STUDY PROTOCOL

LIFE
Low-birthweight Infant Feeding Exploration

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Contents

1 Acronyms

ANC	Antenatal care
BF	Breastfeeding
BMGF	Bill & Melinda Gates Foundation
BMS	Breast Milk Substitute/Formula
CASI	Context Assessments for Successful Implementation
CMO	Chief Medical Officer
DA	Dearness Allowance
DHM	Donor Human Milk
EBF	Exclusive Breastfeeding
FGD	Focus Group Discussions
HIC	High income country
HSPH	Harvard TH Chan School of Public Health
IDI	In-depth Interviews
IUGR	Intrauterine Growth Retardation
IYCF	Infant Young Child Feeding
JNMC	Jawaharlal Nehru Medical College
LAZ	Length-for-age z-score
LBW	Low Birthweight
LIFE	Low Birthweight Infant Feeding Exploration
LMIC	Low- and Middle-Income country
LMP	Last Menstrual Period
MOM	Mother's Own Milk
MUAC	Mid-upper arm circumference
MUHAS	Muhimbili University of Health & Allied Sciences
NEC	Necrotizing Enterocolitis
NICU	Neonatal Intensive Care Unit
OHRP	Office for Human Research Protections
PMI	Maternal perceived milk insufficiency
SOC	Standard of Care
TAG	Technical Advisory Group
UNC	University of North Carolina
VLBW	Very Low Birthweight
WASH	Water, sanitation and hygiene
WAZ	Weight-for-age z-score
WHO	World Health Organization
WLZ	Weight-for-length z-score

2 Definitions of technical study terms

Term	Operational definition for LIFE study
Birthweights	
LBW	less than 2,500 g/5.5 lbs (UNICEF 2014).
VLBW	<1500 g
LBW band 1	1500-1799 g
LBW band 2	1800-2199 g
LBW band 3	2200-2499 g
Feeding behaviors	
Complementary feeding	Feeding of any liquids or foods other than breastmilk or formula to an infant after 6 months of age.
Predominant breastfeeding	Breast milk as predominant source of nourishment, but may also have received liquids, ritual fluids and ORT, drops or syrups (Haroon et al 2013)
Partial breastfeeding	Giving a baby some breastfeeds and some artificial feeds, either milk or cereal, or other foods (Haroon et al 2013)
No breastfeeding	Infants receiving no breastmilk at all (Haroon et al 2013)
Early initiation of breastfeeding	Provision of mother's breast milk to infants within one hour of birth
Exclusive breastfeeding	Child receiving only breast milk (including milk expressed or from a wet nurse) and no other type of milk or solids but could include vitamins, drops of other medicines and oral rehydration therapy (ORT) (Haroon et al 2013).
Breastmilk expression	Expression of breastmilk through any means, such as hand expression, electric pump, or manual pump.
Parenteral feeding	Any method of intravenous feeding
Enteral feeding	Any method of feeding through the gastrointestinal tract, including via the mouth.
People	
Mother	Biological mother. For this protocol, in the case of a maternal death, this term will refer to the primary caregiver for the infant.
Mother-infant pair	Both biological mother and infant together. In the case of multiples, this phrase includes all infants.
Caregiver	Anyone giving care and feeding to the infant post-discharge, such as fathers, family members, or friends.
Primary caregiver	A single person who gives the majority of care and feeding to the infant post-discharge. Typically, the mother, but can also be a father, family member, or friend.
Clinical staff	Any member of the site staff that provide clinical care to study subjects, such as physicians, nurses, or midwives
Study staff	Anyone hired by LIFE to perform study-related activities
Formula types	

Standard term formula	Designed for term infants, based on the composition of mature breast milk. The typical energy content is 68 kcal/100 ml. The concentration of protein, approximately 1.4-1.5 grams/100ml, and calcium and phosphate are not sufficient to provide the recommended nutrient needs for stable and growing preterm infants.(Cochrane Review: Henderson et al 2007)
Preterm formula	Calorie-enriched (approximately 80 kcal/100 ml), protein-enriched (approximately 2.0- 2.4 grams/100ml), and variably enriched with minerals, vitamins, and trace elements to support intrauterine nutrient accretion rates. These milks are often used for nutrition of preterm infants prior to hospital discharge (Cochrane Review: Henderson et al 2007)
Post-discharge formula	Specifically designed for a preterm infant's post-discharge from hospital. These are less nutrient dense compared with preterm formulae, but are calorie (about 72- 74 kcal/100 ml), protein (about 1.8 grams/ 100 ml) -enriched, and variably enriched with minerals, vitamins, and trace elements compared to standard term formula milk. (Cochrane Review: Henderson et al 2007)
Location within facilities	
Neonatal care facility	Any dedicated space that offers additional neonatal care beyond the healthy mother-infant pairs. This space can have different names, such as NICU, SNCU, neonatal ward, neonatal care facility.
KMC	Kangaroo Mother Care
NICU	Neonatal Intensive Care unit, including levels I, II, or III
Study locations	
Study country	The three countries are Tanzania, Malawi, and India.
Study site	The four study sites are Dar es Salaam, Lilongwe, Karnataka, and Odisha.
Study facility	Each study site has 2 to 5 study facilities, which are individual hospitals or clinics. There are 12 total study facilities: ● Lilongwe: 2 ● Dar-es-Salaam: 3 ● Karnataka: 5 ● Odisha: 2
Study region	The two study regions are Africa (Tanzania and Malawi) and SE Asia (India), which have two study sites each.

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2.1 Defining “failure”

At several points in the course of designing LIFE, the study team debated definitions for the terms “failure to thrive,” “failure to breastfeed,” “nutritionally at risk,” “unsuccessful breastfeeding,” and

similar terms. The idea was to generate a study-wide "bright line" definition of an infant's clinical status that would trigger a referral to further care, providing a safety net for study participants.

After debate, we came to consensus that this concept does not require a consistent definition across all study sites in LIFE. Instead, we will use the existing guidelines at each site, referring infants for further care based on the clinical criteria already in use at each site. This approach has several advantages:

1. It is consistent with an observational study design.
2. It is likely more acceptable to local ethics boards, since each definition reflects local ethical norms
3. It facilitates field work because no systemic changes to clinical care will be required.

As a matter of study design, this decision also aligns with our intention *not* to pool data across sites. Rather, we will analyze data from each site separately, describing the SOC in each, so each site dataset will have a consistent definition.

When we design the follow-on LIFT trial, which will be interventional, we will establish a study-wide, consistent, clear, clinical definition of infants for whom breastfeeding is insufficient, as an inclusion criterion. At the point of designing LIFT, we will use the LIFE data to inform this definition, since we will know much more about breastfeeding behaviors, clinical paths, and eventual outcomes of these LBW infants.

No matter the definition, we note a consensus to avoid the word "failure" in the name of the concept.

3 Background and problem statement

Globally, 15% of all babies, amounting to 20 million infants each year, are born low birthweight (LBW). Compared to normal weight infants, LBW infants are at higher risk of morbidity, mortality, and poor growth (Risnes et al 2011; Larroque et al 2001; WHO 2006). The main causes of LBW are preterm birth, intrauterine growth restriction (IUGR), or their combination. Preterm birth and IUGR may have different causes, and these conditions may carry different risks for morbidity, morality, sub-optimal feeding and growth (Katz et al 2013; Christian et al 2013). LBW contributes directly or indirectly to 60-80% of neonatal deaths (WHO 2011; Lawn et al. 2014). It is such an urgent issue that the World Health Assembly set out to reduce LBW by 30% by 2025 (WHO 2014). Unfortunately, there is a paucity of information around feeding practices and optimal feeding strategies for this population, particularly for LBW infants who struggle with breastfeeding or growth.

In 2011, the World Health Organization released the Guidelines on Optimal Feeding for Low Birthweight Infants in Low-and Middle-Income Countries (LMICs). However, 70% of the guidelines are based on "low or very low" quality of evidence (WHO 2011), and the majority of research regarding mother's own milk (MOM) alternatives has been in high-income, hospital-based settings (Blencowe 2013). There is a pressing lack of information about LBW infants in LMICs: existing feeding patterns; rates and causes of unsuccessful breastfeeding; and effective options for feeding, fortification, or supplementation with micronutrients. Forty global health organizations recently issued an urgent call to action to improve the evidence base, specifically for neonatal care units (Greenslade et al 2017). This study hopes to address some of these key gaps.

Strong evidence does exist that breastfeeding improves outcomes for all infants and particularly for LBW infants. WHO and others recommend *early* initiation of breastfeeding within the first hour of birth and *exclusive* breastfeeding for six months with MOM (Haroon et al. 2013). Rates of early initiation of breastfeeding within one hour of life vary widely across the world, with a global estimate of 42% (UNICEF 2018) and regional estimates ranging from 14% to 93% of infants (Development Initiatives 2017). Only 38% of infants globally are exclusively breastfed in the first six months of life (UNICEF 2015). The rate of early and exclusive MOM feeding in LBW infants is not well known. Data are also lacking on the incidence of poor growth in LBW infants who are exclusively breastfed.

As previously expressed, LBW is associated with a higher risk of mortality, morbidity, and suboptimal neurocognitive development. It is such an urgent issue that the World Health Assembly set out to reduce LBW by 30% by 2025 (WHO Global Nutrition Targets 2014). LBW contributes either directly or indirectly to 60-80% of neonatal deaths (WHO Infant Feeding Guidelines 2011). In addition to this increased risk of neonatal death, LBW infants are at higher risk of early growth retardation, infectious disease, developmental delay, adult cardiovascular disease, and death during childhood (Risnes et al 2011; Larroque et a 2001; WHO Optimal feeding of the low birth weight infant(Technical review 2006). While LBW infants may catch up to normal birthweight infants by 12 months of age (Sridhar 2002; Jaruratanasirikul S 1999), the maximum catch growth may happen in the early period, specifically within the first 3 months of life (Han 2010). This study will allow for a better understanding of growth of LBW infants throughout infancy.

Though there is a lack of published data regarding rates of unsuccessful breastfeeding among LBW infants in low and middle-income countries (LMICs), anecdotal site estimates indicate that 3%-15% of LBW infants stop or have difficulty breastfeeding within the first six weeks. Globally, this means that 1 to 3 million LBW infants are nutritionally at risk each year and could benefit from alternative feeding methods. For those unable to exclusively feed with MOM, WHO recommends donor human milk (DHM)

from a human milk bank as the next best alternative with breast milk substitute or formula (BMS) as the last alternative (WHO 2011).

The evidence for appropriate methodology or implementation of these other feeding strategies is lacking. The recommendations regarding DHM are based on a few studies from high income countries among VLBW infants in the first days of life during initial NICU hospitalization with different standards of healthcare compared to most LMIC. DHM has been used to prevent necrotizing enterocolitis (NEC) and infection in very preterm newborns, but it is unclear if there is any benefit of DHM after the initial hospitalization in terms of longer term breastfeeding rates or health outcomes. WHO guidelines also only offer limited insight into dosages/volumes recommended for each feeding option and into how micronutrient (vitamin D, calcium, phosphorus, iron, and vitamin A) fortification should be practiced. There is little evidence comparing fortified versus unfortified DHM on outcomes for LBW and preterm infants, and there remains "no evidence of an effect on survival or longer-term growth and development" when looking at preterm or LBW infants fed formula versus DHM (Quigley et al 2018).

In addition to a lack of evidence, DHM poses substantial feasibility challenges: cold chain; facilities for milk acquisition, pasteurization, and distribution; and cultural acceptability. However, BMS has been linked to increased incidence of NEC in early preterm infants and also poses challenges with regard to clean water supply for mixing formula, and sanitation capabilities. For DHM and BMS, access to safe water and soap, and caregivers' consistent ability to perform hygienic practices for ensuring bottle/vessel cleanliness (handwashing, safe drying and storage) are critical to ensure that feeding practices are not responsible for introducing pathogens to infants (Menon). Many questions remain to be answered regarding appropriate feeding methodologies for alternate feeding options among LBW infants who require alternatives or supplements to MOM.

As all children age, the recommendation for infant feeding transitions from exclusive breastfeeding for the first 6 months of life to a period of complementary feeding. Complementary feeding can be a time of increased morbidity, especially diarrhea or other gastrointestinal illness, and other infectious diseases, due to exposure to foods especially if prepared in an unhygienic way and increased contact with pathogens as infants become more mobile (WHO 2005; Victora 2010). This may be of significant consequence for LBW infants who are already vulnerable.

Current WHO complementary feeding guidelines include guidance for LBW infants born >37 weeks gestations, but recommendations for LBW infants born at earlier gestational ages are lacking (WHO 2005). This is a challenge globally--not just in LMIC. Additionally, there is a lack of data on long term morbidity for LBW infants. In a brief literature search, only 66 articles included complementary feeding and preterm/LBW infants. Moreover, timing of complementary feeding initiation in preterm infants is debated and no clear guidance has been outlined (Vissers et al 2018). Globally, education about complementary feeding to families appears to be limited (Vitta et al 2016) (Kim et al 2017). There is a dearth of information about optimal feeding and growth of LBW infants, particularly in their transition during complementary feeding to infant and family foods and the association between growth trajectories and outcomes in the first 6 months and 12 months of life.

The Low-birthweight Infant Feeding Exploration (LIFE) and the 6-month extension will fill a critical data gap in the field of newborn care regarding feeding LBW infants. We aim to establish the background information required to set up and test the most efficient and feasible infant feeding strategies for LBW infants: first to support breastfeeding, and then to support infants who are nutritionally at risk in the first 6 months of life in LMICs.We will explore all three infant feeding options currently included in the WHO guidelines for LBW infants (WHO 2011), namely mother's own milk (MOM), donor human milk (DHM), and breast milk substitute or formula (BMS), in that order. This work will provide much-needed evidence to inform infant feeding guidelines.

In addition, this 6-month extension will allow for a more comprehensive exploration and understanding of feeding options for LBW infants from 6 to 12 months of age, accounting for timing of introduction of complementary liquids and foods, changes in feeding types, growth and health outcomes over the entire infancy period. This will contribute significantly and allow for high quality data to describe the burden of disease across sites. Specifically, we will be able to describe the following for LBW infants:

- Growth trajectories from 0 to 12 months of age
- Patterns and timing of complementary feeding and continued breastfeeding/breastmilk consumption
- Common infant morbidities and timing of mortality
- Maternal demographics, well-being and environmental factors affecting infant feeding and growth

We will also use the results of this work to design ways to support exclusive breastfeeding (including strategies for feeding with MOM), and to support other options, when mother's own milk (MOM) is unavailable or infants are nutritionally at risk. To do this, we will engage in discussion and consensus-building activities among study staff and key stakeholders, using the collected study data to inform feasible, acceptable Infant and Young Child Feeding (IYCF) strategies for LBW infants that include specific options for those who are nutritionally at risk. The strategies will be tailored to the country as much as possible. A primary product for this later stage will be a white paper documenting key findings from the research and proposing feeding strategies for LBW infants in study sites.

4 Study goal and objectives

The overall study goal is to understand feeding options for LBW infants in LMIC settings, including current feeding practices, health outcomes, and potential interventions. The study will take place in four study sites located in three countries: Tanzania, Malawi, and India. Each study site will encompass 2 to 5 individual study facilities. As shown in Table 1, the study will achieve its overall goal through three objectives.

Table 1: Study goal and objectives

Goal	Understand feeding options for LBW infants in LMIC settings, including current feeding practices, health outcomes, and potential interventions.		
Objectives	**1:** Understand the current practices and standard of care (SOC) for feeding LBW infants	**2:** Define and document the key outcomes (including growth, morbidity, and lack of success on MOM) for LBW infants under current practices	**3:** Assess the acceptability and feasibility of a system-level IYCF intervention and the proposed infant feeding options for LBW infants

To meet the first study objective of understanding practices, routine care and standard of care (SOC) for LBW infant feeding, we will use a mixed methods approach. First, we will conduct focus group discussions (FGD) with clinicians, mothers, family members, and religious leaders to understand current SOC and cultural practices around infant feeding. Second, we will enroll 35 mothers and up to 60 infants per site (approximately 35 pairs) for close in-facility feeding observation before discharge, focusing on the behaviors and interactions between mother, infant, and clinic staff regarding infant feeding. These feeding observations may be conducted in the neonatal care unit and Neonatal Intensive Care Unit (NICU), if applicable. Third, we will enroll and follow a prospective cohort of approximately 300 mothers and their LBW infants at each site. Caregivers of all LBW infants at each site will be approached for consent. All enrolled infants will be followed prospectively for up to six months through a regular schedule of clinic visits, and mothers will be regularly interviewed on their normal feeding practices and related topics. Anthropometric measurements will be taken across the six months of follow up. Fourth, a set of structured facility observations will assess the current patterns of infant feeds and maternal milk in the health facility to understand how infant feeds are currently provided under SOC.

For the 6-month extension:
To meet the first study objective of understanding practices, routine care and standard of care (SOC) for LBW infant feeding, we will use a mixed methods approach. First, we will conduct in-depth interviews (IDIs) with mothers to understand current SOC and cultural practices around infant feeding from 6 months to 1 year and the role of the home environment. Second, we will enroll and follow a prospective cohort of approximately 300 mothers and their LBW infants at each site who have been followed already for the first 6 months of their lives. Caregivers of all LBW infants at each site will be approached for re-consent at or before their infant(s) reach 6 months of age. All enrolled infants will be followed prospectively for up to six months through a regular schedule of 2 visits, and mothers will be regularly interviewed on their normal feeding practices and related topics. Anthropometric measurements will be taken across the six months of follow up at two time points for the infant and at one time point for the mother.

The second study objective is to provide descriptive epidemiology of a variety of LBW infant feeding markers and outcomes. The main data source for these outcomes will be the prospective observational cohort. We aim to follow all cohort infants for measurement up to 12 months outcomes, including breastfeeding behavior, feeds given (complementary), anthropometrics and a variety of infant and maternal health outcomes. Further, a retrospective chart review of LBW infants at each site will document, to the extent possible, the same infant feeding behaviors and outcomes as measured in the prospective cohort. Additional data on feeding and outcomes will be collected in up to 2 sites (Tanzania

and Malawi) short phone interviews that will take place between the two study visits (when children are about 7.5 and 10.5 months of age).

The third study objective is to assess the feasibility and acceptability of a variety of IYCF strategies. Of particular interest are strategies for those LBW infants who cannot successfully breastfeed, including options for providing MOM (such as through expression or storage), DHM (such as from human milk banks), and BMS (such as formula or preterm formula), all possibly with or without supplementation or fortification. We will primarily use qualitative methods. FGDs with mothers, clinicians, family members, and religious leaders will cover IYCF strategies for MOM, DHM, and BMS. Additionally, in-depth interviews (IDIs) on IYCF acceptability and feasibility will be carried out among three populations: government officials, supply chain experts, and human milk bank experts. We will also conduct facility-level Context Assessments for Successful Implementation (CASI) to determine a facility's capacity and capability to implement a new intervention. This will provide insight into the level of support required to implement future IYCF interventions. The DHM readiness assessment will provide information on whether the establishing a DHM bank for study purposes (if identified as a suitable intervention to test) would be possible, feasible, acceptable and safe.

For 6 -month extension:
The main interest around the third study objectives are strategies for those LBW infants who are nutritionally at risk or whose growth is faltering and who may have problems with feeding (breastmilk and/or complementary foods). We will used mixed methods including the prospective cohort data and IDIs with mothers of these infants.

5 Main study activities

To meet these objectives, this mixed-methods study will collect data from a variety of sources. The seven main data collection activities are shown in Table 2, along with target sample sizes and the corresponding study objectives. Each of these activities have different inclusion/exclusion criteria, sample sizes, consent processes, and study procedures, all of which are discussed in turn in this protocol.

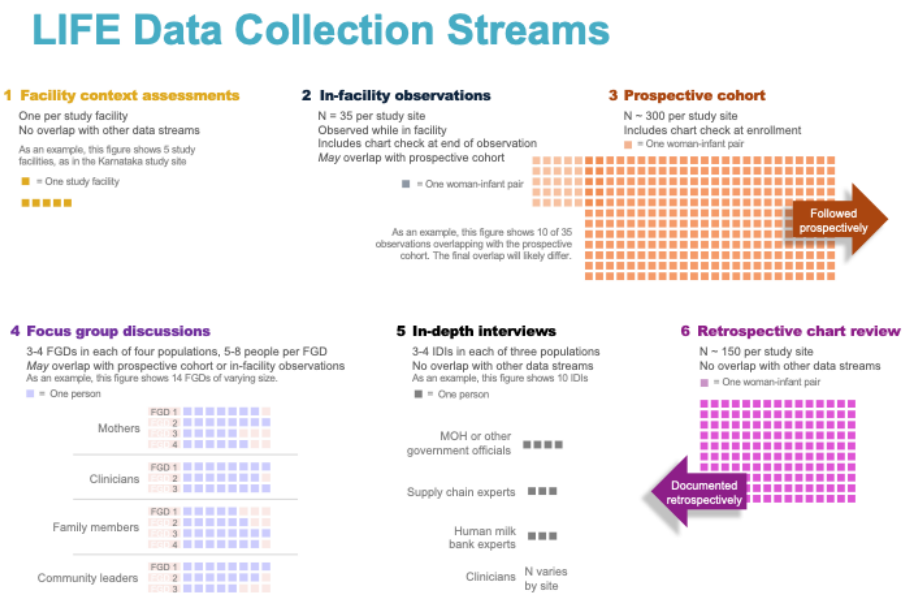
Table 2: Main study activities

		Target N per site				Meets objective		
Activity	Population	Tanzania	Malawi	India - Karnataka	India - Odisha	1	2	3
1. Facility context assessment	All facilities at each study site (clinicians)	3 (90)	2 (60)	5 (250)	3 (90)			✓
2. In-facility observations	Mother/infant pairs	35-45	35-45	35-45	35-45	✓		
	Clinicians	90	60	250	90	✓		
3. Prospective cohort/ 6-month extension	Mother/infant pairs	300	300	300	300	✓	✓	✓
4. Focus group discussions (5-8 participants each)	Health care workers	2-4	2-4	2-4	2-4	✓		✓
	Mothers	4	4	4	4	✓		✓
	Family members	2-4	2-4	2-4	2-4	✓		✓

	Religious leaders, community leaders, or traditional healers	2-4	2-4	2-4	2-4	✓		✓
5. In-depth interviews (individuals or in pairs)	Clinicians	24-60	24-60	24-60	24-60	✓		✓
	MOH and other government officials	2-4	2-4	2-4	2-4			✓
	Supply chain experts	2-4	2-4	2-4	2-4			✓
	Human milk bank experts	2-4	2-4	2-4	2-4			✓
	Mothers (extension)	16-20	16-20	16-20	16-20	✓		✓
6. Chart reviews	Mother/infant pairs	155	155	155	155		✓	
7. Literature reviews	n/a					✓	✓	✓
8. Donor Human Milk Assessment Surveys	All facilities at each study site (stakeholders)							✓

Aside from the literature reviews, these activities require collecting data from a variety of populations. Figure 1 illustrates the relative sizes and participants in the six activities collecting data from human subjects. As shown in the figure, the in-facility observations and the prospective cohort may overlap; women may be enrolled in both, although they are not required to do so. In addition, for the 6-month extension, participants will overlap in the prospective cohort follow up and qualitative IDIs.

Figure 1: Size and possible overlap of six study populations excluding extension



5.1 Language of main study activities

English is a common language across all four sites, but some study activities will be conducted in the local languages. Table 3 presents the standard languages to be used in LIFE, along with which activities will be carried out in which language. This specifies the study materials that must be translated from English into various languages, and those that will not require translation.

Table 3: Language of main study activities

		Dar-es-Salaam	**Lilongwe**	**Karnataka**	**Odisha**
Standard languages for use in LIFE:		Swahili, English	Chichewa, English	Hindi, Kannada, Marathi, English	Hindi, Oriya, English
Activity	Population	Language for each activity			
1. Facility context assessment	All facilities at each study site	English	English	English	English
2. In-facility observations	Mother/infant pairs and clinicians	Swahili	Chichewa	Hindi, Kannada, Marathi	Hindi, Oriya
3. Prospective cohort	Mother/infant pairs	Swahili	Chichewa	Hindi, Kannada, Marathi	Hindi, Oriya
3.1 Prospective Cohort Extension	Mothers/ Infant pairs	Swahili	Chichewa	Hindi, Kannada, Marathi, English	Hindi, Oriya, English
4. Focus group discussions	Mothers	Swahili	Chichewa	Hindi, Kannada, Marathi	Hindi, Oriya
	Family members	Swahili	Chichewa	Hindi, Kannada, Marathi	Hindi, Oriya
	Health care workers	English/Swahili	English	English	English
	Religious leaders, community leaders, or traditional healers	Swahili	Chichewa	Hindi, Kannada, Marathi	Hindi, Oriya
5. In-depth interviews	Clinicians	English/Swahili	English	English	English
	MOH and other government officials	English	English	English	English
	Supply chain experts	English	English	English	English
	Human milk bank experts	English	English	English	English
5.1 In-depth interviews (extension)	Mothers	Swahili/ English	Chichewa/ English	Hindi, Kannada, Marathi/ English	Hindi, Oriya/ English
6. Chart reviews	Mother/infant pairs	n/a	n/a	n/a	n/a
7. Literature reviews	n/a	n/a	n/a	n/a	n/a
8. Donor Human Milk Assessment Surveys	All facilities at each study site	English	English	English	English

6 Preliminary activities/Data Collection Preparation

6.1 Hire staff

Each of the study sites as well as Ariadne Labs will and may need to hire additional team members to implement this study and include the 6 month extension. Some of the hires in the study sites will be required to speak local languages as well as English, since rapid translation of some materials will be needed.

6.2 Develop study materials

Using the protocol as a guide, we will develop a series of semi-structured interview guides for in-depth interviews and focus groups. We will use a qualitative knowledge management tool for systematically and thematically organizing key messages from the IDIs/FGDs. We will also develop a template for documenting observations in the clinics or at home visits that will include highly structured elements of behaviors and the environment, and open-ended elements to capture reflections and narrative descriptions of the observations.

6.3 Train research staff

Once recruited or brought in from other projects, the study staff will need to be trained on the specific research questions, study methodologies and tools, and data collection processes. We will develop a standardized training curriculum using adult learning techniques for use across sites to ensure staff are successfully able to collect and enter data. Trainings will be conducted in-country. All trainings for study staff will include background on the study aims and objectives, overview of data collection methodologies and tools, a discussion of research ethics (informed consent, confidentiality, etc), and data quality assurance mechanisms. Training on qualitative methods will additionally cover principles of rapid qualitative analysis, strategies for interviewing and note taking, and summarizing of IDIs/FGDs for entry into the knowledge management tool. Training for the prospective cohort methodology will focus on survey administration techniques; use of app-based data collection tools where applicable; the data collection tools and their components include assessment of gross and fine motor skills and proper measurement techniques for anthropometric outcomes (weight, length, etc) and newborn gestational aging tests (i.e. Dubowitz, etc.). Training on in-facility observations and context assessment will focus on effective observer techniques and consistency in data collection to ensure inter-observer reliability. Training on chart review will focus on techniques for reliable, accurate abstraction of data from medical records.

Most sites include staff members that have previously collected quantitative and/or qualitative data in other research studies, which should aid in clarification of activities and responsibilities including this 6-month extension. We will evaluate the trainees to ensure that they are adequately prepared to initiate data collection and data entry activities, and we will co-conduct interviews/focus groups (either mock, pilot, or real) until we feel that they are able to both conduct the discussions and do the rapid qualitative analysis independently. If there is turnover after the initial training then we plan to revisit the site and re-train new staff members. Staff that do not pass the evaluation will have remedial training or not be allowed to collect data.

6.4 Data capture system

We will engage with on-the-ground partners and stakeholders to use existing resources and infrastructure to build and adapt technical data collection processes to serve study aims. We will also work with the local analytic teams, Ariadne Labs, and others to construct/adapt appropriate data architecture to support the study.

6.5 Develop Data Quality Assurance plan

We plan to develop a robust data quality assurance plan to assess and ensure data accuracy. See Section 12 for additional information.

6.6 Procure materials for data collection

Over the course of the data preparation phase, computers, tablets, and other materials will be procured.. Further, anthropometric measurement tools will be acquired, as needed, to ensure standardization across sites. Depending on costs, we will consider central purchasing.

7 Facility Context Assessments

7.1 Context assessments

Structured context assessments will take place at each site at the end of the study. This assessment will use the Context Assessment for Successful Implementation (CASI) Tool, a Likert-scale survey developed by Ariadne Labs that assesses five domains of facility context that are associated with implementation success.

7.2 Inclusion/exclusion criteria

All facilities in the LIFE study will be included. These include up to 3 facilities in the Dar es Salaam site, 2 in the Lilongwe site, 5 in the Karnataka site, and 3 in the Odisha site.

The CASI will be completed by front line clinicians who participate in newborn care and IYCF, as well as department and facility leaders.

7.3 Sample size

Our aim is to have the CASI tool completed by a census of all clinicians and leaders at every site involved with labor, delivery, and the NICU. If this is not possible, then we will strive to have as many subjects as possible in each facility. We expect a maximum of 150 clinicians/leaders per site.

7.4 Data collection

The CASI will be self-administered by each facility. Respondents will not provide their names on the tool, but they do indicate their role at the facility.

The tool will be completed either on paper or electronically if the facility has access to computers and Internet service. The tool will be translated into the local language at each facility.

7.5 Data analysis

Responses will be scored and interpreted by the study team and results will be shared with the facilities.

8 In-facility observations

The goal of the in-facility feeding observations is to better understand early feeding practices for LBW infants and the lactation support available for mothers as they initiate breastfeeding. Observations will be carried out by trained observers using a structured instrument.

8.1 Consent from facility leadership

We propose to obtain overall agreement for participation from each health facility and regional health authority, as required by each study site. These may include national ethics committees, institutional ethics committees, ministries of health, district health officers, chief medical officers, medical superintendents, medical directors, or other appropriate officers at each site prior to the start of the study.

8.2 Participation and consent by clinical staff

In the preparations for the study, all clinical staff will be informed that observations will be taking place during the study period, so they will be aware. New staff will be oriented to the research when they are hired.

Consent will be taken from individual staff members at the beginning of the study period. Staff members will have the option to opt out of being observed at any time. We expect up to 150 clinicians per site to be approached for participation.

We will seek full approval of this arrangement from facility leadership as part of that consent process.

8.3 Inclusion/exclusion criteria

Inclusion criteria for the mother/infant pair for in-facility observations are:

Infant birthweight between 1500 and <2500 g
We will use the facility-based birthweight taken at the time of delivery for the eligibility checklist.

Mother's consent
Only women who give written consent at the time of birth will be included.

Exclusion criteria for the mother/infant pair are:

Infant very low birthweight
Children who are very low birthweight (<1500g) will have different clinical needs and interventions. Thus, these infants will be excluded.

Congenital abnormality that interferes with feeding
Infants will be excluded if they have a congenital abnormality that (1) directly interferes with feeding and (2) is readily apparent at birth, requiring no special diagnostic tools. The specific abnormalities for exclusion are:

- Cleft lip or palate
- Hydrocephalus

- Gastrointestinal tract anomalies including gastroschisis, omphalocele, or anal atresia
- Neural tube defects
- Congenital cardiac defects
- Suspected Trisomy 21
- Suspected TORCH infection such as congenital rubella, CMV, toxoplasmosis, or syphilis.

Young mother

In Tanzania and India, women under 18 will be excluded.

In Malawi, women aged 16 to 17 who are married are considered legally emancipated and able to give independent consent, so they may participate. Women who are 16 to 17 and are unmarried will be excluded. All women and girls under 16 will be excluded.

Infant death

If the infant dies within the screening window for observation enrollment (within 6 hours after birth) before the mother can be approached for consent, the mother-infant pair will be excluded. We will also exclude infants that are part of multiple births where the other baby(ies) have died (even if the surviving infant is otherwise eligible).

Maternal death

The study will exclude cases of maternal death that occur during labor and delivery or within the 6-hour post delivery enrollment period.

Out-of-facility birth

Infants born outside of the facility cannot be observed at birth.

Infant >6 hours old at screening

Infants must be enrolled in the in-facility observation study within 6 hours of birth. If the infant is older than 6 hours at the time of screening, s/he will be ineligible for the study.

Mother-infant pairs with the following traits will still be eligible for the study, given that no exclusion criteria are met.

Critical or severe illness jeopardizing early survival

For the observations, we would like to understand a broad range of the feeding experiences of LBW infants. This small sample will yield rich information on just the first few hours and days of life, so we can include infants who may be at high risk of mortality within the first week of life. Their experiences will be informative about the range of SOC at a site.

Critical or severe illness jeopardizing early survival includes:

- Severe neonatal encephalopathy
- Ventilator or inotropic support
- Significant cardiorespiratory instability (hypotension, poor perfusion, or persistent hypoxemia while on the maximal respiratory support available in that setting)

Gestational age

As birthweight will be the main screening criteria for eligibility, we will enroll infants of any gestational age, as long as they meet the other inclusion criteria.

Multiples

Twins or triplets will be considered for enrollment in the study. Multiples have higher rates of LBW and greater demands on a lactating woman, so their experiences will be important to track.

Maternal HIV Status

Women who are HIV infected are eligible. We will encourage the adherence to Option B+ standards so that women receive HIV antiretroviral therapy in concordance with WHO and national guidelines.

Language

Mothers who do not speak English will still be eligible for the observations.

8.4 Screening and recruitment

Study staff will screen for eligibility by periodically checking the facility register for eligible participants. Clinical staff may also notify study staff when there are potentially eligible participants as clinical staff weigh newborns as part of normal practice. When potential participants are identified, study staff will use the checklist to ensure eligibility.

The timing of screening and recruitment is important. We do not want to disturb the mother's first few hours with her newborn, yet we want to begin observations fairly quickly. The target time for approaching a woman for screening will be between 1 and 6 hours after the birth. If it is not possible to screen the woman within 6 hours of giving birth, she will not be approached for inclusion in the observations.

Women will not be screened or recruited before giving birth, even if they are known to be delivering prematurely and therefore at high risk for having a LBW baby. The reason for this is that having a different recruiting method for premature infants and term infants will likely bias the sample of observations toward premature infants.

8.5 Informed consent

Women will provide written informed consent to participate in the in-facility observations. Each participant will consent to:

- observations of the care she receives from clinical staff,
- observations of the feeding behaviors of her and her infant,
- verbal interviews with study staff roughly every three hours until discharge, and
- access to her medical chart and her baby's medical chart by study staff including HIV status, if available.

In the case of illiteracy, we will use a thumbprint for the woman's signature, with an additional signature from an impartial witness to the consent process.

In the case that a mother consents to participate but asks a surrogate to answer questions for her, the action required will be country-specific in alignment with site requirements:

- Tanzania: Mother will complete and sign an additional "caretaker consent form", authorizing a surrogate to share information about her.

- India: Mother's signed consent is sufficient; no further action required.
- Malawi: Only allowed in the case that the mother is in the ICU and unable to answer: the trusted guardian will sign a separate consent form (same version as mother's consent form)

8.6 Observation schedule

From the scientific perspective, the richest and most useful data would arise from continuous observation with complete documentation of every feed or feeding behavior from birth until the infant is discharged from the facility. From a logistical and resource perspective, this is clearly not possible. Intermittent observations will be used, on a schedule that balances feasibility with capturing the highest priority information.

8.6.1 During daytime shifts

As shown in Table 4, the observation schedule will involve continuous observation in the first three hours, plus regular "spot checks" until discharge. If the facility has a regular feeding schedule for infants, then the spot checks will coincide with that schedule, in order to observe as many feeds as possible. If the facility does not have a regular feeding schedule, then facility staff will alert the observer when a feed is taking place. If the facility staff are not present at the time, then a mother or family member may also alert staff that a feed is taking place. If not notified of a feed within 3-4 hours of the previous spot check, the observer will perform an unprompted spot check. In this way, the spot checks will be mainly prompted by feeding behavior, but will take place at a minimum every 3-4 hours, when the observer will ask about any feeding behavior since the previous spot check.

Observers will use structured checklists to record information on all infant feeds. Since the first hours of the child's life will not have been observed, the observer will ask the mother and clinicians about the timing and nature of any feeds that happened prior to observation. If any feeding behavior takes place during the spot checks, the observer will observe and record it.

If the infant enters the NICU or other sick baby unit (SBU), the observer will follow the infant to the NICU/SBU. If the mother and infant are separated, the observer will follow the infant. When the mother is interviewed before discharge, the observer will ask about her nutrition and lactation during the period of separation from the infant.

In the intervals between spot checks, the observer will collect information on WASH for feed preparation in the facility (up to 5 observations), using structured observations of clinicians performing feeding vessel preparation and storage.

Table 4: Observation schedule

Directly after written consent is given:

1.	Before initiating the baseline interview with the mother, conduct the maternal chart check, (include HIV status) and baby chart check
2.	Interview the mother about her demographics, delivery experience, and feeding behavior since the infant's birth. Take baseline anthropometric measurements for the baby.

3.	Observe the mother/infant pair for the first three hours continuously, noting all feeds and feeding behavior of infant and mother. If the mother and infant are separated, observe the infant for the first three hours continuously, noting all feeds.
4.	Perform periodic "spot-checks," with brief questioning of the mother or clinicians, and observation of any feeding behavior. When possible, perform spot checks when feeds occur, or at a minimum every 3-4 hours.
5.	Between spot checks, observe and record WASH behavior for feed preparation in the facility generally.
6.	At discharge, record information about the baby and mother status and take anthropometric measurements for the baby.

8.6.2 During nighttime shifts

Study observers will not be hired to work on overnight shifts. Each morning, study staff will check the mother's and baby's charts to complete as much data as possible from the overnight records. If the mother-infant pair is still in the facility, then the 3-hour spot checks will resume for the day until discharge.

8.7 Sample size

Each site will carry out 35 to 45 observations, with a minimum of three at each facility but with no greater than 45 mothers. This accounts for participants lost to follow up. This equates to 35 mothers and up to 60 infants to account for twins; thus, a total of up to 95 individuals per site. Although the total number of mothers and infants above add up to 105, we will never exceed 95 individuals given the twinning rates in the sites. The goal of this sample is to get a deep understanding of the kinds of feeding experiences that LBW infants might have in each site. The sample size was determined by timeline and budget constraints; sites felt that this number of intensive in-facility observations was achievable.

This sample may not be representative of all LBW infants at a site. However, the rich descriptive results from these cases will give an informative picture of the range of experiences at each site.

8.8 Data analysis

As most of the observational data is being used for description, we will use means, medians and standard deviations to describe continuous variables. Categorical variables will be analyzed with frequencies and proportions, as appropriate.

For anthropometric analyses of the observed infants, we will use the Intergrowth charts to calculate stunting (length-for-age z-score, LAZ), underweight (weight-for-age z-score, WAZ), and wasting (weight-for-length z-score, WLZ). To determine Z-scores, we will use the INTERGROWTH-21st standards for preterm infants (<37 weeks gestation), and the WHO postnatal growth standards for term

infants (>37 weeks gestation) (Villar et al 2015). Further, we will compare the routine care provided to standard of care guidelines.

Any further analyses will be post-hoc and described separately.

9 Prospective cohort

The goal of the prospective observational cohort is to better understand feeding practices for LBW infants, their growth and their health outcomes over the first 12 months of life. A series of clinic visits (when possible) will be conducted as well as home visits, if required.

9.1 Inclusion/exclusion criteria

Inclusion criteria for the mother/infant pair are:

Infant birthweight between 1500 and <2500 g

Using the standard method of weighing described in Appendix A, all newborns will be weighed to screen for study eligibility.

Residence within catchment area of facility

Participants must reside within the catchment area of the facility, because home visits by study staff may be necessary, and travel time and funds are limited. The catchment areas will be defined by each site.

Mother's consent and/or legally accepted representative (surrogate's consent)

As part of the screening and enrollment process, we will conduct a thorough informed consent process following United States Office for Human Research Protections (OHRP) and national guidelines. Only women who give written informed consent for enrollment will be included.

Exclusion criteria for the mother/infant pair are:

Infant very low birthweight

Children who are very low birthweight (<1500g) will have different clinical needs and interventions. Thus, these infants will be excluded.

Congenital abnormality that interferes with feeding

Infants will be excluded if they have a congenital abnormality that (1) directly interferes with feeding and (2) is readily apparent at birth, requiring no special diagnostic tools. The specific abnormalities for exclusion are:

- Cleft lip or palate
- Hydrocephalus
- Gastrointestinal tract anomalies including gastroschisis, omphalocele, or anal atresia
- Neural tube defects
- Congenital cardiac defects
- Suspected Trisomy 21
- Suspected TORCH infection such as congenital rubella, CMV, toxoplasmosis, or syphilis.

Severe neonatal encephalopathy jeopardizing early survival

For the prospective cohort, we would like to understand LBW growth and health for the first six months of life. Unlike for the in-facility observations, infants who die in the first few days will not contribute much to our understanding of the first six months, when our interventions would take place. For this reason, we will exclude infants who are at high risk of mortality within the first week of life. Specifically, we will exclude infants with severe encephalopathy as determined by modified Sarnat criteria.

Some infants with other seriously life-threatening illnesses may not be diagnosed in the early hours and days. This is because some conditions require expensive or invasive diagnostic tests, and others, such as sepsis, may be undetectable for a period of time. If these infants meet all other criteria, then they would be enrolled in the prospective cohort even though they are at higher risk of mortality. Most of these cases will die before 72 hours, since neonatal mortality is concentrated early. In those cases we will replace the infant in the cohort sample. (See section below on "replacement of infants who die in the first 72 hours.")

Plans to leave the study area before end of data collection

In order to conduct the study, we will need to be able to follow mother-infant pairs. If a mother-infant pair plans to leave the study area within 6 months, per maternal self-report, they will not be enrolled. As part of the study set up, we will seek additional insight from local partners on practices of returning to maternal home for birth of child.

Young mother

In Tanzania and India, women under 18 will be excluded.

In Malawi, women aged 16 to 17 who are married are considered legally emancipated and able to give independent consent, so they may participate. Women who are 16 to 17 and are unmarried will be excluded. All women and girls under 16 will be excluded.

Maternal death

The study will exclude cases of maternal death that occur during labor and delivery (or at any time before the consenting process would begin for this study.) Although the LBW infants of deceased mothers are particularly vulnerable and important to study, we anticipate very few, if any, maternal deaths in samples of roughly 300 in each site. Also, given the logistical and ethical complexity in recruiting and following these cases, they would likely require separate study processes, which would take effort and scarce study resources to prepare for, even if no deaths occur.

Maternal deaths will count as an exclusion criteria for the prospective cohort if they occur before enrollment . In the event that a mother dies at any time after enrollment, the infant will still be retained in the cohort.

Infant death

The study will exclude infants that die before enrollment can occur, as well as infants that are part of multiple births where the other baby(ies) have died (even if the surviving infant is otherwise eligible).

Infant >72 hours old at screening

Infants must be enrolled in the prospective cohort study within 72 hours of birth. If the infant is older than 72 hours at the time of screening, s/he will be ineligible for the study.

Mother-infant pairs with the following traits will still be eligible for the study, given that no exclusion criteria are met.

Gestational age

As birthweight will be the main criteria for eligibility, we will enroll infants of any gestational age, as long as they meet the other inclusion criteria.

Multiples

Twins or triplets will be considered for enrollment in the study as multiples have higher rates of LBW and greater demands on a lactating woman. For the extension, they will still be considered as long as they are in the current prospective cohort. If only one infant in a multiple birth qualifies for inclusion, that infant may be enrolled and followed as a singleton would. If both infants qualify, both may be enrolled.

Maternal HIV Status

Women who are HIV infected are eligible. We will encourage the adherence to Option B+ standards so women are receiving HIV antiretroviral therapy in concordance with WHO and national guidelines.

Language

Mothers who do not speak English will still be eligible for the study.

Out-of-facility birth

Infants may be born anywhere and still be eligible for the study, as long as they present at a study facility for weight measurement within 72 hours of birth. See details below on identification and recruitment of these infants.

Prospective Cohort Extension study: This component will consist of enrolling mother/infant pairs.

Inclusion criteria for the mother/infant pair are:

- Currently enrolled in the LIFE study (follow-up from birth to 6 months of age) and fulfills all original inclusion criteria (infant birthweight between 1500g and <2500g, residence in catchment area of facilities in which born)
- Mother's and/or legally accepted representative (surrogate's) consent obtained for herself and infant for another 6 months of follow up

Exclusion criteria for the mother/infant pair are:

- Not previously enrolled in the LIFE study in addition to all original exclusion criteria (birthweight outside range, congenital abnormalities at birth, neonatal encephalopathy at birth, young mother, maternal death during labor and delivery, infant death before 72 hours of life, infant is more than 72 hours old at enrollment)

9.2 Recruitment of infants born in the facility

All LBW infants will be screened at study facilities to determine their eligibility for the prospective cohort. LWB infants will be identified directly after birth in the same way as for the in-facility observations, and study staff will be notified (described above).

Some of the inclusion/exclusion criteria are best assessed through a brief chart check or discussion with clinicians. Once a LBW birth is identified, study staff will record on the screening instrument:

- Mother's age
- Congenital abnormalities, as defined in I/E criteria
- Infant critical or severe illness, as defined in I/E criteria

If the mother/infant pair is still eligible after these checks, then study staff will approach the woman to check the final criteria, which are whether she plans to leave the study area in the next six months. If she is still eligible, then we will progress to the consent process.

The target time for approaching a woman for the prospective cohort will be between 1 and 6 hours after the birth, but not within the first hour after the birth. There is a maximum time cutoff of 72 hours for recruitment to the prospective cohort; women can be enrolled at any time up to their discharge from the facility or 72 hours (whichever was first).

If an infant is born overnight when no study staff are present, then study staff will review the register or medical records when they arrive for their shift in the morning. Women can be approached for inclusion at that point. To avoid bias, we will pick the odd number admissions to select children for enrollment (1,3,5,7th admissions overnight) for inclusion.

6-month extension: This is not applicable see section 9.4

9.3 Recruitment of infants born outside the facility

In some sites, a subset of women give birth outside of a facility, typically at home, and bring their infants to the facility soon after birth. We would like to recruit these women into the prospective cohort, but will not be able to weigh their infants at birth. Given our definition of LBW, we will be recruiting infants who are at least 1500 g at birth and at most 2499 g at birth. However, infants typically lose weight quickly in the first days after birth, so these thresholds must be adjusted for women who arrive at facilities later.

We will recruit infants born outside the facility as long as they present at the facility within 72 hours of birth. We will evaluate for the inclusion criteria of LBW based on the elapsed time since birth and the baby's weight at first visit, according to Table 5.

The values in Table 5 are based on a review of several studies of weight loss among infants in the first few days, including weight curves for term vs. preterm infants, LBW vs. normal weight, and breastfed vs. formula fed (Bertini et al 2015; Flaherman et al 2015; Miller et al 2015; Anchieta et al 2004; Shaffer et al 1987; Christensen et al 2006). Weight curves for C-section births were excluded from consideration. In this literature, estimates of median weight loss at 72 hours range from -2% or -3% for term, normal weight, formula fed infants, to about -10% for LBW, exclusively breastfed infants. This range is large, and the variation in observed weights increases with each day of life, producing more uncertainty in the estimates by day three.

For these reasons, it will not be possible to know an infant's birthweight precisely based on weight at day three. Also, to support the field work for the study, we require a fairly simple algorithm for classifying infants who are a few days old as having been LBW at birth. Taking these considerations into account, we took approximations of median weight loss rates in the first three days from the literature, based on initial birthweight and other factors, and constructed the threshold weights presented in Table 5. We accept the imprecision of this algorithm for estimating birthweight in order to allow recruitment from the population of women who give birth at home.

Other than the weight criteria, all other inclusion/exclusion criteria, informed consent, and study activities for out of facility births will be identical to those for facility births.

6-month extension: This is not applicable see section 9.4

Table 5: Weight thresholds for inclusion of infants up to 72 hours old born outside the facility

		Weight thresholds for inclusion in study		Percent change from assumed birthweight	
Time since birth	Exactly	Minimum weight	Maximum weight	In minimum weight	In maximum weight
None	0 to < 6 hours	1500	< 2500	n/a	n/a
12 hours	6 hours to <18 hours	1483	< 2467	-1.3%	-1.1%
24 hours	18 hours to <30 hours	1467	< 2433	-2.7%	-2.2%
36 hours	30 hours to <42 hours	1450	< 2400	-4.0%	-3.3%
48 hours	42 hours to <54 hours	1433	< 2367	-5.3%	-4.4%
60 hours	54 hours to <66 hours	1417	< 2333	-6.7%	-5.6%
72 hours	66 hours to 72 hours	1400	< 2300	-8.0%	-6.7%

9.4 Recruitment for 6-month extension

All mother-infant pairs who are currently being followed up for the first 6 months of their infants' lives are eligible to take part in this study and will be approached for re-consent to an additional 6 months of follow-up at or before the 6-month visit. Mothers and infants will not be screened but rather all current participants will be approached and asked to re-consent for continued follow-up in the study. Study staff will read the recruitment script before asking the participant for consent.

9.5 Replacement of infants who die in the first 72 hours

As described in the exclusion criteria for the prospective cohort, it will not be possible to identify all infants with severe or critical illness before discharge. Because not all diagnoses can be made definitively before discharge, some critically ill infants may end up in the prospective cohort, and may, unfortunately, die early.

These cases will not contribute much data to the study on their growth and feeding patterns, and may erode our ability to achieve our target sample size. We aim for 350 mothers at enrollment with 300 still

enrolled at 6 months. The drop of 50 mothers is due to expected loss to follow up as well as deaths. However, this margin may not be large enough to cover all the deaths if we include infants who have serious illnesses that are not apparent at discharge. In that case, we may end up with more infants than expected dying in the first few days, and fewer than 300 infants by six months.

For these reasons, we will consider an enrolled infant who dies within the first 72 hours to be excluded from the cohort, and we will recruit an additional infant in its place. In effect, this puts an additional inclusion criterion on this cohort, which is surviving the first 72 hours of life. If the infant dies at any time after 72 hours, he or she will not be replaced in the sample.

Baseline data from infants who die in the first 72 hours will not be included in the formal analyses, but may be included in post-hoc explorations.

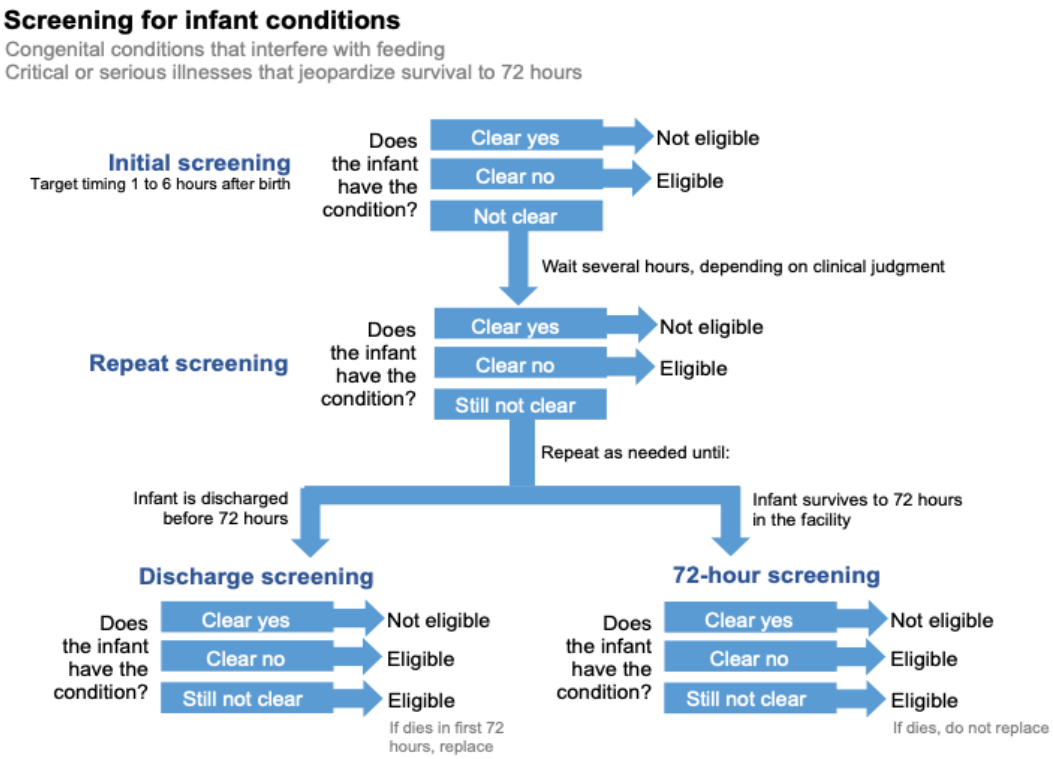
9.6 Assessment of congenital abnormalities and serious illnesses in infants

Some of the congenital abnormalities and serious illnesses will be readily apparent at birth, such as cleft lip. However, at first screening, it may not be possible for the clinicians to conclusively state whether the infant has some of the other congenital abnormalities or serious illnesses in our exclusion criteria. Some conditions may take a day or several days to fully develop.

Figure 2 shows how cases will be handled. Infants who clearly have the condition or illness at the first screening will be ineligible, and if they clearly do not, they will be eligible. In the event that it is unclear, then the study staff will revisit the mother-infant pair several hours later, the amount of time depending on the clinical judgment of the facility staff. If at that repeat screening the infant's condition is still not clear, study staff will again delay screening and revisit later.

In the end, the final screening will take place when the infant is discharged or when 72 hours has elapsed since birth, whichever comes first. In either case, as shown in Figure 3, if the condition is still unclear, then the infant will be treated as eligible. If the infant is enrolled at discharge before 72 hours, and then happens to die within the 72 hour mark, that infant will be replaced in the prospective sample. If the infant is enrolled at 72 hours, it will be kept in the prospective sample even if it dies soon afterward.

Figure 2. Screening for infant conditions



9.7 Overlap between observation and prospective cohort recruitment

There is no requirement for women in the observation group to participate in the prospective cohort, or vice versa. The consents are entirely separate. If a woman is approached for the observation and declines to participate, she may still be approached later for the prospective cohort.

9.8 Informed consent

The goal is to follow LBW infants for up to 12 months, with a regular schedule of visits to collect both survey and anthropometric data, review of medical chart, including HIV status, for visits occurring at the study facility, as well as observation of feeding when relevant. We will use a full informed consent process, involving a description of the study, discussion of risks and benefits, ability to withdraw at any time, and other required information. This consent will be documented with the woman's signature. All women will receive a copy of the consent documentation.

In the case of illiteracy, we will use a thumbprint for the woman's signature, with an additional signature from an impartial witness to the consent process.

In the case that a mother consents to participate but asks a surrogate to answer questions for her, the action required will be country-specific in alignment with site requirements:

- Tanzania: Mother will complete and sign an additional "caretaker consent form", authorizing a surrogate to share information about her.
- India: Mother's signed consent is sufficient; no further action required.

- Malawi: Only allowed in the case that the mother is in the ICU and unable to answer: the trusted guardian will sign a separate consent form (same version as mother's consent form)

If a mother dies during the study period, we will ask permission from the family to continue following the infant. A new informed consent process will be conducted with the infant's primary caregiver.

6-month extension: Study staff will ask the mother to reconsent around before their 6-month study visit as part of their original follow-up in the LIFE study. The recruitment script will be read before reviewing the consent form. The study staff will give the participant time to review, ask questions **and think about** whether or not they would like to **consent** to their participation**.**

9.9 Incentives

Women in the prospective cohort and extension may be asked to come to the facility when they otherwise would not. They will be provided a standard travel allowance for each visit that they make to the facility for a study visit, in alignment with local site and ethics review board requirements.

6-month extension:

(*Insert site specific location: Tanzania and or Malawi):* For the follow up phone calls at around 7.5 and 10.5 months: Participants will not incur any costs associated with receiving the phone calls. As such, there will be no compensation to participants for phone calls during these time points.

9.10 Benefits to participation

The benefits to study participants may include additional monitoring of a child's growth and earlier referral to care as part of participation in the cohort study.

9.11 Maternal or infant death during study period

In the case of a maternal death during the study period, we will ask permission from the family to continue following the infant. A new informed consent process will be conducted with the infant's primary caregiver.

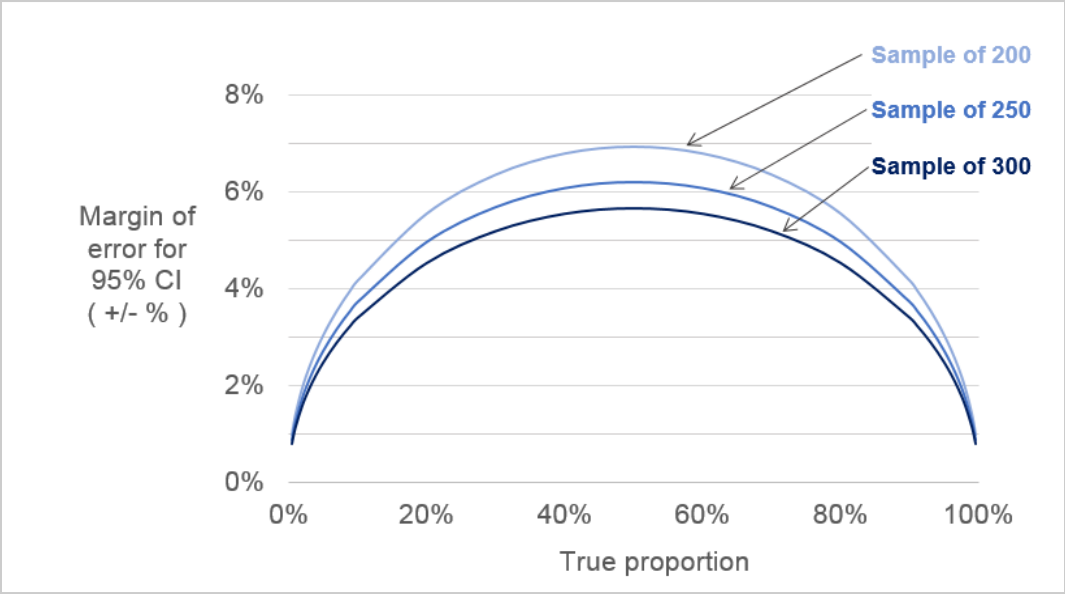
In the case of an infant death during the study period, we will not continue to collect study information on the mother; however, we will ensure that the mother is receiving adequate care, through appropriate referral mechanisms.

Verbal autopsies of the infant would be desirable, but they were not budgeted for as part of this study. In Tanzania, the study team will have to perform a verbal autopsy at 40 days post-death to meet government regulations, so they will be available for that site. If possible, we will gather information from verbal autopsies.

9.12 Sample size

The sample size will be as large as possible given constraints in time, recruitment rates, and budget. We do not plan to do any hypothesis testing using this observational data. The main statistical results will be point estimates and confidence intervals for certain rates, such as the percent of LBW infants who do not successfully breastfeed, require tube feeding, or the percent who develop certain conditions. The final sample size will determine the precision of the estimate, as shown in Figure 3. For example, with a sample of 250 infants, a true proportion of 5% can be estimated with precision of +/- 2.7%. The 95% confidence interval would be 2.3% to 7.7%.

Figure 3: Margin of error in estimation of a single proportion with a single sample



While we would like the precision of our estimates to be as high as possible, we have no statistical requirement for a particular number. Instead, we propose aiming for 350 enrolled mothers at each site, with an estimated final sample of 300 mothers in each site due to loss to follow up and withdrawal. This target will give a reasonable level of precision on the point estimates and appears logistically achievable in the study period.

The sample of mothers will be different from that of infants due to multiple births; thus we expect up to 850 individuals be enrolled in the study if there are many sets of twins. We do expect twins to occur with some frequency in our samples, since we are selecting for LBW infants, and many multiples are LBW. The overall rates of multiple births in the general population are 7.2 per 1,000 births in India, 20.8 in Malawi, and 18.8 in Tanzania (Smits and Monden, 2011). We expect higher rates of multiples in our study population, so to be conservative, we double these estimates and assume our sample will include 14.4 multiples per 1,000 births in India, 41.6 in Malawi, and 37.6 in Tanzania. If these rates hold, and assuming both twins are eligible and enroll, then our sample of 300 women would include 304 infants in India, 313 infants in Malawi, and 311 infants in Tanzania. This will not greatly change the precision of the estimates shown for a sample of 300 in Figure 3.

The presence of twins will also introduce an element of clustering, since the outcomes within a set of twins may be highly correlated. The clustering will make the confidence intervals larger, with the degree of enlargement depending on two factors: 1) how many sets of twins are both enrolled in our sample, and 2) how correlated the outcomes are within twins (intraclass correlation coefficient, or ICC). This degree of enlargement can be expressed as the design effect (DEFF), or the increase in the sample size that is required to detect the same difference at the same level of statistical power, given the clustering in the sample.

Other research using large samples of births that include some proportion of multiples (Yellin et al, 2017) shows that the DEFF can be calculated as:

$$DEFF = 1 + \rho\ \gamma_p$$

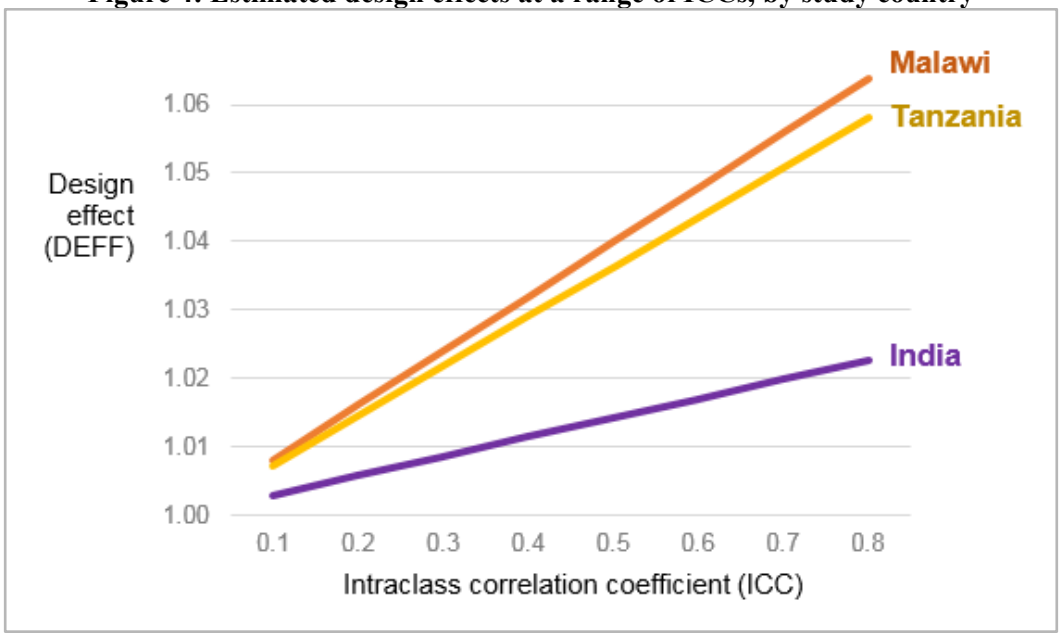
where ρ (rho)= ICC of the outcome within twins, and γ_p (gamma) is the proportion of the total sample of infants who are twins.This proportion is defined as:

N = total number of infants in sample (as opposed to the total sample of mothers)
M_S = total number of singletons in sample
M_P = total number of pairs of twins in sample
$\gamma_p = 2M_P / N$

To estimate the DEFF for this study, we draw ICC estimates from the literature. A review of birth outcomes research (Yellin et al, 2018) presents ICCs for twins for three outcomes of interest to LIFE: 0.26 and 0.85 for admission to the NICU; 0.25 and 0.36 for any sepsis; and 0.08 and 0.17 for proven necrotising enterocolitis. We may not be able to predict the ICCs we observe in the LIFE sample from these because these estimates show such a wide range, and were drawn from developed settings.

Because we may not be able to predict the ICCs in LIFE, we estimated DEFF for each study country at ICCs ranging from 0.1 to 0.8 (Figure 4). India's DEFFs will likely be lower than the other sites' because its baseline twinning rate is lower. In the most extreme case of ICCs in the 0.8 range, the largest DEFFs we would expect in this study would be about 1.06, or a 6% increase in sample size needed for the same precision at the same level of power. In a sample of 312 infants given a DEFF of 1.06, the precision of the point estimate would be as though we had a sample of 294 (312/1.06). This will not introduce a large distortion to the precision estimates.

Figure 4: Estimated design effects at a range of ICCs, by study country



9.13 Response rates

The four study sites vary in annual birth volume and rate of LBW births (Table 6). The enrollment period for the prospective cohort will be approximately four months long (see Gantt chart), and each site expects

to have an estimated number of LBW babies born in that period. The table shows the response rates needed in each site in order to enroll 300 women into the cohort.

Table 6: Birth volume and response rates

Site	No. of study facilities	Annual birth volume		4-month study period	
		All births	LBW births (estimated)	LBW births (estimated)	Required response rate[1]
Dar es Salaam	3	35,997	5,550	1,850	16%
Lilongwe	2	20,000	1,280	427	70%
Karnataka	5	21,000	2,100	700	43%
Odisha	2	19,600	3,320	1,107	27%

[1] Percent of LBW births that need to be enrolled in order to achieve a total sample of 300; please note we may have a longer enrollment period than 4 months if required.

9.14 Schedule of data collection

We will collect a variety of information from the mother-infant pairs enrolled in the prospective and extension cohort throughout the study, as shown in Table 7. Table 7.1 shows the data collection time for the prospective cohort 6-month extension. The additional phone check-ins (are around 7.5 and 10.5 months) are only applicable for Tanzania and/or Malawi.

Table 7: Data collection schedule for prospective cohort

	Base-line	Weekly for first 6 weeks				Monthly			
Weeks and months of infant's life	0	1	2	4	6	2	3	4	6
Demographics and pregnancy history	✓	✓							
Gestational age	✓								
Infant anthropometrics	✓	✓	✓	✓	✓	✓	✓	✓	✓
Maternal anthropometrics	✓				✓				✓

Infant feeding/maternal lactation questionnaire	✓	✓	✓	✓	✓	✓	✓	✓	✓
Infant health questionnaire	✓	✓	✓	✓	✓	✓	✓	✓	✓
Maternal health questionnaire	✓	✓	✓	✓	✓	✓	✓	✓	✓
Home Water/Sanitation Assessment	*As required*								
Sick visit or inpatient data	*As required*								
Mortality	*As required*								

Table 7.1: Data collection schedule for prospective cohort extension

	Monthly			
Months of infant's life	7.5	9	10.5	12
Demographics and home environment		✓		✓
Well-being		✓		✓
Infant anthropometrics		✓		✓
Maternal anthropometrics				✓
Infant feeding/maternal lactation	✓	✓	✓	✓
Infant health	✓	✓	✓	✓
Maternal health	✓	✓	✓	✓
Home Water/Sanitation Assessment		✓		✓
Mortality	✓	✓	✓	✓

9.15 Location of follow up visits

Mothers will return to the facility with their infants for each follow-up visit. Based on site requirement, clinic-based visits or home visits will be conducted in the early neonatal period. If the mother misses a facility visit, then study staff will make a home visit.

6-month extension: same procedures apply though home visits will be more prevalent.

9.16 Data collection at sick visits

If an enrolled infant falls ill, the mother may bring the child to the facility for a sick visit, unrelated to the study. The sick visit will present an opportunity to collect additional data on the illness as well as lactation and growth of the infant.

To collect this data, we would like to identify the mother-infant pair as enrolled in the study. Each site will establish a simple process to achieve this. For example, in Malawi, study participants will have a study appointment card attached to their health passport, listing the participant study number, visit schedule, and contact details of lead study staff. Also, study patients will be advised to come to the study clinic when they are sick, or to contact the study team if they seek care elsewhere or are hospitalized. In India, enrolled patients will be advised to return to the facility in case of illness, and also given a trial card with instructions to other physicians to inform the study investigator if they seek care elsewhere.

For all infants presenting for a sick visit: we will ask the mother questions about the infant's illness and any changes in feeding due to that illness, and we will weigh the infant at the time of the visit / admission to health facility. In the event that an infant is admitted to the health facility, we will ask the mother additional questions about the infant's stay in the hospital, and study staff will review chart documentation to gather additional data about the infant's diagnosis. We will also weigh the infant at the time of discharge from the health facility.

Prior to any data collection occurring at sick visits or health facility admission, the study team will confirm it is appropriate to proceed with data collection with the baby's health care provider. The study team will also confirm the mother is comfortable with the sick study visit prior to proceeding. The study team will develop an internal SOP regarding culturally appropriate criteria for approaching families in the health facility.

6-month extension sick visit: These sick visits are not applicable and will not be implemented during the additional 6 months of follow up. Additional information on illnesses will be collected in the revised prospective extension cohort follow up tool rather than a separate sick visit tool.

9.17 Data collection at home visits

In the event that a mother cannot come to the facility for a follow up visit, a home visit will be made by study staff. As above, visits in the early neonatal period will be conducted at the facility or home per site requirements. The mother, father, and other family members will be informed in advance of these visits so they are not surprised. At the home visit, study staff will complete the same measures as for the facility visit, unless they are not possible due to lack of equipment.

The home environment, a critical factor in the safety of infant feeding, will be important to understand when designing a safe IYCF intervention. During the home visit, study staff may separately collect supplemental information on WASH in the home environment for a sample of mothers at selected follow-up timepoints. This potential data collection would be restricted to documenting observable measures and equipment. We would not collect biological samples, unused supplies, or swabs of containers.

In addition, the mother will be asked to use the illness diary (morbidity diary) that will be provided to the mother during the initial 6-week visit. The purpose of the diary is to record details on specific illnesses experienced by the baby (diarrhea, vomiting, fever and respiratory infections) in between study visits. At each follow-up visits, the mother will bring the completed illness diaries to the study team and will be given a fresh set of blank illness diaries for the next visit.

6-month extension: Same procedures will be followed with some modification to the questions.

9.18 Study measures

9.18.1 Demographics, pregnancy history and home environment

At baseline and at 9 and 12 months, we will ask the mother to respond to a questionnaire with basic socio-demographic information such as age, education level, marital status, and home environment. These variables and response categories will likely differ somewhat by site.

The questionnaire will also cover a detailed pregnancy history, access to antenatal care (ANC), supplementation during pregnancy, food and water insecurity, household water, sanitation and hygiene (WASH) conditions, and related topics. Not all questions will be asked in the last 6 months versus the first.

Some demographic and household WASH questions will be asked at the Week 1 follow-up visit to minimize the number of questions asked to the mother at baseline (shortly after delivery)

9.18.2 Gestational age (baseline visit only)

Measuring gestational age is notoriously difficult. Prematurity and intrauterine growth restriction are the primary causes of LBW. We anticipate that there will be physiologic differences between these groups, but also acknowledge the challenges in accurate identification of gestational age in this study population due to limitations in prenatal care and use of early ultrasound. The availability and quality of gestational age data will be highly variable between study sites. Only one site (India-Karnataka site) will have access to early pregnancy ultrasonography, because of the presence of an existing trial for antenatal corticosteroid. In the remaining sites, the majority will likely not have access to ultrasound dating and will rely on last menstrual period (LMP) or Symphysis fundal height, as per standard of clinical care. We will capture what gestational age information is available from the woman's antenatal card, ultrasound history, etc. In cases where ultrasound data is available (either through clinic records or maternal self-report), we will consider this to be the first preference for gestational age dating. We will also ask the mother the date of her last menstrual period (LMP), and use this information as second preference for gestational age dating if ultrasound results are not available.

Given the poor quality and availability of antenatal dating in some sites, the LIFE study will also need to rely on neonatal assessment for gestational age dating. In a recent systematic review, 18 different neonatal assessments for gestational age dating were identified in the literature (Lee 2017). The most commonly used, simpler and feasible assessments, such as the Ballard or Capurro, were inaccurate, dating pregnancies to +/- 4 weeks of ultrasound dating. The Dubowitz exam was found to be the most accurate, dating 95% of pregnancies within +/- 2.6 weeks of an

ultrasound based date. However, the Dubowitz exam includes 21 signs including neurologic and external physical signs, and the feasibility of training, standardization and use in LMIC requires further programmatic research.

We propose to use the Dubowitz exam for gestational age dating in the LIFE study, and in the formative phase will assess the feasibility of training and standardization of this assessment across the four study sites. The Every Newborn Action Plan Metrics Group prioritized the assessment of the feasibility of implementation of the Dubowitz exam as a research priority for gestational age dating in LMIC.

For multiples enrolled in the study, we will determine gestational age for one baby and infer the same gestational age for the other twin or triplets.

9.18.3 Infant anthropometrics

Infant growth measures will include weight, length, head circumference, and mid-upper arm circumference (MUAC) according to the protocol in Appendix A. Trained members of the study team will be conducting the measurements per site.

9.18.4 Maternal anthropometrics

Maternal height, weight and mid-upper arm circumference will be captured at enrollment. Maternal weight and MUAC will be captured at 6 weeks postpartum, 6 month study visit and at the end of the 12-month study.

9.18.5 Infant feeding questionnaire

We will administer baseline and follow-up questionnaires on infant feeding/maternal lactation. An extended list of questions will be asked at 9 and 12 months and a shortlist may be asked during the check in phone calls. The infant feeding questionnaire will cover topics such as:

Description of feeds

- Feeds provided:
  - Breastmilk (Mother's own milk, Other mother's milk -), colostrum (at baseline), Donor Human Milk
  - Formula
  - Supplements
  - Other feeds (water, cow's milk, etc.)
  - Liquids
  - Semi solid / solid foods and specific food groups
- Method of feeding (direct breastfeeding, cup/palladai/spoon, bottle)
- Who advised/decided on the type of feed (clinic staff, mother, etc)
- Provider of feeds (mother, clinic staff, etc.)
- Timing of introduction of complementary feeds and reasons
- Frequency of feeds
- Self-efficacy of complementary feeding

Breastfeeding (this information will not be collected during the extension as it is not applicable anymore):

- Time to first latch or milk feed (global standard: within 1 hour of birth)
- Day (post-birth) achieved full milk production/established lactation ("coming to volume")
- Exclusive breastfeeding duration
- Number of breastfeeding or expression sessions per 24 hours
- Expressed Breast Milk storage method (refrigerator, freezer, etc.)
- Breastfeeding adequacy measures, like frequency of feeds (average number of expressions/full breastfeeding episodes per day), duration of feeds, and direct observation if the infant breastfeeds during the interview (WHO IMCI criteria, LATCH score, PIBBS scale). In addition, we will use the NeoEAT-Breastfeeding Screener Tool to assess symptoms of problematic infant breastfeeding based on maternal self-report.
- Maternal Perceived milk insufficiency (PMI)
- Incidence of infection (e.g. mastitis) or troubles breastfeeding (e.g. engorgement)
- Reason for stopping/difficulty breastfeeding (e.g. mother decision; medically directed)
- Breastfeeding self-efficacy and social support
- Use of kangaroo mother care; separation of mother and infant
- IYCF support provided/program/education availability
- Influence of family/friends (social norms) on breastfeeding behavior

9.18.6 Infant health questionnaire

Information on infant health will be captured through a questionnaire to the mother or primary caregiver (i.e. self-report from mother/caregiver), and clinical records (when available). The infant health questionnaire will include the topics below. We will also pilot infant morbidity in-home diaries for mothers to record daily details on selected morbidities like diarrhea, vomiting and respiratory infections) that are most likely to influence infant feeding and growth. Diaries will be introduced at the Week 6 visit (when transitioning to monthly follow-up visits), and mothers will be instructed to fill the diaries to use as a recall aid for subsequent visits. An extended list of questions will be asked at 9 and 12 months and a short list may be asked during the phone calls.

Infant severe morbidities and need for clinical care

- *Diarrheal disease*
  Including history of:
  - Persistent diarrhea (diarrhea that lasts for >14 days, often seen in infants who are malnourished)
  - Acute diarrhea (diarrhea lasting 14 days or less)
  - Severe diarrhea: diarrhea that is associated with signs of clinical dehydration including sunken eyes, lack of thirst, tenting with skin pinch, etc
  - Hospitalizations for diarrheal illnesses (Bhandari 2003)
- *Pneumonia/acute respiratory infection* - possible though maternal self-report and clinic records. Definition: cough or difficulty breathing plus either fast breathing or chest wall indrawing (WHO 2013).
- *Sepsis or other severe infection*

    - Possible serious bacterial infection in a young infant: evidence of severe clinical illness in an infant aged 7-59 days per IMCI criteria
    - Very severe febrile illness: Fever + danger signs in a child 60 days or older per IMCI criteria
    - Positive blood culture or CSF culture at any age. This will be documented based upon maternal self-report, patient personal health records, or clinic record.
- *Malaria*, diagnosed by rapid test or parasitological confirmation will be documented based upon maternal self-report, patient personal health records, or clinic record.
- *Necrotizing Enterocolitis (NEC):* Definitions (based on clinic records and maternal report):

  (1) confirmed NEC – modified Bell's stage II or greater with pneumatosis or portal venous gas on abdominal x-ray as well as intestinal (abdominal distention, blood in stool, abdominal tenderness) and clinical features (temperature instability, apnea, bradycardia, lethargy, metabolic acidosis, thrombocytopenia). (Kliegman and Walsh 1987)
  (2) suspected NEC – two of the following: abdominal distention, blood in stool, systemic features of lethargy/apnea/hypotonia. (Quigley 2018)
- *Seizures, neonatal encephalopathy* (based on clinical records)
- *Hyperbilirubinemia/Kernicterus* (based on clinical records)
- *HIV diagnosis:* A subset of children whose mothers' test negative in prenatal testing may have subsequent exposure due to maternal peripartum HIV infection. We anticipate that these children will be at higher nutritional risk. Additionally, some percentage of children who are HIV exposed will seroconvert during the six-month follow up period. We will encourage testing of all children who do not grow adequately for HIV and will also encourage testing of all children with known HIV exposure at intervals per the country standard of care.
- *Hospitalization*: composite hospitalization for any reason. Clinic stay duration and discharge timing will be captured as well as supplementary feeds (e.g. nasogastric feedings, fortified feedings).
- *Malnutrition*: Severe acute malnutrition (SAM)/ moderate acute malnutrition (MAM)

Infant mortality (asked at all data collection time points, including follow up phone calls)

- Date and time of death
- Cause of death, if available via medical record, or verbal autopsy in Dar es Salaam.

9.18.7 Maternal health questionnaire

The questionnaire for the mother will cover self-reported incident conditions, i.e. illnesses.

It will also assess symptoms of anxiety and depression using the PHQ-2 survey, which has 2 questions with Likert responses. The PHQ-2 survey has been translated and validated in Chichewa and Swahili, Hindi, Kannada, Marathi, and Oriya. An extended list of questions will be asked at 9 and 12 months and a shortlist may be asked during the phone calls.

9.18.8 Follow up phone survey
(*Insert local partner name if applicable)*

In order to minimize loss to follow up, there will be additional touch points between the 6- and 9-month visit (at around 7.5 months) and the 9- and 12-month visits (at around 10.5 months). This will be done in the form of a phone call. These calls will last approximately 15-20 minutes. Where a participant cannot be reached after numerous attempts, a home visit may be made. A short survey will be administered at these touch points asking mainly about the status of the infant and mother and about feeding.

9.19 Ethics

9.19.1 Risks to participants

This study does not involve an experimental treatment or intervention. It does involve identification of a vulnerable population of infants who are low birthweight and who are nutritionally at risk given anthropometrics and growth trajectory. These patients will be managed per the current SOC and routine processes at each study site. A goal of this study is to gain better understanding about the SOC for management of this population. We anticipate that the SOC will vary by site.

The risk to subjects will be minimized through adherence to local SOC. When an infant is identified as at risk, they will be referred to health officials to receive the local SOC feeding therapies. For the prospective cohort, will continue to follow enrolled participants through 12 months postpartum, including if the infant is admitted to a facility.

The proposed study activities pose minimal, if any, added health risk to study participants. The population in this study is intrinsically at high risk for morbidity and mortality due to underlying LBW, but we do not anticipate that the study would contribute to these underlying risks.

Because HIV positive mothers will be eligible for inclusion in the study, the risk of mother-to-child transmission will exist. However, HIV testing and treatment among pregnant women is high in the study sites, and thus the risk of transmission of HIV through breastfeeding is low.

Less severe risks may include burden of travel and loss of economic productivity for families enrolled in the prospective cohort who report to the clinics for follow-up, as clinic attendance may be costly from both a monetary and time perspective

There could also be inconvenienced or experience discomfort in direct observation of feeding practices immediately after childbirth and subsequently. We will mitigate this by obtaining informed consent, emphasizing the right to opt out at any time, and employing female observers and providers.

9.19.2 Informed consent

The informed consent process for each of the data collection streams is described in their respective sections of this protocol.

9.20 Data analysis

As most of the quantitative data collected is being used for descriptive epidemiology of LBW infants, we will use means, medians and standard deviations to describe continuous variables. Categorical variables

will be analyzed with frequencies and proportions, as appropriate. Confidence intervals around all measures will be constructed to take any clustering into account.

For anthropometric analyses, we will use the INTERGROWTH charts to describe growth velocity and trajectories for the infants, including stunting (length-for-age z-score, LAZ), underweight (weight-for-age z-score, WAZ), and wasting (weight-for-length z-score, WLZ). To determine Z-scores, we will use the INTERGROWTH-21st standards for preterm infants (<37 weeks gestation), and the WHO postnatal growth standards for term infants (>37 weeks gestation) (Villar et al 2015).

Where possible, we will relate these anthropometric measures with reported infant feeding, using correlations for continuous variables or cross-tabulations of categorical variables. Any further analyses will be post-hoc and described separately.

6-month extension:
As with the original LIFE study, we will utilize mixed methods to fully understand the growth trajectories and care for LBW infants during the first 12 months of life. We will use the full cohort from 0 to 12 months of age to assess growth trajectories and velocities over time, evaluating the impact of complementary feeding (food types and timing) on infant anthropometrics, and assessing morbidities over time.

We will use descriptive methods for initial description of the LBW cohort with means, medians and standard deviations for continuous variables. Categorical variables will be analyzed with frequencies and proportions, as appropriate. Confidence intervals around all measures will be constructed to take any clustering into account.

For anthropometric analyses, for preterm infants we will use the INTERGROWTH-21st postnatal growth standards for preterm infants to describe growth velocity and trajectories for the infants up to 12 months of age, including stunting (length-for-age z-score, LAZ), underweight (weight-for-age z-score, WAZ), and wasting (weight-for-length z-score, WLZ). To determine Z-scores, we will use the INTERGROWTH-21st standards for preterm infants (<37 weeks gestation), and the WHO postnatal growth standards for term infants (>37 weeks gestation).

We will use longitudinal multilevel models to assess the contribution of infant feeding and morbidity as well as maternal and household characteristics. Such models will also allow us to test whether there are differences in growth patterns related to gestational age and size for gestational age at birth, and whether the effects of care, feeding, and morbidity differ according to birth characteristics.

10 Qualitative research

As shown in Table 8, we will conduct FGDs and/or IDIs with a variety of stakeholders including clinicians, first-time and experienced mothers, family members, religious leaders, community leaders, traditional health care providers, supply chain and milk bank experts, and Ministry of Health officials. We might also speak with individuals that have been identified through the interviews as having a specific set of expertise or experience relating to the domains below. We recommend a minimum and maximum number of FGDs or IDIs for each of the populations that allows sites flexibility for religious leaders, community leaders, and traditional healers based on their context and which group is the most appropriate

to approach for these discussions. Each discussion will cover the domains listed – as well as others as they arise – corresponding directly to one or more of our study objectives.

Table 8: Focus groups and in-depth interviews, with domains for discussion

Focus group discussions	8-12 total	Domains for discussion	Meets objective 1	2	3
Mothers of preterm LBW infants aged 0-3 months	1	Beliefs and norms around IYCF practices Feasibility and acceptability of IYCF options Preferences around IYCF support Barriers and facilitators to IYCF	✓		✓
Mothers of full-term LBW infants aged 0-3 months	1				
Mothers of preterm LBW infants aged 4-7 months	1				
Mothers of full-term LBW infants aged 4-7 months	1				
Male family members of LBW infants	1-2	Beliefs and norms around IYCF practices Feasibility and acceptability of IYCF options Role of family and community in IYCF practices	✓		✓
Female family members of LBW infants	1-2				
Religious leaders	2-4	Beliefs and norms around IYCF practices Barriers and facilitators to IYCF implementation Feasibility and acceptability of IYCF options *Religious and community leaders only:* Role of religious or community leaders in IYCF Religious or community beliefs and rules around IYCF	✓		✓
Community leaders					
Traditional healers					
Health Care Workers: Nurses, nurse midwives, midwives	8-20	Current SOC for LBW infants Feasibility and acceptability of IYCF options Barriers and facilitators to IYCF implementation Prior IYCF strategies and lessons learned	✓		✓
In-depth interviews	38-84 total	Domains for discussion	1	2	3
Health Care Workers: Doctors	8-20	Current SOC for LBW infants Feasibility and acceptability of IYCF options Barriers and facilitators to IYCF implementation Prior IYCF strategies and lessons learned	✓		✓
Community health workers	8-20				
MOH and other govt. officials	2-4	Commitment to LBW infant feeding strategies Possible risks and their mitigation Prior IYCF strategies and lessons learned	✓		✓

		Recommendations for implementation			
Supply chain experts	2-4	How BMS and bottled water can be distributed reliably in the community How external factors affect the supply chain Barriers and facilitators to a reliable supply chain			✓
Human milk bank experts	2-4	How DHM can be distributed reliably in the community How external factors affect the supply of DHM Barriers and facilitators to establishing human milk banks			✓
In-depth interviews(extension)	16-20 total	Domains for discussion	1	2	3
Mothers of LBW infants aged 9-12 months, male, growing well	4	Feeding practices Home environment Maternal and infant well-being Growth of infant	✓		✓
Mothers of LBW infants aged 9-12 months, female, growing well	4				
Mothers of LBW infants aged 9-12 months, male, not growing well	4				
Mothers of LBW infants aged 9-12 months, female, not growing well	4				

10.1 Focus group eligibility criteria

Mothers

These four focus groups will include mothers of LBW infants only. Two groups will include mothers of LBW infants aged 0-3 months, and two for mothers of LBW infants aged 4-7 months. The groups will be further stratified by preterm and full-term infants.

Mothers with healthy birthweight infants or VLBW infants will be excluded. Mothers with HIV will be eligible for participation. Mothers under the age of 18 will be excluded in India and Tanzania, and unmarried mothers under the age of 16 will be excluded in Malawi.

Mothers' participation in the prospective and observational studies will not impact their eligibility for participation in the focus group discussions. Should sites find it difficult to recruit mothers for focus group discussions then they will be able to conduct individual interviews instead.

Mother's family members

Family members with the following relationship to a mother of a LBW infant up to 7 months old are eligible to participate: husbands, guardians, mother's parents, mother's in-laws, grandmothers, sisters, and sisters-in-law. The focus groups will be separated by gender: fathers, husbands, and grandfathers in their own group, and sisters, sisters-in-law, and grandmothers in a separate group. In India, the female focus groups will be further separated into family members from the mother's generation (sisters, sisters in law) and those from the older generation (mothers-in-law, grandmothers.)

To be eligible, relatives must play a role in providing opinions, care, or support for the mother related to IYCF. Eligible family members do not need to be family members of the participating mothers in the project; they only need to be family members of a current mother with a LBW infant. Family members are eligible to participate even if the mother has died or if the mother is not participating in the focus groups, observation, or prospective components of the study.

Religious leaders, community leaders, or traditional healers

The aim of these focus groups is to capture the social norms and pressures on breastfeeding behavior in the community beyond women's immediate families. The intent is that the three categories of people can inform the study of religious, social, and naturopathic norms in each site.

For each focus group, sites can decide the best composition for the group. For example, the FGDs can be segregated by role (i.e. only religious leaders in a single group) or by gender (i.e. female community leaders and female traditional healers in a single group), but the compositions are not limited to these options. While both men and women will be eligible for these focus groups, they need not be separated by gender. All sites will conduct between 2-4 FGDs and determine which roles are appropriate to participate in focus groups based on what is relevant for that context.

Religious leaders will be eligible if they are recognized leaders of the community's primary religion(s). Community leaders or elders will be eligible if they are viewed as opinion leaders or having significant influence over IYCF practices in the area. Traditional healers are those in the community who provide non-Western medical care, and these individuals will be eligible for participation if they provide care directly to mothers or infants related to IYCF practices, or advise other people who carry out, support, or influence infant feeding.

Health care workers

Health care workers who are currently involved in providing support for IYCF will be eligible. They must either provide care directly to mothers or infants, provide advice to people who feed or support/influence feeding, or support or supervise other clinicians that directly provide care to mothers or infants. Health care workers who could be involved in the future in providing support for IYCF will also be eligible. Examples of healthcare workers include nurses, nurse midwives, and midwives. We will exclude health care workers who have been at their position for less than six months

10.2 Language of focus group discussions

Focus group discussions with health care workers will be in English. Focus group discussions with mothers, family members, religious leaders, community leaders, and traditional healers will be conducted in the local language. As shown in Table 3 above, these FGDs will be carried out in Chichewa in Lilongwe, and in Swahili in Dar es Salaam. In Odisha, there are two main local languages, Oriya and Hindi, so we will split the focus groups by language. For example, of the two FGDs with mothers of infants 0-3 months old, one will be conducted in Hindi and the other in Oriya. Similarly, in Karnataka, there are three local languages, Kannada, Marathi, and Hindi, and we will split FGDs by language.

10.3 In-depth interview eligibility criteria

Clinicians

Clinicians who are currently involved in providing support for IYCF will be eligible. They must either provide care directly to mothers or infants, provide advice to people who feed or support/influence feeding, or support or supervise other clinicians that directly provide care to mothers or infants. Clinicians who could be involved in the future in providing support for IYCF will also be eligible. Examples of doctors include OB/GYN, neonatologists, outpatient physicians, and pediatricians. The nurse cohort may include nurses, nurse midwives, and lactation nurses. We are also interested in speaking with community health workers that are connected to a study facility. While we provide clinician examples here, in-depth interviews may not be limited to these types of providers.

We will exclude clinicians who have been at the facility for less than six months.

Of note, some sites might determine that the nurse cohort or other health care worker cadres are better suited as a focus group discussion instead of an in-depth interview. In this case, the eligibility criteria remain the same, and interview facilitators will use the same interview guide with clearly marked questions for the focus group discussions.

Ministry of Health and other government officials

Any members of the MOH or other government officials whose responsibilities include supporting (e.g., human resource or budget) or participating in IYCF studies, quality improvement initiatives, or implementation programs, or whose support would be necessary for the success of an IYCF program, are eligible for inclusion.

We will exclude officials who have been in their post for less than six months.

Supply chain experts

Individuals who are involved in or provide oversight for the local supply chain of resources potentially needed for an IYCF intervention, or who has expert knowledge about local supply chains, is eligible for inclusion. These may be government employees or staff of independent NGOs, either domestic or international.

The interviews will include people knowledgeable about centralized, systemic distribution of supplies needed for an IYCF intervention, such as managers of drug stores, vaccine depots, or public health supplies. We will also include individuals in the local study facility that order or receive the supplies needed for an IYCF intervention.

Human milk bank experts

Anyone who has knowledge or experience with local human milk banks or the establishment and operation of human milk banks in other LMICs is eligible for inclusion. This could include those who have established HMB, have tried to, or are currently planning their development. This could include global leaders or those in-country that have experience with HMB or are familiar with the concept. Examples of in-country individuals could be community leaders involved with nutrition or newborn or maternal health.

Mothers (6-month extension)

Mothers chosen and consented for IDIs will include those currently enrolled in the prospective cohort. Additionally, their infants need to be between 9 and 12 months of age. These mother-infant pairs will be divided by growth status (faltering vs not faltering based on z-scores) and by sex of the infant.

10.4 Recruitment

The recruitment method for each population will be somewhat different, as described below:

Clinicians and health care workers

Site clinicians and health care workers will be approached in person by study staff members when convenient. Identification of clinicians might come from site leadership members or other clinicians that participate in the focus group discussions.

Mothers of LBW infants

Recruitment of mothers of LBW infants for the focus group discussions will be done by study staff members during clinic hours. They will approach women at a convenient time when they are at the facility. This could be before discharge or when the woman comes in for a postnatal visit for either herself or her baby.

Family members of mothers of LBW infants

Recruitment of family members of mothers of LBW infants will be done by study staff members. Individuals can be recruited in two primary ways: 1) through the mothers that participate in the FGDs or 2) if they accompany the woman to a postnatal visit at the facility. A secondary method of recruitment is through the facility's Community Advisory Board that provides outreach to community members.

Religious and community leaders

Religious and community leaders will be recruited by either study staff members or Community Advisory Board members.

Traditional healers

Traditional healers will be recruited by study staff members or Community Advisory Board members.

MOH and other government officials

Recruitment of Ministry of Health or other government officials will be done by senior study staff members and project partners. We will recruit by the individual's function in the government, and their relation to IYCF policies. Recruitment might also be done by snowball sampling as interviewees might identify others with expert knowledge in IYCF practices, policies, and guidelines.

Supply chain experts

Recruitment of supply chain experts will be done by study staff members. We will request contact information for individuals that are involved with the distribution of IYCF supplies as well as facility staff members that order and receive IYCF supplies. We will employ snowball sampling for the identification and recruitment of supply chain experts.

Human milk bank experts

In India only, human milk bank experts will be recruited by study staff members. We will request contact information for individuals to conduct interviews with and employ snowball sampling for further recruitment.

Mothers (6-month extension)

Mothers will be recruited by study staff in person when they are at facility for a clinic visit or via phone. They will approach women at a convenient time when they are at the facility. All mothers who will be approached will be current participants in the prospective cohort study. Study staff members will approach these individuals and read the consent form and recruitment script in private. If recruited for the IDIs, the study staff will schedule a time to conduct the interview either at home or before/after a scheduled facility visit.

10.5 Informed consent

The study team will obtain written informed consent from all participants before the start of the interview or focus group discussion.

10.6 Conduct of groups and interviews

If key informants are unable to participate in FGDs then they will be interviewed individually or in pairs.

All parties participating in a focus group discussion or interview will be provided a study fact / recruitment sheet prior to the interview or focus group.

All IDIs and FGDs will be conducted by a facilitator and 1-2 note takers in a private location that is convenient for participants. If possible, the interviews may be done at the facility or participant's office, as appropriate. At the study start, trainers may also attend to observe the facilitator to ensure the quality of data collection. All discussions will be audio recorded. Notes for FGDs and IDIs will be taken in either English or the local language based on the note taker, and any notes taken in the local language will be translated into English immediately following the conversations. Discussions will be analyzed using rapid qualitative analysis, which involves taking notes during interviews and focus groups then writing summaries of key messages immediately following the conversation based on recall of the conversation and the notes.

For the extension: The interview guides will be analyzed using thematic coding of the interview transcripts

10.7 Data analysis

Messages will be organized by themes/topics in the knowledge management tool. Content of each transcript or theme/topic will be reviewed on a regular basis (after every several interviews/focus groups). Learnings from earlier conversations can inform iterations of interview and focus group guides to explore some areas more in-depth or new areas of interest. A comprehensive analysis of the key messages will be done at the end of the data collection phase to inform intervention, study, and implementation recommendations.

Audio recordings of IDIs and FGDs may be transcribed and translated for potential future analyses and to ensure that a complete data set is available. All IDIs performed during the extension will be transcribed and translated.

10.8 Schedule of data collection

Each site is planning for a total of 8-12 focus groups. The focus groups will be broken down by respondent type, with 2-4 focus groups (consisting of approximately 5-8 participants per group) with each of the following cohorts: mothers, family members, and recognized community members (includes community leaders, traditional healers, and religious leaders). We expect a maximum of 384 individuals participating in focus groups across all sites.

Each site is planning for 6-12 semi-structured in-depth interviews with non-clinicians. The interviews will be broken down by respondent type, with 2-4 interviews among each of the following cohorts: government officials, supply chain experts, and human milk bank experts. Sites will also conduct in-depth interviews with clinicians with a maximum of 40 clinicians and or community health workers at each study facility. For example, this could be up to 24 clinician interviews in Malawi's two study facilities. Sites will determine the number of clinicians and clinician roles included in the interviews based on who would be most appropriate to speak with in their context and the number of clinicians on staff. Dependent on the number and size of facilities, as well as individual staffing characteristics, there will be up to 38-84 data collection events per site over the period allotted in the study timeline. The timing of data collection will depend on the subjects' and interviewers' availability, and sites and the study team will be flexible with the proposed timing. We expect a maximum of 288 individuals participating in in-depth interviews across all sites. For the 6-month extension, in total, up to 20 mothers per study site will participate in the IDIs. We expect a maximum of 80 mothers participating in the in-depth interviews across all sites.

10.9 Ethics

The risks to participants in the FGDs are the possibility of feeling uncomfortable sharing their opinions or hearing the opinions of others, or having others from the group think poorly of them because of their opinions or the experiences they choose to share. However, participants will be told that they do not need to answer any questions that they are not comfortable answering, and participants can choose what they do and do not want to share with the group. All participants will be asked to keep the discussion confidential and not share the identities of participants, but we will not be able to ensure compliance with this request. Again, participation, lack thereof, or withdrawal of consent will not affect clinical care of patient participants, or employment of clinical staff participants.

The risk to participants in the IDIs is the possibility of feeling uncomfortable sharing their opinions. However, participants will be told that they do not need to answer any questions that they are not comfortable answering and participants can choose what they do and do not want to share.

Participants in IDIs and FGDs will receive reimbursement for their participation, as identified by sites (Table 9)

Table 9: Reimbursements for participation in FGDs and IDIs

Study Site	Incentive
Karnataka	Participants will receive loss of wages, travel allowance and dearness allowance (DA) for each study visit, not to exceed US $15
Odisha	Participants will receive loss of wages, travel allowance and dearness allowance (DA) for each study visit, not to exceed US $15
Lilongwe	Participants will receive the Malawi Kwacha equivalent to US $10 for participating. This amount is to cover the costs of transport expenses to and from the interview / focus group. Snacks and sodas will also be provided for focus group participants.
Dar es Salaam	Participants will receive travel allowances for participating in the focus group, not to exceed US $25.

Participants in IDIs and FGDs will not receive direct benefits from participating, other than transportation reimbursement in some sites. They may feel personal satisfaction knowing that they are contributing to the development of an IYCF that may benefit themselves or others in their community.

11 Desk research

11.1 Chart review

The goal of the chart review is to document the routine and SOC at each site prior to the start of the study. Although LIFE is non-interventional, the extra attention that the study will bring to infant feeding issues in the facilities may affect the SOC we observe. In particular, having an observer present may alter clinicians' or mothers' behaviors in ways that are difficult to measure. The chart review data will allow us to compare the SOC we observe in LIFE to what was occurring just before LIFE began. This will augment our picture of the true SOC.

We will review 155 retrospective charts of LBW births at each study site; the number of individuals included will be up to155 mothers (to account for errors) and their infants (up to 200 infants, accounting for twins). Cases will be selected by the inclusion/exclusion criteria (see below), taking all qualifying cases that occurred as chronologically close as possible to the start of the LIFE study. Since the purpose is to understand routine and SOC before the LIFE study, we will not review any cases admitted after the study launch.

We will abstract information on infant feeding volume and frequency, status at discharge and information given, as well as any available health outcomes (anthropometrics, morbidities, etc.). As much as possible, the data collected in the chart review will mirror that collected in the in-facility observations.

11.1.1 Inclusion/exclusion criteria

These criteria for the chart review are nearly the same as those for the in-facility observations. See that section above for definitions of each.

The inclusion criteria for the mother/infant pair for the chart review are:

Infant birthweight between 1500 and <2500 g
Discharged before the launch of LIFE at site (specific date TBD)

Exclusion criteria for the mother/infant pair are:

Infant very low birthweight
Congenital abnormality that interferes with feeding
Young mother
Maternal death (occurring at any time before the baby's discharge from the facility)
Out-of-facility birth
Infant death at facility within 72 hours of birth

Mother-infant pairs with the following traits will still be eligible for the chart review, given that no exclusion criteria are met.

Nighttime birth
These cases are excluded from the in-facility observations because of the difficulty of having study staff present overnight. However, no such restriction applies to the chart review cohort, so we will take the opportunity to document these infants' experiences.
Critical or severe illness jeopardizing early survival
Gestational age

Multiples
Maternal HIV Status
Language

11.2 Literature reviews

We will conduct two literature reviews to support the study aims. The first will be carried out in the early study period, while the protocols are with ethics boards. This literature review will cover topics in infant feeding research in the three study countries, including documented SOC for LBW infants, how clinical guidelines are applied in SOC, previous IYCF interventions involving LBW infants, facilitators and barriers to implementation of interventions, current procurement practices for BMS and DHM, and volume requirements for BMS and DHM.

The second will be carried out as data collection for the prospective cohort is winding down and data analysis is beginning (see study timeline). This "umbrella" literature review will survey other literature reviews and meta-analyses on LBW infant nutrition in LMICs, as well as reviews of IYCF interventions in LMICs. The results of this review will inform the country-level analysis workshops, synthesis of findings, and overall design of the IYCF intervention.

12 Donor Human Milk Readiness Assessment Surveys

12.1 Donor Human Milk Readiness Assessments Surveys

There are a number of different tools that will be used for this activity depending on whether a site has a DHM bank, had government buy-in to a DHM bank and the comfort of the study staff. There are 4 different tools: (1) a validated DHM assessment of an existing DHM bank in India-Karnataka, (2) a validated India-specific DHM readiness assessment tool for other facilities in India, (3) a general validated DHM readiness assessment tool to be used outside India and (4) a qualitative guide to use for observation of flow and discussion with key informants at facilities.

This activity is designed to collect information needed to inform future research activities that may utilize existing donor human milk banks or establish donor human milk banks for research. PATH and /or the study staff will provide consent forms to participants and will ask for verbal consent before proceeding with the survey questions.

12.2 Inclusion/exclusion criteria

- Frontline clinicians who participate in maternal and newborn care as well as department and facility leaders/ stakeholders at LIFE study health facilities in India, Malawi and Tanzania.
- Those who are willing to participate and provide verbal consent

12.3 Sample size

For the validated tool: to assess the functioning of the existing DHM bank at one facility in India-Karnataka – completed by PATH in consultation with facility staff. The total number completed tools will be one for only one facility with a DHM bank in India-Karnataka.

For the mixed methods validated tool: to assess DHM readiness that will be completed per study facility - completed by PATH with the help of up to 14 key stakeholders. The total number completed tools per site will be up to 5. The total number of tools completed across all sites will be up to 12.

For the qualitative tool: to assess DHM readiness, the questions in the tool guide will be asked of up to 10 key informants based on their availability during the tour of each study facility. This will only be used in Tanzania and in up to 3 facilities in total.

12.4 Data collection

This is a one-time data collection exercise in the form of either: (1) a largely qualitative facility readiness assessment tool with some qualitative questions for facility staff or (2) a facility tool observing the flow of milk along with key informant interviews in the study facilities.

This could take anywhere from 1hr to a day depending on the tool administered, key informants involved and size of the study facility.

Participants will not be formally recruited and do not need to give their name or any identifying information. Some key stakeholder at the facility level will be alerted about when data collection will take place and those who are available at the time of the facility visit/tour will be consulted and read the recruitment script. They will be given a copy of the consent form and asked to verbally consent.

After reading the recruitment script and confirming eligibility, study staff/ PATH team will administer the consent script to verbally consent the clinicians/leaders, ensuring that they describe the trial, risks, benefits, what will be involved and answer any questions that the participants may have. Data collectors (the PATH team) will speak with clinicians who are present at the study facility at the time of the assessment/ tour to supplement observations of readiness and answer questions about the facility.

12.5 Data analysis

We will conduct a descriptive analysis of the flow and infrastructure of facilities related to the possible establishment of DHM banks. We will also conduct thematic coding of qualitative data.

13Monitoring and Quality Assurance

Designated study personnel at Ariadne Labs and country sites will be responsible for the data monitoring activities, which will occur frequently throughout the study. Planned meetings will occur on a regular basis to review study implementation and address any concerns regarding data collection tools or data quality. Given the formative, multi-site nature of the study, we will work closely with sites to adapt current processes and data infrastructure to ensure accuracy and reliability of data collection across study

sites. We will integrate cycles of change into our technology development to enable adaptability as the study evolves.

13.1 Quantitative data streams

Data quality will be ensured through the following mechanisms:

- Standardized data collection methodologies through adapting existing systems or utilizing new systems, at our partner sites, with the option for web-based, mobile, tablet, or netbook app if possible, which allows for offline data collection and syncing when connectivity is available.
- For sites using the CommCare electronic data capture application from Dimagi, for one or more data streams, data will be encrypted and stored according to each country's data privacy regulations. Data transfer will use industry-standard HTTPS or Secure File transfer protocols.
- For sites that use their own data collection tools for one or more data streams, we will establish controls for data security and transfer in accordance with all site requirements.
- Front end checks in data entry templates will be implemented in the electronic data capture applications whenever possible. For example, the in-Facility Observation, Chart Reviews, and Prospective Cohort data collection forms where possible will have built-in validation and logic checks, data type restrictions, and drop-down or multiple choice options to minimize data entry errors.
- Back-end checks to enable comparison of multiple data sources, which will aid in validating accuracy and concordance of collected data.
- Development and review of a Data Quality Assurance protocol in coordination with sites and study collaborators to assess data validity, reliability, and accuracy. The DQA protocol will set forth procedures for the data entry range and logic checks mentioned above, data cleaning protocols, and ongoing data review and feedback. We will explore the possibility of conducting double entry validation to ensure data quality and coaching of poor performing data collectors.
- Construction of Universal ID's based on local data, using a longitudinal system to register patients at initial point of contact and then follow up for multiple points of reassessment.

All data transferred to Ariadne Labs will be stored on an Amazon Web Services server in a secure environment approved by Harvard University Information Technology (HUIT). Additionally, data may be stored on the secure Harvard shared drive. The information security controls for all stored data will equal or exceed HUIT requirements for the applicable level of data sensitivity classification, such as the following best practice controls:

- all data will be encrypted in transit and at rest
- servers containing sensitive data will not be exposed to the internet
- access will be restricted to authorized personnel by role via proxy servers using AWS security groups and private subnets.
- users will be required to login and passwords will be a minimum of 8 characters and be alphanumeric.
- vulnerability scanning will be performed daily and all user activity will be logged.

We will also establish reporting services, which will provide data back to mobile or web users through a web-portal or notification service. Reports will be generated in three broad categories:

- Monitoring and managing Operations, such as site progress reports, day-to-day task lists for field workers, lost to follow up rates and risks, etc.
- Monitoring and managing Data Quality
- Supporting execution of activities

We will align these processes with the site and local IRB requirements.

13.2 Qualitative data streams

With a multidisciplinary team including representatives from each test site, we will develop semi-structured interview guides for the focus groups and individual interviews that will be primarily consistent across the sites but with some site-specific questions. The guides will be pilot tested in each site and revised as needed. Along with these guides, we will develop a knowledge management tool for qualitative data to ensure consistency and completeness in reporting. Staff will be trained on how to document key themes and engage in rapid qualitative analysis, and how to enter this content into the knowledge management tool.

These data will be reviewed on an ongoing basis and feedback and/or additional re-training will be provided, as needed. In order to ensure that IDIs and FGDs are being conducted consistently and appropriately across all sites, all IDI/FGD facilitators will be trained to lead these discussions (see training section above), and we will expedite a small number of transcripts from each facilitator to be transcribed and translated for review by our team. For this review process, we will develop and implement a quality control checklist for best practices for conducting IDIs and FGDs, and if we find that facilitators are deviating from the checklist, we will provide feedback and if necessary, re-train staff. Additional review of translated transcripts will be conducted until each facilitator is performing at a satisfactory level. IDI transcripts and FGD notes will be translated into English by the facilitators/notetakers/transcriber immediately following each interview/FGD, and these translated notes will be reviewed on an ongoing basis by our team.

14 Partners and experts

We will bring together a range of partners and experts to design, monitor, and implement LIFE (Table 10). We will work with three country partners that will engage in on the ground implementation in Malawi (1 site), Tanzania (1 site), and India (2 sites). We will engage with a range of partners to provide world class expertise in donor human milk and milk bank design in LMICs, nutrition, feeding, neonatology, etc. to provide design, monitoring and implementation input into all aspects of the research. We will also develop relationships with state and national governments, as well as the World Health Organization, to ensure buy-in and support for the work and results.

Table 10: Study partners

Partner	Role and area of expertise
Bill and Melinda Gates Foundation	Funder & Steering Committee Member
Harvard TH Chan School of Public Health/ Ariadne Labs	Project Leadership. Ariadne Labs will provide both scientific and management leadership for the project design, monitoring and implementation of this project, overseeing all partners and ensuring their coordination.

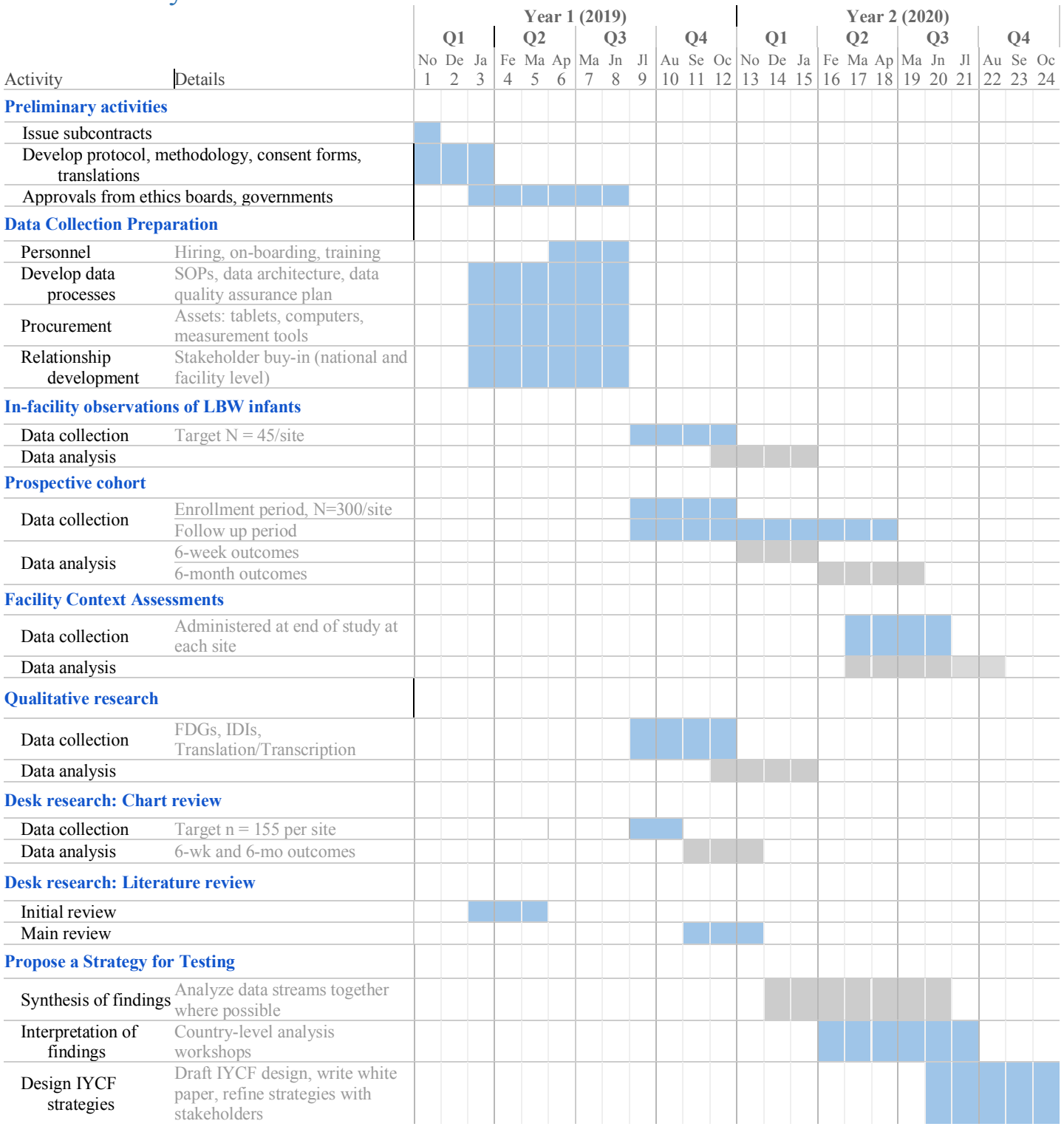
	Ariadne Labs is a joint center between Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health. Ariadne Labs' mission is to create scalable health care solutions that deliver better care at the most critical moments in people's lives, everywhere. Ariadne Labs' solutions and tools are simple, practical and designed to have global impact. The work of Ariadne Labs is rooted in rigorous scientific methodology and best practices in industry for implementation. Each body of work is built on a foundation of innovative use of informatics, measurement, implementation and improvement science, and professional program management. Ariadne Labs has unique experience and success in designing and managing successful large scale, complex trials in maternal and newborn health. Areas of expertise include: large scale coordination; complex trial implementation in Asia and Africa; applied solutions and driving scale; data capture and feedback; community and facility-based follow up; formative and qualitative research; and practical field applications of solutions.
Jawaharlal Nehru Medical College (JNMC), Belgaum	Site Implementation. The central team of investigators from the Women's and Children's Health Research Unit (WCRU) of JNMC Belgaum will lead the implementation of the project in 2 geographies/sites in India (5 facilities in Karnataka State and 3 facilities in Odisha State). The facilities in Karnataka with capacity to eventually run an efficacy trial are located in different parts of the state. Clinical and cultural practices are known to be significantly different between the facility sites, as such it is recommended to capture data from the 5 sites. Some of the data collection support processes have been centralized to do this in an efficient way. The WCRU at JNMC has vast experience of implementing both facility and community based clinical trials in the area of maternal and newborn health since 2001, when the unit was established with NICHD funding. Complementary work has included establishing five regional data centers towards a maternal newborn health registry, training research faculty, and strengthening IRB and study management systems. The center is skilled in community-based interventions and research. The proposed team includes expertise in neonatology care for preterm and LBW infants; milk bank design; and large-scale implementation of clinical trials in LMIC. SCB Medical College will act as the partner in Odisha State for implementation. Their site is experienced in coordinating with JNMC for the implementation of two recent WHO clinical trials.
MUHAS/ HSPH, Tanzania	Site Implementation. The MUHAS-Harvard team will lead the implementation of the project across 3 facilities in Dar-es-Salaam, Tanzania. The MUHAS-Harvard partnership recently celebrated the 25th year of collaboration during which the team has conducted more than 20 randomized trials of nutrition and infection interventions and many other bilateral research and training activities in Tanzania and Boston. These trials have evaluated the effect of maternal and child micronutrient supplementation, antibiotics, antiretrovirals and other maternal and child health interventions on child survival, growth and broader development. The site investigators have also participated in large collaborative multi-country trials. The partnership has developed a strong follow-up system that uses a combination of facility and community based tracking activities. The proposed team includes expertise in nutrition, infant feeding supplementation, and implementation of efficacy trials in Tanzania.

UNC Project Malawi (UNCPM)	Site Implementation and expert technical assistance . The UNC Project Malawi team will lead the implementation of the project across two facilities in Lilongwe, Malawi. Additionally, four faculty from UNC-Chapel Hill will provide subject matter expertise in neonatology, infant feeding and human nutrition. UNC Project Malawi (UNCPM) is a clinical research site (CRS) for the UNC Global HIV Clinical Trials Unit. It is a biomedical research institution in Malawi. Its mission is to identify innovative, culturally acceptable, and affordable methods to improve the health of the people of Malawi through research, health service strengthening, prevention, training and care. It has three main areas of work; research, training and clinical care service support. UNCPM is a >20-year collaboration between the University of North Carolina at Chapel Hill and the Malawi Ministry of Health. UNCPM has been an active Clinical Research Site for the National Institutes of Health Division of AIDS since 2001. Current ongoing research studies are from grants mainly sponsored by the NIH, Centers for Disease Control and Prevention (CDC), Bill and Melinda Gates Foundation, and the EDCTP. The institution manages grants in the excess of $12 million annually. In the past 5 years, the institution has enrolled more than 12,000 participants onto 40 randomized clinical trials. The proposed team includes expertise in the conduct of research in Malawi, pediatrics, and infant feeding strategies for low birthweight infants.
Emory University	Expert technical assistance. Provide expert guidance in the nutrition, WASH, Infant and Young Child Feeding, and behavioral health and education aspects of the project and provide inputs as required for the tasks outlined under the scope of work, and aid in formative research, program implementation and evaluation and write up. The Hubert Dept Global Health at the Rollins School of Public Health at Emory University seeks to improve health services and delivery systems around the world through research, teaching and service. The proposed team includes expertise in maternal nutrition, breastfeeding and child nutrition and WASH.
Brigham and Women's Hospital	Expert technical assistance. Provide expert guidance in the care of vulnerable infants aspects of the project and provide inputs as required for the tasks outlined under the scope of work, and aid in formative research, program implementation and evaluation and write up. The Brigham and Women's Hospital (BWH) Global Newborn Health Lab is dedicated to improving the survival, health, and potential of mothers and newborns in low-income countries. Research at the BWH Global Newborn Health Lab is focused on the prevention, management, and improvement of outcomes for the major newborn illnesses in low-income countries. The Lab aims to (1) improve epidemiologic evidence on the major causes of newborn morbidity and mortality in low-income settings and (2) design and evaluate innovative, high-impact, and scalable public health interventions that target the major causes of neonatal morbidity and mortality in low-income settings. Dr. Lee has expertise in pediatric and newborn care, as well as clinical trials in LMIC. Dr. Lee will provide guidance on outcomes determination and the safety-monitoring plan.
PATH	Expert technical assistance. Provide expert guidance in the facility assessment, flow of the milk, and human milk bank aspects of the project and provide inputs as required for the tasks outlined under the scope of work.

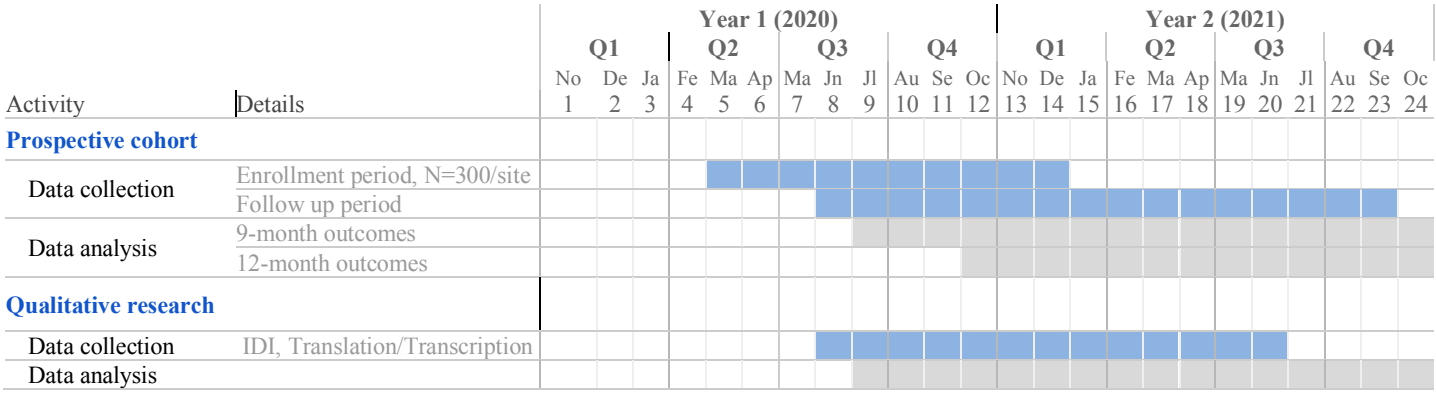
PATH is a global team of innovators working to accelerate health equity so all people and communities can thrive. They advise and partner with public institutions, businesses, grassroots groups, and investors to solve the world's most pressing health challenges.

The proposed team includes expertise in IYCF, breastfeeding and human milk banks.

15 Study timelines



15.1 Study Extension Timeline



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17 Appendix A: Protocol for measuring infant weight, length, head circumference, and MUAC

All measures will be taken by trained study personnel.

Weight will be measured using a digital weighing scale with 10 gram precision consistent across sites. The scale will be placed on a flat, hard surface and will be tared to zero prior to each measurement. The infant is to be naked with no clothing or diaper, and should be calm prior to the measurement. The infant will be weighed three times and three weights will be documented.

Length will be measured using an infantometer with measurements to the nearest 1mm that is placed on a raised, flat surface. The infant is to be naked with no clothing or diaper, and should be calm prior to the measurement. One person will hold the infant's legs and move the foot board. An assistant will stand at the headboard and position the infant's head so that it touches the top of the headboard and is in the Frankfort Vertical Plane, such that a vertical line from the ear canal to the lateral corner of the eye is perpendicular to the horizontal board. The infant will be gently straightened. The measurement will be recorded to the nearest 1mm. The measurements will be taken two more times and the three values will be averaged.

Head circumference will be obtained using a non-elastic tape with measurements to the nearest 1mm. The infant will be held on the mother's lap. Study personnel will anchor the tape immediately above the infant's eyebrows, wrapping it around the head with positioning over the fullest protuberance of the skull in the back. A second measurer can help with appropriate positioning. The tape will be pulled such that is it is gently taut around the infant's head, and a measurement will be read to within 1mm. The measurement will be taken two more times and the three values will be averaged.

Mid upper arm circumference will be measured three times using a special tape at the midpoint between the tips of the infant's left shoulder and elbow, per standard guidance. To measure:

1. Ask the mother to remove any clothing that may cover the child's left arm. If possible, the child should stand erect and sideways to the measurer.
2. Estimate the midpoint of the left upper arm.
3. Straighten the child's arm and wrap the tape around the arm at the midpoint. Make sure the numbers are right side up. Make sure the tape is flat around the skin.
4. Inspect the tension of the tape on the child's arm. Make sure the tape has the proper tension and is not too tight or too loose. Repeat any steps as necessary.
5. When the tape is in the correct position on the arm with correct tension, read the measurement to the nearest 0.1 cm.
6. Immediately record the measurement.

18 Appendix C: List of hospitals where data collection will take place

Country Site	Hospital/Clinic Names where Data collection will take place
Tanzania Site	Muhimbili National Hospital
	Temeke District Hospital
	Mbagala Rangi Tatu Hospital
	Amana Regional Referral Hospital
Malawi Site	EMMW- Kamuzu Central Hospital
	Bwaila Hospital
India/Karnataka Site	KLES Dr Prabhakar Kore Charitable Hospital, Belagavi
	S S Institute of Medical Sciences & Research Centre, Davangere
	Bapuji Child Health Institute & Research Centre, Davanagere
	Women & Children Hospital, Davanagere
	Chigateri District Hospital, Davanagere
India/Odisha Site	Srirama Chandra Bhanja (SCB) Medical College and Hospital, Cuttack
	Sardar Vallavbhai Patel Post Graduate Institute of Paediatrics, Cuttack
	City Hospital Oriya Bazar, Cuttack

19 Appendix D: List of Co-PIs

Co-PIs	Institution
Linda Vesel PhD, MPH	Harvard School of Public Health (HSPH)
Shivaprasad Goudar, MD, MPHE	Jawaharlal Nehru Medical College (JNMC), Belgaum

Tisungane Mvalo, MD	UNC Project Malawi (UNCPM)
Dr. Karim Manji, MBBS, MMED, MPH, FTAAS, FRCP(Lon) FRCPCH (Lon)	MUHAS/ HSPH, Tanzania
Chris Sudfeld, ScD	MUHAS/ HSPH, Boston
Melissa Young, PhD	Emory University
CC Lee, MD, MPH	Brigham and Women's Hospital
Irving F Hoffman, PA, MPH	University of North Carolina