but a comprehensive analysis considering multiple issues around the trial.

However, the recommendation to stop the trial after the results of an interim analysis will not be guided only by statistical considerations, but also other considerations such as external new information.

These conditions will be considered for the preterm and SGA infant groups independently.

## 20. Monitoring

### 20.1. Monitoring standards and procedures

The purposes of trial monitoring activities are to verify that:
-The rights and well-being of human participants are protected.
- The captured trial data are accurate, complete, and verifiable from source documents.
-The conduct of the trial is in compliance with the currently approved protocol/amendment(s), with ICHGCP, and with the other relevant local and international standards

There will be 6 levels of trial monitoring: site, WHO, CC, external, laboratory, and supplementation. Detailed monitoring SOPs will be developed.

The investigators will permit trial-related monitoring, audits, ERC review, and regulatory inspections, and will provide direct access to source data and documents.

Prior to commencement of recruitment, the CC will conduct training using the study SOPs with all study site research teams including: screening, informed consent, recruitment, data collection, follow up and other study procedures. This training will emphasise the need for accurate and thorough data reporting, and vigilance in identifying, detecting and reporting any possible adverse events, safety concerns or protocol deviations.

Noncompliance with the protocol, SOPs, ICHGCP, and/or other relevant standards by an investigator or institution will lead to prompt action by the sponsor to secure compliance. If necessary, formal audits may be done. If the monitoring and/or auditing identifies serious and/or persistent noncompliance, the hospital or site participation may be terminated, and relevant entities advised.

### 20.2. Monitoring by the site

Each study team will have their own supervisor who will train and monitor their teams and who will monitor adherence to all study SOPs. This will include scheduled and unscheduled visits, review of skills and quality of data collected, and feedback and supportive supervision.

The site PIs will oversee site monitoring, review monitoring data and conduct in person monitoring visits. They will focus on study processes, particularly screening, enrolment, supplement administration, and outcome measurement, as well as supervising quality checks on CRFs, database query resolution, SAEs and protocol deviations.

The site PIs will also monitor real time and monthly cumulative data by hospital including: eligibility criteria, recruitment, primary and secondary outcomes, protocol deviations, safety, progress and quality indicators.

### 20.3. Monitoring by the coordinating centre

Monitoring activities of the coordinating centre will include:

-Data monitoring by site and hospital – including eligibility criteria, recruitment, primary and secondary outcomes, progress, and quality indicators . The CC will also review all reports from the sponsor and external monitor and coordinate with the sites to implement appropriate actions.

-Regular online monitoring meetings with each site

-In-person site visits to monitor quality on the ground. These visits will be largely to monitor clinical processes, rather than document reviews. In particular, these visits will focus on screening, enrolment, supplementation, and outcome measurement. Additional in-person visits will also be organised as needed in response to specific urgent or emergency level issues.

### 20.4. Monitoring by the sponsor

Monitoring activities of the WHO sponsor coordinating team will include:

-Monitoring of safety and protocol deviations. WHO will monitor all SAEs, AEDIs, AEs and protocol deviations. WHO will also organise reporting of safety and protocol deviations to the DSMB and WHO ERC according to the DSMB and WHO ERC requirements.

-In person site visits to monitor quality on the ground. These visits will be largely to monitor clinical processes, rather than document reviews. In particular, these will focus on screening, enrolment, supplementation, and outcome measurement. Additional in person visits will also be organised as needed in response to specific urgent or emergency level issues.

### 20.5. External monitoring

External monitoring will be conducted by a contracted clinical research organisation (CRO) that is skilled in trial monitoring in LMICs. The CRO will have the responsibility to review enrolment and consent procedures including review of consent forms, adherence to probiotic supply chain and distribution procedures and quality of outcome assessment.

Monitors will be selected based on demonstrated monitoring experience, content area expertise and availability to conduct multiple visits. Monitors will not be employed or affiliated with institutions

participating in PROPS trial. The monitor's qualifications will be documented.

When selected, monitors will be trained on the protocol, informed consent forms, and all other relevant study documents (including SOPs).

The timing and frequency of the monitoring visits will be decided after appointment of the monitor. The frequency of monitoring visits may also change depending on site performance, rate of recruitment, and whether any issues have been identified. This will be decided in coordination with the sponsor as the trial progresses.

Findings from the external monitoring visits will be summarized in written reports with any recommendations for change documented. Reports will be reviewed by the sponsor, and action taken where necessary.

### 20.6. Specimen and laboratory monitoring

A dedicated infectious disease consultant team will be contracted to audit, build capacity and monitor the hospitals and laboratories in all sites including the following:

-supplies needed for specimen collection, processing and analysis
-specimen collection procedures
-transport of specimens
-laboratory procedures (both hospital and centralised laboratories)
-storage of specimens
-communication with clinical teams about results
-antibiotic therapy required and clinical management of infants

## 21. Training and standardisation and capacity building

This multi-country, multi-centre trial will involve 5 expert research sites working collaboratively with the CC and WHO.

Prior to study initiation, all staff will be trained in the study objectives, study strategy and in good clinical practice (GCP), including maintaining ICHGCP standards. Additionally, each team will undergo intensive training in their area of work (e.g. screening, consenting, anthropometry measurements, clinical procedures, intervention delivery, laboratory, data management). All country and hospital investigators will receive research ethics training and obtain and maintain a valid GCP certificate.

Research staff will be required to complete a standardized in-person training workshop with investigators and project managers, prior to commencement of site recruitment or new staff starting to work on the trial. Refresher courses will be conducted regularly.

Study teams will conduct pilot data collection prior to study commencement, to ensure they are able to accurately complete forms.

Research capacity strengthening will also be achieved through participation in this trial, including:
-Site research teams will actively participate in the development of study protocol, study forms, manuals and planning of trial operations;

-All key personnel working on the trial will be qualified by education, training, and experience to perform his or her respective task(s), and will complete GCP training;
-Training and workshops will be provided for local research teams on conducting trial activities;
-Training and workshops will be provided for site data managers in data management and biostatistical procedures;
-Once the trial is completed, the study will support dissemination activities (including conference travel) for country investigators and co-investigators to present findings.

## 22. Ethical and regulatory issues

This trial will follow the standards set by the World Medical Association Declaration of Helsinki on ethical principles for medical research.[96]

### 22.1. Research ethics approval

Ethical and regulator approval will be obtained from all sites, and ethical approval will be obtained from the WHO ethical review committee (ERC).

Any modifications to the protocol which may impact on the conduct of the study, potential benefit of the study participants or may affect their safety, including changes of study objectives, study design, study population, sample sizes, study procedures, study outcomes, study analyses, or other significant aspects will require a formal amendment to the protocol. Such amendments will be agreed upon by study PIs and submitted to WHO ERC and other relevant local ethical review boards prior to implementation.

Administrative changes of the protocol are minor corrections and/or clarifications that have no effect on the way the study is to be conducted. These administrative changes will be agreed upon by the study co-investigators and documented accordingly through version control. The WHO ERC and other ethical bodies will be notified of these at the discretion of the study PIs.

### 22.2. Informed consent

Written individual informed consent in the local languages will be obtained from the mother of the infant prior to the inclusion of the infant in the trial. Age of consent and assent and all procedures will adhere to the country level regulations in each site. A senior research team member will approach the mother to ask for consent. The mother will be given a copy of the information sheet and will be allowed time to read the sheet. The researcher will read out the sheet if the mother is unable to read or would like assistance. The researcher will also ask the mother if they understand the information in the form. If the mother appears not to understand any parts of the form, the researcher will explain each sentence until the mother clearly understands. The mother will be asked to sign the consent form. If the mother is unable to sign, a thumb imprint will be taken and witnessed by an impartial literate witness.

There will be two separate consent procedures (i) consent for screening and (ii) consent for the main trial. The main trial consent also includes consent for collection and analysis of biological specimens (faeces and blood) which will be collected only for the purposes outlined in this protocol. We will not be

using the specimens for other studies. We will also not be using any participant data for additional studies.

As described in section 7.1, after screening, mothers will be given as much time as possible to reflect on participation. The research team will explain that consent is needed before the baby is 48 hours old because probiotics may have maximum benefit in very young babies under 2 days of age. The research team will explain that if the mother is unable to decide if will not influence any care she receives from the study hospital.

### 22.3. Safeguards to protect any recognized vulnerability of the study participants

Mechanisms to protect privacy, confidentiality and safety, apply to all infants and their families, regardless of background. The mothers of the infants may be from disadvantaged groups (such as adolescents, migrants, poor women and women with chronic illnesses). Informed consent/assent forms and information forms will be available in simple, easy to understand terms, as well as translated into relevant local languages. If the mother is not literate, forms can be read to them by an impartial individual. All mothers will be given time to reflect on participation, and are free to refuse to participate, both confidentially and without prejudice.

### 22.4. Perceived risks and benefits of the study

As highlighted in the study background section, there is equipoise regarding the efficacy and safety of probiotics in low-resource settings for infants. This trial aims to establish whether probiotic supplementation is beneficial and/or harmful for both preterm and SGA infants . If proven effective, newborns and their families will benefit as probiotics can be scaled up across LMICs. Conversely, if demonstrated to be ineffective for or harmful then this is important for the families and the global community. While probiotics are relatively inexpensive, avoiding unnecessary or harmful use can also reduce healthcare costs.

### 22.5. Insurance

A reputable international trial insurance provider will be engaged to provide trial insurance for the period of the trial. Thus, all randomized participants will be covered by indemnity for non-negligent harm through trial participation.

### 22.6. Access to treatment or counselling for health conditions

Families will be encouraged to attend study facilities for health services wherever possible. The study teams will explain to mothers that they must seek care when they are concerned about problems with themselves and their infants. In particular, study teams will highlight the important danger signs and signs of infection that require care seeking. The study teams will ask families to inform them when they are seeking care from the study facility or other health facilities. The study team members will be carefully trained to assist in referral of all infants who are identified as being unwell during the trial.

Trial participants will not be disadvantaged in access to (or prevented from accessing) clinical care at any time during the trial. If any additional problems are identified during the trial, this will be brought to the attention of the treating clinician.

Trial participants in both groups will have access to all other therapeutic interventions available in the facilities.

### 22.7. Reimbursement or compensation to study participants

The study will pay for any test that is needed for the study. However, the study will not be able to pay for any other extra tests and will not be able to pay for additional hospital investigations or treatment including surgery.

The scheduled follow up visits will take approximately 15 to 30 minutes. Compensation for time spent during data collection will be non-monetary compensation in all sites and will be provided according to site specific protocols and customs [e.g. soap, phone credit, baby gift, utility items, thank you cards].

### 22.8. Payments to the trial organisers

Trial organisers do not receive any incentives for enrolling participants to the study. During the trial period, they will receive their normal salary from their employers or a personal stipend supporting postgraduate studies and living expenses.

### 22.9. Responsiveness of the project to community needs and priorities

There is a need for this trial in order to resolve questions of efficacy and safety relating to probiotic supplementation in low-resource settings, particularly given the potential role of probiotics in preventing avoidable infant mortality and morbidity. All sites are located in low-resource settings as described in section 2 above and in hospitals with a high caseloads of preterm and SGA infants.

### 22.10. Confidentiality

All study-related information will be stored securely at the study site. All participant information will be stored in locked file cabinets in areas with access limited to research study staff only who are GCP-certified.

All reports, data collection, process, and administrative forms will be identified by a coded ID [identification] number to maintain participant confidentiality. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from study records identified by code number. All local databases will be secured with password- protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in separate, locked files in limited-access secure areas.

### 22.11. Declaration of interests

All investigators will be required to complete a WHO Declaration of Interests form which includes declarations of financial and other competing interests. If the declarations constitute a conflict of interest, this investigator will be asked to stop participating in the trial.

## 23. Engagement with communities, local health facilities and the ministry of health

This study involves participants and their families as partners in research. The site teams have long standing links in the study areas and with the local leaders, villages, towns and cities. As per usual procedures the local PIs will hold meetings with local leaders to discuss the best methods of community engagement, information sharing and dissemination of findings after study completion. Local meetings will be held to explain the study and to ask for input including question and answer sessions. The site teams will also conduct end of study dissemination activities in each site.

Health service practitioners from the local health facilities will be part of the study teams and involved in development, planning, implementation and analysis. Study procedures will be complementary to existing health services. Permission has been sought from each health facility before implementation of the study.

At the time of screening, the mothers of the infant will be informed transparently and respectfully about the trial, including the risks and the freedom to choose not to participate. The mothers will also be allowed to consult with their family/ community members prior to consenting to participation in the trial.

## 24. Communication and dissemination activities

The investigators are committed to widespread communication and dissemination activities for this trial.

The approved trial protocol will be registered in a publicly availably international clinical trials registry (CTR) before the study commences. Regulatory approval for use of the probiotic strains will be obtained from all authorities according to national requirements. The study protocol will be published in an open access peer-reviewed journal as soon as possible after the trial procedures are developed. The statistical analysis code will also be provided as open access repository as soon as it is developed and tested.

If there are important protocol modifications such as changes to eligibility criteria, the interventions, these will be communicated to study participants, trial registries, regulators and journals.

Ministry of Health staff and other health service providers will be briefed about the study before it commences to ensure clear understanding of the trial's procedures from the outset.

Dissemination will follow a process of sharing results with the participants and local health care providers, Ministry of Health staff and the international community. On completion of the trial, the study teams will share the findings with the study participants through meetings with their local health care providers and community meetings where possible. National service providers including health departments and programme managers will be briefed and presentations will be made to ensure all interested parties understand the findings and consider implications for national and local programmes.

The primary trial findings will be prepared as peer-reviewed manuscripts, and published in a high impact, open-access, international journals and disseminated. Findings will also be reported at national and international conferences. Derivative products (including evidence summaries, policy briefs and other tools to facilitate implementation) will also be developed and disseminated. All trial investigators

will be eligible to be authors if they fulfill ICJME criteria for authorship. We will write all drafts of manuscripts ourselves and will not use professional writers.

## 25. Post trial access to study supplements if proven effective

Currently, these is only a conditional recommendation on the use of probiotics in preterm infants, and there are no current WHO guidelines on the use of probiotics in term SGA infants. In the event the study demonstrates that probiotic supplementation provides significant public health benefits for these vulnerable infants, the findings from this trial will be used to update WHO recommendations and develop new recommendations. The findings will also be used to develop LMIC national and hospital level clinical practice guidelines.

If the probiotic supplement is proven to be efficacious and safe, then WHO/MCA will work with the WHO prequalification department and UNICEF supply division to increase availability and access to the probiotic product in the study countries and all LMICs.

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## 27. Annexes

### 27.1. Annex 1 – PROPS clinical case definitions

| **Suspected sepsis** | |
|---|---|
| **< 2 months** | **2 months to < 6 months** |
| - During the current episode of acute illness, a history of one or more of:<br>not feeding well or not able to feed at all<br>movement only when stimulated or no movement at all<br>high body temperature (≥ 38 °C)<br>low body temperature (< 35.5 °C)<br>severe chest indrawing<br>convulsions<br>fast breathing (≥ 60 breaths per minute) in infants aged 0–6 days<br>drowsiness or unconsciousness<br>grunting<br>central cyanosis*<br>severe jaundice*<br>severe abdominal distention* | -During the current episode of acute illness, a history of fever or hypothermia plus a history of one or more of:<br>Seriously ill with no apparent cause<br>Purpura, petechiae<br>Shock |
| Adapted from:<br>WHO Pocketbook 2013[97]<br>IMCI 0-59d Chartbooklet 2019[98] | Adapted from:<br>WHO Pocketbook 2013[97] |

*The signs should not be able to be attributable to another diagnosis

| Suspected necrotising enterocolitis aged < 6 months | | | | |
|---|---|---|---|---|
| **Stage** | **Classification** | **Systemic signs*** | **Abdominal signs** | **Radiographic signs** |
| IA | Suspected | Temperature instability, apnea, bradycardia, lethargy | Gastric retention, abdominal distention, emesis, heme-positive faeces | Normal or mild intestinal dilation, mild ileus |
| IB | Suspected | Same as IA | Grossly bloody faeces | Same as above |
| IIA | Definite, mildly ill | Same as IA | Same as above, plus absent bowel sounds with or without abdominal tenderness | Intestinal dilation, ileus, pneumatosis intestinalis |
| IIB | Definite, moderately ill | Same as IA, plus 'mild' metabolic acidosis** or thrombocytopenia | Same as above, plus absent bowel sounds, definite tenderness, with or without abdominal cellulitis or right lower quadrant mass | Same as IIA, plus ascites |
| IIIA | Advanced, severely ill, intact bowel | Same as IIB, plus one or more of the following: hypotension, bradycardia, , combined respiratory acidosis and metabolic acidosis, neutropenia, or disseminated intravascular coagulation | Same as above, plus signs of peritonitis, marked tenderness, and abdominal distention | Same as IIA, plus ascites |
| IIIB | Advanced, severely ill, perforated bowel | Same as IIIA | Same as IIIA | Same as above, plus pneumoperitoneum |
| References: Modified Bell staging criteria for necrotising enterocolitis[99] | | | | |

* Hospital defined normal values will be used

** To be defined

| **Suspected diarrhoeal disease** | |
|---|---|
| **< 2 months** | **2 months to < 6 months** |
| -A young infant has diarrhoea if the faeces have changed from the usual pattern and are frequent and watery (more water than faecal matter). The frequent semi-solid faeces of a breastfed baby are not diarrhoea. The faecal frequency should be more than 3 times per day. Frequent passing of formed faeces is not diarrhoea, nor is the passing of loose, pasty faeces by breastfed babies. | -Passage of 3 or more loose or liquid stools per day. Frequent passing of formed stools is not diarrhoea, nor is the passing of loose, pasty stools by breastfed babies. |
| **Suspected severe diarrhoeal disease** | |
| **0 to 59 days** | **2 months to < 6 months** |
| As per case definition for diarrhoeal disease<br>Plus two or more of the following signs of dehydration | As per case definition for diarrhoeal disease<br>Plus two or more of the following signs of dehydration |
| -lethargy or unconsciousness | -lethargy or unconsciousness |
| -sunken eyes | -sunken eyes |
| -unable to drink or drinks poorly | -unable to drink or drinks poorly |
| -skin pinch goes back very slowly (>= 2 s) | -skin pinch goes back very slowly (>= 2 s) |
| | |
| References:<br>WHO Pocketbook 2013[97]<br>WHO Integrated Management of Childhood Illness (IMCI) 0-59d Chartbooklet 2019[98] | References:<br>WHO Pocketbook 2013[97] |

## 27.2. Annex 2 – Timeline for study implementation

| Trial month | -10 | -9 | -8 | -7 | -6 | -5 | -4 | -3 | -2 | -1 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Development of materials and approvals** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Development of protocol, CRFs, ICFs | x | x | x | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Protocol, CRFs, ICFs submitted to and approved by WHO ERC | | | | x | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Protocol, CRFs, ICFs and product package submitted to and approved by site ethics committees and regulators | x | x | x | x | x | x | x | x | x | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| **Data management system development, statistical analysis and monitoring** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Data management and statistical systems development, monitoring and analysis | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| **Main trial** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SOP development and training | x | x | x | x | x | x | x | x | x | x | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Run in period for pretesting and trial activities | | | | | | | | x | x | x | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Start of enrolment, supplementation and follow up of infants | | | | | | | | | | | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Follow up of infants | | | | | | | | | | | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | | | | | | | | | | |
| End of enrolment and supplementation | | | | | | | | | | | | | | | | | | | | | | | | | | | | | x | | | | | | | | | | |
| End of follow up | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | x | | | | |
| Final cleaning and first results available | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | x | x | x | x |
| **Product** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Product released from manufacturer and to sites | | | | | | | | | | x | | | | | | | | | | | x | | | | | | | | | | | | | | | | | | |
| End of shelf life of product (24m) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | x | | | | | | | a |

## 27.3. Annex 3 – Numbers of infants expected in the study

| | Bangladesh | Ethiopia | Kenya | Nigeria | Pakistan | Total numbers |
|---|---|---|---|---|---|---|
| Preterm < 37 weeks | 2,200 | 2,000 | 2,000 | 2,000 | 1,300 | 9,500 |
| No of preterm infants with sepsis | 550 | 500 | 500 | 500 | 325 | 2,375 |
| No of preterm infants with positive blood cultures | 165 | 150 | 150 | 150 | 98 | 713 |
| | | | | | | |
| Term SGA | 900 | 900 | 900 | 900 | 900 | 4,500 |
| No of SGA infants with sepsis | 135 | 135 | 135 | 135 | 135 | 675 |
| No of SGA infants with positive blood cultures | 41 | 41 | 41 | 41 | 41 | 203 |

## 27.4. Annex 4 – SPIRIT matrix

| Spirit terms | Activity | Activity details | STUDY PERIOD | | | | | | | | | | | Form | Team |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Enrolment | Allocation | Post-allocation | | | | | | | Close-out | | |
| | | | TIMEPOINT | minus t1 | 0 | t1 | t2 | t3 | t4 | t5 | t6 | t7 | t8 | tx | |
| | | | Chronological age in da | <2 | <2 | <2 | | | | | | | | | |
| | | | Age since enrolment in | 2 | 2 | 2 | 28 | 35 | 56 | 84 | 112 | 140 | 168 | 182 | |
| | | | Age in weeks | 0.3 | 0.3 | 0.3 | 4 | 5 | 8 | 12 | 16 | 20 | 24 | 26 | |
| | | | Age in months | 0.1 | 0.1 | 0.1 | 0.9 | 1.2 | 1.8 | 2.8 | 3.7 | 4.6 | 5.5 | 6.0 | |
| ENROLMENT: | ENROLMENT: | | | | | | | | | | | | | | |
| Informed consent | Informed consent for screening | Informed consent for screening | | X | | | | | | | | | | ICS | Screening team |
| Eligibility screen | Screening | Screening | | X | | | | | | | | | | SCR | Screening team |
| Informed consent | Informed consent for main trial | Informed consent for main trial | | X | | | | | | | | | | ICM | Screening team |
| Allocation | Allocation | Allocation | | | X | | | | | | | | | RAN | Screening team |
| | Baseline health data collection | Baseline infant and maternal health | | | X | | | | | | | | | BAS, ITH, PHC, FDP | IOA team |
| | Baseline infant anthropometry | Baseline infant anthropometry | | | X | | | | | | | | | ANT | IOA team |
| INTERVENTIONS: | INTERVENTIONS: | | | | | | | | | | | | | | |
| | Product delivery and compliance* | Product delivery and compliance | | | | ◆ | — | | | | | | | PDC | Intervention delivery team |
| ASSESSMENTS: | ASSESSMENTS: | | | | | | | | | | | | | | |
| | Safety 1** | AESI and other safety ascertainment | | | | ◆ | — | ◆ | | | | | | SAF | Intervention delivery team |
| | Safety 2 | SAE initial ascertainment | | | | ◆ | — | ◆ | | | | | | SAI | All |
| | Safety 3 | SAE review | | | | ◆ | — | ◆ | | | | | | SAR | All |
| | Safety 4 | SAE close out | | | | ◆ | — | ◆ | | | | | | SAC | PIs |
| | Hospital 1 | Hospital daily review | | | | ◆ | — | — | — | — | — | — | ◆ | HDM | Study clinicians |
| | Hospital 2 | Hospital case ascertainment initial illness | | | | ◆ | — | — | — | — | — | — | ◆ | HCA | Study clinicians |
| | Hospital 3 | Hospital case details at discharge | | | | ◆ | — | — | — | — | — | — | ◆ | HDC | Study clinicians |
| | Specimens1*** | Faecal specimen collection | | | | ◆ | — | | | | | | | SPE | Screening team at enrolment |
| | Specimens2 | Blood culture specimen collection | | | | ◆ | — | — | — | — | — | — | ◆ | SPE | Study clinicians |
| | Specimens3 | Laboratory specimen reception | | | | ◆ | — | — | — | — | — | — | ◆ | SPR | Laboratory team |
| | Specimens4 | Laboratory results | | | | ◆ | — | — | — | — | — | — | ◆ | LAB | Laboratory team |
| | Outcome assessment1 | Infant illness and health care | | | | | X | | X | X | X | X | X | X | ITH, PHC, FDP | IOA team |
| | Outcome assessment3 | Final infant illness and health care | | | | | | | | | | | | X | ITH, PHC, FDP | IOA team |
| | Outcome assessment2 | Final infant anthropometry | | | | | | | | | | | | X | ANT | IOA team |
| | Endpoint ascertainment1 | Adjudication of trial endpoints | | | | | X | | X | X | X | X | X | X | ADJ | Adjudication committee |
| | Endpoint ascertainment2 | Censorship | | | | ◆ | — | — | — | — | — | — | ◆ | CEN | IOA team |
| OTHER ASSESSMENTS: | MONITORING: | | | | | | | | | | | | | | |
| | Protocol deviation1 | Protocol deviation | | ◆ | — | — | — | — | — | — | — | — | ◆ | PDE | All |
| | Protocol deviation2 | Sponsor review of protocol deviations | | ◆ | — | — | — | — | — | — | — | — | ◆ | PDS | Sponsor |
| | Monitoring and supervision | Monitoring and supervision | | ◆ | — | — | — | — | — | — | — | — | ◆ | SUP | Monitoring and supervision |
| | PROCESS EVALUATION: | | | | | | | | | | | | | | |
| | Process evaluation | Process evaluation | | | | | ◆ | — | ◆ | | | | | PRO | Process evaluation |

**Notes**

First 24 hours = d1, 24-47h= d2, 48-75= day 3 etc

* Infants are dosed from day 1 or 2 to day 29 or 30 (ie for 28 days)

** Safety monitoring is for 7 days after each dose, it continues for 7 days after the last dose ie the last dose is day 29 or 30 so the last safety visit will be 29 + 7 = day 36 or day 37

*** Faecal specimens collected at enrolment, day 7, day 28, and month 6