# **RESEARCH PROTOCOL**

# CAMBODIA INTEGRATED NUTRITION, HYGIENE, AND SANITATION IMPACT EVALUATION - ENDLINE

## I. Introduction

Despite strong economic growth and rising living standards in the last two decades, high levels of undernutrition persist in Cambodia. Most efforts to reduce incidence of stunting have sensibly focused on providing children with better nutrition during the critical period when stunting manifests. However, many studies have shown that better nutrition alone cannot wholly eliminate growth delays. Acute and persistent infections associated with unsafe water, poor sanitation, and inadequate hygiene may impact gut health and therefore overall nutrition and growth. Thus, complementary water, sanitation and hygiene (WASH) interventions aimed at reducing diarrheal disease and exposure to fecal bacteria may be an important means to securing optimal nutritional outcomes for children.

There is growing interest in the potential for <u>combined</u> nutrition and WASH interventions to yield complementary and even synergistic effects on child linear growth and enteric infections, along with the many downstream impacts of reduced undernutrition. The limited empirical evidence and understanding of the links between enteric infections and nutrition strongly suggests this would be the case (see figure below), but the effectiveness of these approaches has not necessarily been formally investigated by impact evaluations. Thus, this impact evaluation will add value by providing an evidence base for informed decision-making and future programming, as well as contribute to the global body of knowledge.

## 2. Study Overview

The impact evaluation of the USAID-funded Cambodia Integrated Nutrition, Hygiene, and Sanitation (NOURISH) Project consists of a cluster randomized controlled trial design to rigorously test the effectiveness of integrating at-scale WASH and nutrition services in reducing stunting beyond what standalone nutrition or WASH programs can achieve. The underlying theory of change is that integration of WASH and nutrition services leads to greater health outcomes than either WASH services or nutrition services (i.e. the integrated activity is greater than the sum of its parts).



### Figure I: Theory of Change

The design consists of randomizing the roll-out of large-scale nutrition and WASH interventions in 55 rural communes across three provinces of Cambodia: Battambang, Pursat, and Siem Reap, into four groups. These activities pre-date the proposed research, described in this document, which is limited to an endline survey. Previous randomization allows for the identification of individual and combined causal effects of nutrition and WASH interventions: WASH alone (primarily community-led total sanitation coupled with supply chain support for sanitation and hygiene products, and social and behavior change communication), nutrition alone (complementary feeding, community-based education, and conditional cash transfers linked to the utilization of key health and nutrition services during the first 1,000 days), and combined WASH/nutrition compared with a control group.

A baseline survey conducted by USAID in September 2016 collected outcome measurements at the beginning of the project to establish a baseline estimate of outcome indicators. At endline, the evaluation will measure differences in height and weight in children, as well as prevalence of enteric infections and anemia, after exposure to these interventions. Other outcomes include incidence of diarrhea, sanitation and hygiene practices, and nutritional status that result from these interventions.

# 2.1. Objective

The objective of the impact evaluation is to test whether the approach to integrating WASH and nutrition services is effective and should be further promoted. Furthermore, the impact evaluation will investigate whether this approach is more effective than independent nutrition or WASH programs. The endline survey aims to measure differences in nutritional outcomes in children I - 28 months across the four intervention groups. The evaluation will answer the following questions:

- 1. Do nutrition interventions, as delivered at scale, lead to improved child health (as measured by linear growth, enteric infections, and incidence of diarrheal disease)?
- 2. Does expanded access to sanitation lead to improved child health?
- 3. Is the combined effect on child health of sanitation/hygiene and nutrition interventions delivered together greater than the additive effect of the two interventions delivered independently?

# 2.2. Methodology

The sampling frame consists of households within 491 villages in 55 communes across three provinces of Cambodia: Battambang, Pursat, and Siem Reap. The endline will collect data from two different samples within these same communes. The main sample will be randomly selected from IDPoor households with at least one child 1-28 months old (classified as "Eligible Households" in Table I below). The secondary sample consists of any household within these communes. Table I below shows an overview of the study target population from which the study sample will be selected.

Provinces	# Target Districts	# Target Communes	# Target Villages	# Total Households	Eligible Households
Battambang	8	22	180	48,725	4,956
Pursat	4	6	83	16,963	1,375
Siem Reap	10	27	228	43,434	3,675
Total	22	55	491	109,122	10,006

#### Table 1: Overview of Study Population

The study sample size was calculated so the study has sufficient power for a minimum detectable effect size (MDES) of 0.19 for differences in height-for-age (HAZ) scores between treatment groups and a MDES of 0.18 for differences between each treatment group and the control group. This translates to a 23.4 percent change in HAZ scores between treatment groups, and a 22.2 percent change in HAZ scores between treatment and control groups. The following assumption were used to estimate the sample size:

- Power: 80 percent.
- Significance level: 95 percent (using a two-sided test).
- Baseline HAZ: mean of -0.96 with a standard deviation 1.187, estimated from the study baseline.
- Intra-cluster correlation coefficient (ICC): 0.014 on the HAZ outcome variable at the commune level, estimated from the study baseline
- Increased allocation of eligible communes to the control arm, 19 communes (one-third of the total), to enhance statistical efficiency of multiple hypothesis testing.

The resulting sample size is a total of 4,015 children (consisting of 73 observations per cluster) from eligible households selected randomly across the study area. Eligible households include those who are IDPoor and have at least one child I - 28 months of age. A two-stage cluster sampling method will be used to select a random sample. In the first stage, the total sample in each province will be divided by all number of villages in each province to select number of households in each village. After dividing the calculated 4,015 sample size by the 491 villages, the average number of households per village is eight. In the second stage, a list of households in each village will be developed by consulting with village leaders prior to the data collection. IDPoor households with children between I and 28 months of age will be identified. If the number of eligible households in the village is equal or less than eight, then all will be surveyed. If the number is greater than eight, then a simple random sample will be used to select eight households. All field supervisors will be trained to perform this role with direct supervision by the sampling specialist.

Provinces	Estimated Eligible Households	Percent of Sample	Required Sample
Battambang	4,956	49%	١,967
Pursat	I,375	14%	563
Siem Reap	3,675	37%	I,485
Total	10,006	100%	4,015

 Table 2: Required Sample for the Main Survey

In addition, the secondary sample will collect reliable point-estimates of sanitation coverage at the group level. Thus, the required sample size was calculated based on a conventional approach for proportions, using 95% confidence level with a margin of error of +/-4%.

$$N = p(1-p) * \left[\frac{Z}{ME}\right]^2 * 4 = 0.39(1-0.61) * \left[\frac{1.96}{0.04}\right]^2 * 4 = 1,473$$

where:

p = proportion of sanitation coverage of 0.39, estimated using study baseline data Z = 1.96 (for 95% confidence level) ME = margin of error of +/-4%.

Given the 491 villages, three additional random households per village will be required, for a total of 1,473 additional households. The three households will be randomly selected from the list of households developed in consultation with village leaders.

# **2.3. Data Collection and Analyses**

The endline data collection consists of collecting anthropometric measurements, stool samples, and anemia screening for children 1-28 months living in IDPoor households and a 30-minute survey to their primary caregiver. In addition, three other households in the same villages will be surveyed and asked about sanitation coverage and health care access. This will consist of a short 10-minute survey with only a small subset of questions from the main survey.

### 2.3.1. Survey Instrument

Household data will be collected using interviewer-assisted structured questionnaires. The survey questionnaire was developed by the evaluation team and the majority of questions are based on validated questions in the Cambodia Demographic and Health Survey (CDHS) questionnaires.

Modules	
l.	Basic Information from Primary Caregiver
II.	Family Size, Pregnancy, and Child Births
III.	Participation in Community Nutrition and WASH activities
IV.	Child Health (Diarrhea and other illness)
V.	Child Dietary Diversity
VI.	Young Children Feeding Practices
VII.	Household Water and Sanitation
VIII.	Household Characteristics

### **Table 3: Questionnaire Modules**

### 2.3.2. Pilot Test of the Questionnaire

The questionnaire will be translated from English to Khmer and back-translated to ensure correct translation. Prior to training the enumerators, the Co-Principal Investigator along with selected surveyors from our survey firm partner, will pilot the questionnaire for language, understanding, skip patterns, and time duration. Any confusing language will be corrected. The questionnaire will be shortened if needed at this time, prior to the start of the training. The pilot will also test the anthropometric measurement protocols for clarity, efficiency, and careful handling of children.

### 2.3.3. Selection and Training of Enumerators

Our survey firm partner, supervised by the PI, will identify, hire and train a team of qualified enumerators and experienced in field and anthropometric data collection. Three-day training will be conducted with the enumerators and supervisors to make them well-versed about the study protocol, key principles of interviewing, and fundamentals of anthropometric measurement. The training will include practice in the classroom using the questionnaire and weighing and measuring children, and practice in the field to identify households, select respondents, and conduct interviews and measure children.

### 2.3.4. Location of Interviews

The interviews, anthropometry measures, and anemia screening will be conducted at the eligible households. All efforts will be made to find a private and quiet place for the interview, away from other people.

### 2.3.5. Duration of Interview

Each interview, including anthropometry and anemia screening, will take no longer than one hour. Referrals, questions or concerns raised may take longer and are not counted within this time. If the pilot test shows that the survey takes longer, then questions will be removed or streamlined. We recognize the time constraints of families, especially women, in the project areas.

### **2.3.6. Data Collection Procedures**

Field data collection will be conducted by 40 well-trained data collectors working in pairs with direct supervision by five field supervisors, a field coordinator and anthropometry trainer, and a sampling specialist. One data collector out of each pair will be trained and qualified to conduct the anemia screening. The project leader will oversee the field data collection until the end.

The data collectors will first introduce themselves to the participants, explain the survey objective and procedure, and seek written informed consent from the mother/caregiver prior to the start of any data collection. A unique identification number will be given to each participant.

#### Anthropometry Measures:

For eligible children (1-28 months), the survey will collect anthropometric data through trained and experienced data collectors and anthropometrists from a local survey firm (to be contracted). The data collectors will measure the weight and height of the child following FANTA Guidelines.<sup>1</sup>

Anthropometric measurement is comprised of weight and length. Weight will be measured using Uniscale (UNICEF recommended scale) in Kilogram with precision to one decimal point. Length will be measured using a length board (UNICEF / WFP recommended) in Centimeter with precision to one decimal point. Two data measurements are required, one from the measurement taker and another on from an assistant. Both measurement taker and assistant will back check the recording during and after measurement to ensure accuracy of reading and quality of recording. *Children who are found to be wasted* (WHZ < -2) or severely wasted (WHZ < -3) will be referred to the appropriate existing treatment programs.

#### Anemia Screening

Anemia screening will be conducted for women of childbearing age (15-49), including pregnant women, and all eligible children. The anemia test consists of taking two images of the participant's fingernail beds using an app-based measurement platform developed at Georgia Tech<sup>2</sup>. Studies have shown that the degree of pallor in the fingernail beds correlates with physiologic levels of hemoglobin in the blood and determines whether or not a person is anemic. Because this is not a diagnostic method and may only yield a general range of hemoglobin, we will use these data only to generate means and not to indicate a specific individual's health status.

#### Survey and Stool Sample Container

Following these tests, the enumerator will proceed to conduct the survey with the mother or primary caregiver at their home. In addition, the enumerator will leave a sterile fecal collection container to the primary caregiver of the eligible child (see the stool collection SOP), instructing them to collect feces from

<sup>&</sup>lt;sup>1</sup> Cogill, Bruce. 2003. Anthropometric Indicators Measurement Guide. Washington, DC: Food and Nutrition Technical Assistance (FANTA) Project, FHI 360

<sup>&</sup>lt;sup>2</sup> Mannino, R.G., Myers, D.R., Tyburski, E.A., Caruso, C., Boudreaux, J., Leong, T., Clifford, G.D., and Lam, W.A. 2018. Smartphone app for non-invasive detection of anemia using only patient-sourced photos. *Nat Commun* 4;9(1):4924. doi: 10.1038/s41467-018-07262-2.

the same evening or the following morning's defecation events, either in a diaper or in a child potty. At the end of the visit, a small gift will be given in appreciation for their time and support.

Staff from the field team will return to the household on the following day to retrieve the containers. At pick-up, fecal specimens will be mixed with a preservative, collected and transported at room temperature. The study team consulted with several laboratories in Cambodia, but due to missing capacity, the analysis of stool samples cannot be conducted in Cambodia (see Annex). Specimens will be bagged and sealed on ice or with frozen refrigerant packs in an insulated box to be transported to the Brown Global WASH Lab in the School of Civil and Environmental Engineering at the Georgia Institute of Technology in the United States.

### 2.3.8. Data Management and Analyses

To reduce the lag time between data collection and data analysis, as well as to minimize data entry error and conduct real-time data quality checks, the study will collect data using tablet devices. This technology also enables linking the survey data with GPS coordinates and observation photos taken during the visit. Personal identifiers are only required in order to locate the household for the pick-up of the stool sample containers the following day. Confidentiality of all study participants will be protected, with personal identifying information stored separately from the rest of the data, under lock and key, described in the IRB application. All data will be encrypted and uploaded to a password-protected server, and only the head of the survey firm/supervisor and the PI (in addition to any other key GT staff to be determined and added to this IRB) will have access to this information. No names of children will be recorded, and only the name of the respondent will be collected, this only in hard copy on the contact sheet and on the consent form.

### Survey Data:

Data will be analyzed in STATA by the evaluation team using log-binomial (prevalence data) and linear regression (continuous outcomes after log-transformation) models to compare outcomes between intervention groups. Clustering within communes will be accounted for by generalized estimating equations or random effects, depending on whether the data meet the assumptions for these methods. To account for baseline imbalances between intervention arms, all analyses will be adjusted using baseline primary outcome data.

### **Stool Specimens:**

The following analysis will be conducted:

- Enteric infections using gastrointestinal pathogen panel (PCR):
  - Presence of the following pathogens:
    - Campylobacter
    - Clostridium difficile, Toxin A/B
    - Escherichia coli O157
    - Enterotoxigenic E.coli (ETEC) LT/ST
    - Salmonella
    - Shiga Toxin-producing E.coli (STEC) stx1/stx2
    - Shigella
    - Vibrio cholerae
    - Yersinia enterocolitica
    - Cryptosporidium
    - Entamoeba histolytica

- Giardia
- Adenovirus 40/41
- Norovirus GI/GII
- Rotavirus A
- Presence of soil-transmitted helminths using Kato-Katz:
  - Ascariasis
  - Hookworm
  - Trichuriasis

### Anemia Screening:

Fingernail images will be run through an algorithm that utilizes the color of the fingernail beds and conjunctiva to assess the degree of anemia. This algorithm uses images taken of the patient's fingernail beds and analyzes the raw image data. Smartphone images utilize the Red, Green, and Blue (RGB) color model to display images. These 3 colors are assigned intensity values from zero to 255 and are added together in each pixel of the image to generate every color in the image. Successful proof-of-concept experiments have shown that raw blue intensity values correlate with the concentration of hemoglobin in the blood.

## 3. Data Quality Assurance

The survey team will follow standard operating procedures for data collection including verification procedures conducted both at the site and at its headquarters as well as data quality query. Specifically, our survey firm partner will employ the following set of quality control procedures:

- A field manager and supervisors will ensure all enumerators follow the agreed timeline and procedures; when issues arise, the field manager will be involved to find a proper solution.
- The supervisors will accompany the enumerator teams for at least 10 percent of the interviews to be conducted.
- Controllers/inspectors will be independent from the interview process and will conduct backchecks of at least 10 percent of the completed surveys. Surveys to be back-checked will be selected randomly, stratified by enumerator to ensure each one is checked on a largely equal basis. During the repeat interview, several validation questions will be asked: interview date, interview duration, answers to several questions, interview location, etc. If everything is fine, all interviews of the particular enumerator are approved. If there is any doubt, 100 percent of questionnaires of the particular enumerator are checked.

Field data collection staff will provide additional oversight and monitoring of the quality of data collected by the enumerators and the quality of supervision performed by the survey supervisors:

- Back-checks on a random sample of surveys (at least 5 percent of all completed surveys) using a subset of the survey questions. Any errors found will be consulted with the enumerator and immediately rectified. In some cases, interviewers will have to make a repeat visit or a repeat call to the respondent to clarify some answers.
- Accompaniments of enumerators during interviews at regular intervals. Observe the enumerators' familiarity with and comprehension of the questionnaire and clarity in asking questions. If the evaluation team perceives any need for additional training, it will coordinate with the supervisors and arrange for immediate additional briefings and training.

# 4. Ethical Considerations

Ethical clearance for the baseline of this study was obtained from the National Ethics Committee for Health Research (NECHR) in the Cambodia Ministry of Health in September 2016. Ethical clearance for the endline of this study is sought from NECHR and Georgia Institute of Technology. All participants will be explained about process and objective of the study in Khmer language and asked for a written informed consent before data collection. A consent script will be carefully read to respondents. This consent form contains information on the purpose of the study, risks and benefits, confidentiality, and the voluntary nature of participation – each person can refuse to participate or withdraw at any time without any negative consequence or penalty.

Face-to-face interviews will be kept confidential and done in a private location. Each participant will be assigned a unique code number; and anonymity will be ensured in the questionnaire and database, with personal identifying information stored separately from the rest of the data. All data will be password protected. Details around confidentiality and access to data have been described in the IRB application in the appropriate sections.

## 5. Risks and Benefits

These have been described in the consent form. In brief, there are no direct benefits to participants. If we identify that the child is underweight or undernourished (definition: I or more standard deviations below the mean WHO reference population value, adjusted for age, on either weight or height), we will recommend that caregivers consult the local health clinic. There are no major risks to participating in this study. There are no perceived risks from the stool sample collection given that it is non-invasive. There is a minor inconvenience of time spent responding to questions. Some questions regarding stunting, defecation and infant feeding practices could potentially be sensitive. However, these questions have been successfully used by other studies in Cambodia and have been found to be acceptable to participants. In general, to mitigate this potential sensitivity, respondents will be informed that they can choose not to participate in the survey, or to refuse to answer any particular question in the survey if they so choose.

To compensate respondents for their time and participation, a small gift equivalent to 6,000 riels (\$1.5 USD) will be offered to each household. We anticipate this to be in the form of phone credit for their preferred carrier.

## 6. Limitations

Potential limitations of the survey include response bias and recall bias. There are many reasons why people may provide less-than-truthful responses to questions. For instance, they may provide socially desirable answers. We will attempt to reduce this potential bias by providing respondents with clear information about why they were being interviewed (including the fact that their responses would have no bearing on their participation or lack of participation in in the project or other services) as well as transparent information about the use of their responses and the fact that they would never be identified individually or by name in any reports. We will also observe the home and add this observation data when it is appropriate. Recall bias, which results from having to ask respondents for information from a period in the past, is inevitable. This is a particular concern for health recalls among women and children. We will attempt to reduce this by using short recall periods.

# 7. Timeline

Below is a detailed timeline for planning and field work.

Cambodia Nutrition and Sanitation Endline - Work Plan																
		July			August		September									
Tasks	Wk I	Wk 2	Wk 3	Wk 4	Wk I	Wk 2	Wk 3	Wk 4	Wk I	Wk 2	Wk 3	Wk 4	October	November	December	January
Sampling frame finalized																
Pilot test survey questionnaire																
Training and field test																
Data collection (surveys, anthropometry, anemia, stool samples)																
Quality control																
Data analysis																
Evaluation Report																
Dissemination of Findings																

# **Annex – Laboratory Capacity for Required Stool Sample Analysis**

The study team reached out to several laboratories in Cambodia to assess their capacity to conduct the required stool sample analysis for this study (see Table below). In addition, the Battambang Provincial Hospital and the Kampong Cham Provincial Hospital only perform direct microscopy on very few stool parasite requests. While they have been receiving external support from the Diagnostic Microbiology Development Program (DMDP) and the US Naval Medical Research Unit (NAMRU), the support has been focused on bacteriology and there is less capacity in parasite detection. None of the laboratories have the capacity to conduct all of the required tests so the team will have to transport the stool samples outside of Cambodia for analysis.

	Institute Pasteur	NIPH	СММ
Gastrointestinal Pathogen Pan	el (PCR)		
Campylobacter	Yes	No	No
Clostridium difficile, Toxin A/B	Yes	No	No
Escherichia coli 0157	Yes	No	No
Enterotoxigenic E.coli (ETEC) LT/ST	No	No	No
Salmonella	Yes	No	No
Shiga Toxin-producing E.coli (STEC) stx1/stx2	No	No	No
Shigella	Yes	No	No
Vibrio cholerae	Yes	No	No
Yersinia enterocolitica	Yes	No	No
Cryptosporidium	Yes	No	No
Entamoeba histolytica	Yes	No	No
Giardia	Yes	No	No
Adenovirus 40/41	Yes	No	No
Norovirus GI/GII	No	No	No
Rotavirus A	Yes	No	No
Methods:	No PCR; only stool culture or stool parasitology		
Soil-Transmitted Helminths (K	ato-Katz or other available	methods)	
Ascariasis	Yes	Yes	Yes
Hookworm	Yes	Yes	Yes
Trichuriasis	Yes	Yes	Yes
Methods:	Stool parasitology and direct examination		
Quality and Reliability of Result	S:		
External Quality Assessment results	Level A by CTCB and ABP program		
International/standard operating procedures	ISO15189 accreditation; only accredited clinical microbiology laboratory in Cambodia		

## **Cambodia Nutrition and Sanitation Endline Survey**

# For Primary Caregiver of Young Children (1 – 28 months)

IDENTIFICATION						
SUPERVISOR NAME:	CODE:					
INTERVIEWER NAME:	CODE:					
INTERVIEW DATE: / / START	TIME:: END TIME::					
PROVINCE:	CODE:					
DISTRICT:	CODE:					
COMMUNE:	CODE:					
VILLAGE:	CODE:					
HOUSEHOLD GPS COORDINATES:						

SCREENING QUESTIONS							
C1. Is there a child aged 1 to 28 months living in this household?	<ol> <li>YES</li> <li>NO → STOP INTERVIEW</li> </ol>						
[ASK TO SPEAK WITH THE MOTHER OR PRIMARY CAREGIVER OF THE CHILDREN 1 TO 28 MONTHS. CHECK DATE OF BIRTH ON CHILD'S BIRTH CERTIFICATE OR YELLOW HEALTH CARD BEFORE STARTING SURVEY.]							
C2. Has the eligible child/children lived in this village for his/her entire life?	<ol> <li>Yes → READ INFORMED CONSENT</li> <li>No</li> </ol>						
C3. Where did child live before?	<ol> <li>In another village within the same commune</li> <li>Outside this commune → STOP INTERVIEW</li> </ol>						

**QID**: |\_|\_|\_|\_|

## Informed Consent Form

Hello. My name is \_\_\_\_\_\_. I am working with KHANA Center for Population Health Research, a research firm based in Phnom Penh, with approval from the Ministry of Health and the Provincial Health Department. We are gathering data for a research project conducted by Georgia Institute of Technology.

- 1. We are conducting a study about child nutrition in Battambang, Pursat, and Siem Reap. The information we collect will help inform development of targeted activities to improve child nutrition in your area.
- 2. Your household was randomly selected to participate in the survey.
- 3. We will ask you some questions about your household and your young children.
- 4. We would also like to weigh and measure all the eligible children in your household.
- 5. At the end of our visit, we will leave a plastic container for your child's next stool and will come back tomorrow to collect it.
- 6. Your participation in this study is expected to last no more than 1 hour today and a brief visit (10 minutes) when we come back to collect the sample.
- 7. All of the answers you give will be **confidential** and your name will not be shared with others outside our research team.
- 8. Your participation in the study is completely **voluntary**, but we hope you will agree to answer the questions since your views are important.
- 9. If I ask you any question you don't want to answer, just let me know and I will go on to the next question or you can stop the interview at any time.

In case you need more information about the survey, you may contact the person listed on this information sheet.

[AFTER READING THIS FORM, GIVE PARTICIPANT THE INFORMATION SHEET]

Do you have any questions?

C4. Do you understand and agree to participate in this study?								
1. YES	2. NO → STOP INTERVIEW							

Signature of Respondent

Date

Signature of Enumerator

Date

I. BASIC INFORMATION FROM PRIMARY CAREGIVER						
We will start this interview with a few questions to get basic information about you.						
Q1. In what month and year were you born? [ASK FOR IDENTIFICATION CARD OR OTHER DOCUMENT TO CONFIRM DATE OF BIRTH]	Gregorian Month 88. Don't Know Month      Gregorian Year 8888. Don't Know Year					
Q2. How old were you at your last birthday?	_  Years					
Q3. What is your religion?	<ol> <li>Buddhist</li> <li>Muslim</li> <li>Christian</li> <li>Other (Specify:)</li> </ol>					
Q4. What is the highest level of school you attended?	<ol> <li>Never attended school</li> <li>Primary (1 – 6)</li> <li>Lower Secondary (7 – 9)</li> <li>Upper Secondary (10 – 12)</li> <li>Higher</li> <li>Other (Specify:)</li> <li>88. Don't Know</li> </ol>					
Q5. What is your current marital status?	<ol> <li>Married or living together</li> <li>Divorced or separated</li> <li>Widow</li> <li>Never married</li> </ol>					
Q6.a What is the highest level of school your (spouse/partner) ever attended?	<ol> <li>Never attended school</li> <li>Primary (1 – 6)</li> <li>Lower Secondary (7 – 9)</li> <li>Upper Secondary (10 – 12)</li> <li>Higher</li> <li>Other (Specify:)</li> <li>88. Don't Know</li> </ol>					
Q6.b In the past 24 months, have you lived outside of this commune?	<ol> <li>Yes</li> <li>No → SKIP TO Q7</li> </ol>					
Q6.c For how many months did you live outside of this commune? [IF MORE THAN ONE TIME, ADD THE TOTAL TIME]	Months					

Q7. How many people currently live in this household, including yourself, other adults and all children who regularly sleep and eat in this household?	People				
Q8. Of the people who currently live in this household, how many are:					
a) Children under 18 years old?	Children				
b) Adults 18 years or older?	Adults				
Q9. How many eligible children aged 1 to 28 months live in this household?					
ELIGIBLE CHILDREN ARE AGED 1 TO 28 MON	THS LIVING IN THE SAME COMMUNE THEIR ENTIRE LIFE				

II.

## BASIC INFORMATION FOR ALL CHILDREN 1 TO 28 MONTHS

I would now like to ask you questions about these children aged 1 to 28 months. Let's start with the order of the children, starting with the youngest.							
	CHILD 1	CHILD 2	CHILD 3				
Q10. What is the order of (CHILD NAME) (youngest = 01, second youngest = 02, etc.)?							
Q11. What is (CHILD NAME)'s gender?	1. Male 2. Female	1. Male 2. Female	1. Male 2. Female				
Q12. Are you (CHILD NAME)'s mother?	<ol> <li>Yes</li> <li>No → SKIP TO Q13</li> </ol>	1. Yes 2. No	1. Yes 2. No				
Q12a. Did you receive antenatal care while you were pregnant with (CHILD NAME)?	<ol> <li>Yes</li> <li>No → SKIP TO Q12c</li> </ol>	<ol> <li>Yes</li> <li>No → SKIP TO Q12c</li> </ol>	1. Yes 2. No <b>→ SKIP TO Q12c</b>				
Q12b. How many times did you receive antenatal care while you were pregnant with (CHILD NAME)?	# of Times	# of Times	# of Times				
Q12c. Where did you give birth to (CHILD NAME)?	<ol> <li>Home, with traditional birth attendant</li> <li>Home, with trained midwife</li> <li>Public health center, clinic or hospital</li> <li>Private clinic/hospital</li> <li>Other (specify)</li> </ol>	<ol> <li>Home, with traditional birth attendant</li> <li>Home, with trained midwife</li> <li>Public health center, clinic or hospital</li> <li>Private clinic/hospital</li> <li>Other (specify)</li> </ol>	<ol> <li>Home, with traditional birth attendant</li> <li>Home, with trained midwife</li> <li>Public health center, clinic or hospital</li> <li>Private clinic/hospital</li> <li>Other (specify)</li> </ol>				
Q13. Was (CHILD NAME) ever breastfed?	<ol> <li>Yes</li> <li>No → SKIP TO Q15</li> </ol>	1. Yes 2. No <b>→ SKIP TO Q15</b>	<ol> <li>Yes</li> <li>No → SKIP TO Q15</li> </ol>				
Q14. Is (CHILD NAME) still being breastfed?	1. Yes 2. No	1. Yes 2. No	1. Yes 2. No				
Q15.1 What is (CHILD NAME)'s birth date?	Day     Month     Year	Day   _  Month     Year   _	Day    Month     Year				
Q15.2 [DOUBLE ENTER BIRTH DATE]	Day    Month   _  Year	Day     Month     Year	Day    Month     Year				

### FORM #1 30-Jul

Q15.3 [SOURCE OF BIRTH DATE]	<ol> <li>Official document (birth certificate / yellow card)</li> <li>Self-reported, no birth certificate / card</li> </ol>	<ol> <li>Birth certificate or yellow card</li> <li>Self-reported, no birth certificate / card</li> </ol>	<ol> <li>Birth certificate or yellow card</li> <li>Self-reported, no birth certificate / card</li> </ol>
Q16.1 What was (CHILD NAME)'s weight at birth?	KG    .    88. Don't Know	KG    .    88. Don't Know	KG    .    88. Don't Know
Q16.2 [SOURCE OF WEIGHT AT BIRTH]	<ol> <li>From yellow card</li> <li>Self-reported, yellow available birth weight not recorded</li> <li>Self-reported, no yellow card → SKIP TO NEXT CHILD or Section III</li> </ol>	<ol> <li>From yellow card</li> <li>Self-reported, yellow available but no recorded weight at birth</li> <li>Self-reported, no yellow card → SKIP TO NEXT CHILD or Section III</li> </ol>	<ol> <li>From yellow card</li> <li>Self-reported, yellow available but no recorded weight at birth</li> <li>Self-reported, no yellow card → SKIP TO Section III</li> </ol>
Q16.3 [WHAT IS THE LAST MONTH THE CHILD'S WEIGHT WAS PLOTTED ON THE CHILD'S YELLOW HEALTH CARD?]	Month	Month	Month
	GO TO CHILD 2 OR IF NO MORE CHILDREN SKIP TO SECTION III.	GO TO CHILD 3 OR IF NO MORE CHILDREN SKIP TO SECTION III.	

## III. ANTHROPOMETRY MEASURES FOR SAME CHILDREN 1 TO 28 MONTHS

Now I would like to take the height and weight measurements of all of these children aged 1 to 28 months. Let's start with the youngest child (CHILD 1).			
Q17. SELECT TYPE OF SCALE		1. Type A 2. Type B	
	CHILD 1	CHILD 2	CHILD 3
[RECORD SAME CHILD ORDER (youngest = 01, second youngest = 02, etc.)]			
[USE THIS WEIGHT VERS	ION FOR SCALE TYPE A ONL	Y]	
Q17.1 [FIRST TIME: RECORD <b>WEIGHT</b> IN KILOGRAMS]	KG   _ .	KG   . _ .	KG   .
Q17.2 [SECOND TIME: RECORD <b>WEIGHT</b> IN KILOGRAMS]	KG   _ .	KG   _ .	KG   .
USE THIS WEIGHT VERS	ION FOR SCALE TYPE B ONL	Y]	
Q17.1.1 [FIRST TIME: RECORD <b>WEIGHT</b> IN KILOGRAMS]	KG   _ .   [MOTHER AND CHILD]	KG   .  . [MOTHER AND CHILD]	KG   _ .   [MOTHER AND CHILD]
	KG   _ .   [MOTHER ONLY]	KG   .  .   [MOTHER ONLY]	KG   _ .   [MOTHER ONLY]
Q17.2.1 [SECOND TIME: RECORD <b>WEIGHT</b> IN KILOGRAMS]	KG   . .   [MOTHER AND CHILD]	KG   . .    [MOTHER AND CHILD]	KG   _ .   [MOTHER AND CHILD]
	KG   _ .   [MOTHER ONLY]	KG   . .   [MOTHER ONLY]	KG   _ .   [MOTHER ONLY]
[CONTINUE WITH HEIGH]	MEASUREMENT BELOW]		
Q18.1 [FIRST TIME: RECORD <b>HEIGHT</b> IN CENTIMETERS]	CM   _ .	CM   _ .	CM   _ .
Q18.2 [SECOND TIME: RECORD <b>HEIGHT</b> IN CENTIMETERS]	CM   _ .	CM   _ .	CM   _ . _
ONLY IF DIFFERENCE BETWEEN MEASUREMENTS IS <u>GREATER THAN 1.0 CM</u> Q18.3 [THIRD TIME: RECORD <b>HEIGHT</b> IN CENTIMETERS]	CM   _ .	CM   _ .	СМ   _ . .

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Q19. CONFIRM CHILD LAYING OR STANDING FOR MEASUREMENT	1. Laying 2. Standing	1. Laying 2. Standing	1. Laying 2. Standing
Q20. CHECK IF (CHILD'S NAME) HAS EDEMA	1. Yes 2. No	1. Yes 2. No	1. Yes 2. No
	GO TO CHILD 2 OR IF NO MORE CHILDREN SKIP TO SECTION IV.	GO TO CHILD 2 OR IF NO MORE CHILDREN SKIP TO SECTION IV.	

# HEALTH OF SAME CHILDREN 1 TO 28 MONTHS

Now I would like to ask you some health questions about the same young child/children we just measured. [ADMINISTER THESE QUESTIONS FOR EACH OF THE SAME CHILDREN 1 TO 28 MONTHS]

IV.

Q21. RECORD SAME CHILD ORDER (FROM Q10): (youngest = 01, second youngest = 02, etc.)				
Did [CHILD NAME] have [SYMPTOM]?	A. TODAY	B. YESTERDAY	C. DAY BEFORE YESTERDAY	D. IN THE LAST 7 DAYS (SINCE THIS DAY LAST WEEK)
Q22. Vomit?	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know
Q23. Fever?	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know
Q24. Abdominal pain?	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know
Q25. Diarrhea?	1. Yes 2. No 88. Don't Know	<ol> <li>Yes</li> <li>No</li> <li>88. Don't Know</li> </ol>	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know
IF ANSWERED "YES" TO Q25 (DIARRHEA): Q25a. For how many days did (CHILD NAME) have diarrhea?			Days	88. Don't Know
Did [CHILD NAME] have [SYMPTOM]?	A. TODAY	B. YESTERDAY	C. DAY BEFORE YESTERDAY	D. IN THE LAST 7 DAYS (SINCE THIS DAY LAST WEEK)
Q26. Three or more bowel movements in one day?	1. Yes 2. No 88. Don't Know	<ol> <li>Yes</li> <li>No</li> <li>88. Don't Know</li> </ol>	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know
Q27. Number of bowel movements each day	 88. Don't Know	 88. Don't Know		
Q28. Watery or soft stool (unformed)?	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know
Q29. Blood in the stool?	1. Yes 2. No 88. Don't Know	<ol> <li>Yes</li> <li>No</li> <li>88. Don't Know</li> </ol>	<ol> <li>Yes</li> <li>No</li> <li>88. Don't Know</li> </ol>	1. Yes 2. No 88. Don't Know

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Q30. Bruising, scrapes or cuts that occurred?	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know
Q31. Toothache / teething	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know	1. Yes 2. No 88. Don't Know
Q33. Since (CHILD NAME) was born, has he/she ever been given antibiotics as treatment for any illness? <i>ENUMERATOR: SOME EXAMPLES OF COMMON ANTIBIOTICS</i> <i>ARE AMOXICILLIN, CLAMOXIL, PENICILLIN, TETRACYCLINE,</i> <i>AMPICILLIN, COTRIMOXAZOLE.</i> <i>PARACETAMOL AND PANADOL ARE <u>NOT</u> AN ANTIBIOTIC.</i>			1. Yes 2. No 88. Don't Know	→ SKIP TO SECTION
Q34. For what reason(s)? MARK ALL THAT APPLY			<ol> <li>Respiratory ir breathing)</li> <li>Cold or flu</li> <li>Sore throat or</li> <li>Diarrhea</li> <li>Vomiting</li> <li>Pain relief</li> <li>Heal skin wor</li> <li>Intestinal infla</li> <li>Fever or high</li> <li>Dengue</li> <li>Malaria</li> <li>Other (specified)</li> <li>Bon't know</li> </ol>	nflammation (difficulty r cough und, burn, cuts, injuries ammation or ulcer temperature y)

ADD NEXT ELIGIBLE CHILD OR IF NO MORE CHILDREN SKIP TO SECTION VI

V. CHILD DIETARY DIVERSITY OF SAME CHILDREN 1 TO 28 MONTHS				
[ADMINISTER THESE QUESTIONS FOR EACH OF THE SAME CHILDREN 1 TO 28 MONTHS]				
RECORD SAME CHILD ORDER (FROM Q10):         (youngest = 01, second youngest = 02, etc.)				
Q35. Now I would like you	Plain water?			
to please describe everything that your	B. Instant formula?			
(CHILD NAME), ate and drank vesterday, during the	Milk such as tinned, powdered or fresh animal milk?			
day or night.	D. Breastmilk?			
MARK ALL THAT APPLY	E. Fresh juice?			
A Think chaut when	F. Plain soup broth?			
(CHILD NAME) first	G. Borbor?			
woke up yesterday. Did (be/sbe) eat	H. Juice drinks or carbonated drinks?			
anything at that time?	I. Any other liquids? (Specify:)			
IF YES: Please tell me	J. Food made from rice, noodles, or other grains?			
everything (he/she) ate at	Beans?			
PROBE: Anything else?	Nuts and seeds such as peanut, cashew nut			
B. What did (CHILD	A. Pumpkin, carrots, squash, or sweet potatoes that are yellow or orange inside?			
NAME) eat next?	N. White potatoes, white yams, or any other foods from roots?			
IF YES: Please tell me	O. Long beans?			
everything (he/she) ate at that time. PROBE: Anything else?	P. Any dark green, leafy vegetables like amaranth leaves, moringa, morning glory, water spinach?			
FRODE. Anything else?	Q. Ripe mangoes, ripe papayas, jackfruit?			
[CONTINUE UNTIL SHE SAYS NOTHING ELSE.	Any other fruits or vegetables? (Specify:)			
DO THIS FOR THE	Liver, kidney, heart, or other organ meats?			
ENTIRE DAY.	Flesh foods like beef, pork, lamb, goat, chicken, or duck?			
AS THE RESPONDENT	U. Wild animals like frogs, snails, crabs, insects?			
CIRCLE THE "1" BY THE	V. Duck or chicken eggs?			
CORRESPONDING	W. Fresh or dried fish?			
	X. Small rice field fish?			
	Small fish powder?			
	. Any foods made from beans, nuts, or seeds?			
	AA. Cheese, yogurt, or other milk products?			
	BB. Any oil, fats, butter, or foods made with any of these?			
	CC. Non-sugary traditional snacks, such as banana coconut sticky rice, bean pork sticky rice, etc?			

	DD. Any sugary foods such as sweets, candies, cakes, or biscuits?		
	EE. Any packaged snacks such	as chips?	
	FF. Condiments for flavor, such	as soy sauce or prahok?	
[ONLY IF SELECTED "Y. SMALL FISH POWDER" IN Q35: MARK ALL THAT APPLY Q35.Y1 You mentioned your child ate small fish powder, did you buy it or make it at home?		<ol> <li>Bought it</li> <li>Made it at home</li> <li>Received as gift</li> <li>88. Don't know</li> </ol>	
Q36. Did (CHILD NAME) eat any solid, semi-solid, or soft foods yesterday during the day or at night? <i>[IF 'YES' PROBE]:</i> What kind of solid, semi-solid or soft foods did he/she eat yesterday?		<ol> <li>Yes → GO BACK TO Q35 TO RECORD FOOD</li> <li>No → SKIP TO SECTION VI</li> </ol>	
Q37. How many times did (CHILD NAME) eat solid, semi- solid, or soft foods yesterday, during the day or at night?		Number of Times 88. Don't know	

ADD NEXT ELIGIBLE CHILD OR IF NO MORE CHILDREN SKIP TO SECTION VI

VI. HYGIENE PRACTICES				
Can you show me the space where you usually prepare food for cooking?				
Q38. OBSERVE WHETHER SURFACE FOR PREPARING FOOD (CHOPPING, CUTTING, ETC) IS A CLEANABLE MATERIAL (WOOD, BAMBOO, GRASS MATS, PLASTIC MATS, OR ANOTHER POROUS MATERIAL IS <u>NOT</u> CLEANABLE)	<ol> <li>Yes, surface for preparing food is a cleanable material</li> <li>No</li> </ol>			
RECORD OBSERVATION				
Q39. OBSERVE WHETHER FOOD PREPARATION SURFACE IS ELEVATED OFF THE FLOOR	<ol> <li>Yes, elevated off the floor</li> <li>No</li> </ol>			
RECORD OBSERVATION				
Q40. OBSERVE WHETHER FOOD PREPARATION AREA IS CLEAN OF VISIBLE DIRT	<ol> <li>Yes, clean of visible dirt</li> <li>No</li> </ol>			
RECORD OBSERVATION	2. 110			
Q41. OBSERVE WHETHER THERE ARE FLIES ON FOOD OR ON FOOD PREPARATION AREA	<ol> <li>Yes, flies observed</li> <li>No flies observed</li> </ol>			
RECORD OBSERVATION				
Q42. OBSERVE WHETHER A HANDWASHING STATION CAN BE FOUND WITHIN 10 METERS OF THE FOOD PREPARATION AREA	<ol> <li>Yes, handwashing station within 10 meters</li> <li>No</li> </ol>			
RECORD OBSERVATION				
Q43. Do you clean your cooking utensils before using them?	1. Yes 2. No → SKIP TO Q45			
Q44. What do you clean your cooking utensils with?	<ol> <li>Water and soap</li> <li>Water and ash/mud/sand</li> <li>Water only</li> <li>No water, dry cloth or towel only</li> </ol>			
Q45.a Do you clean food preparation surfaces ?	1. Yes			
ENUMERATOR: THIS IS THE SAME SURFACE OBSERVED PREVIOUSLY	2. NO → SKIP IO Q46			
Q45.b What do you clean the food preparation surfaces with?	<ol> <li>Water and soap</li> <li>Water and ash/mud/sand</li> <li>Water only</li> <li>No water, dry cloth or towel only</li> </ol>			

Q46. Do you have a container to store food?	1. Yes 2. No <b>→ SKIP TO Q50</b>
Q47. Is the container you use to store food covered? [CONFIRM BY OBSERVATION]	1. Yes 2. No
Q48. Do you clean the container used for food storage between uses?	1. Yes 2. No
Q49. What do you clean it with?	<ol> <li>Water and soap</li> <li>Water and ash/mud/sand</li> <li>Water only</li> <li>No water, dry cloth or towel only</li> </ol>
Q50. Do you cook the food until boiling?	1. Yes 2. No
ASK Q51 – Q54 ONLY IF Q36=YES FOR ANY ELIGIBLE CHIL (CHILD WHO IS NO LONGER EXCLUSIVELY BREASTFEED)	LD. OTHERWISE SKIP TO SECTION VII. ING)
Q51. Do you reheat the food before feeding your young child again? REFER TO ANY ONE OF THE ELIGIBLE CHILDREN NOT EXCLUSIVELY BREASTFEEDING	1. Yes 2. No
Q52. Do you clean the utensil/s used to feed your young child? REFER TO SAME ELIGIBLE CHILD FROM PREVIOUS QUESTION	1. Yes 2. No <b>→ SKIP TO Q54</b>
Q53. What do you clean these utensil/s with?	<ol> <li>Water and soap</li> <li>Water and ash/mud/sand</li> <li>Water only</li> <li>Dry cloth or towel</li> </ol>
Q54. Do you prepare your young child's food separately from the other food? REFER TO SAME ELIGIBLE CHILD FROM PREVIOUS QUESTION	<ol> <li>Yes, always</li> <li>Yes, sometimes</li> <li>No</li> </ol>

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VII. PREGNANCY AND	CHILD BIRTHS
[IS RESPONDENT THE MOTHER OF ANY OF THE CHILDREN AGED 1 TO 28 MONTHS THAT YOU MEASURED? CHECK IF ANSWERED "YES" TO ANY CHILD ON Q12]	<ol> <li>Yes</li> <li>No → SKIP TO Q68</li> </ol>
Q55. Are you pregnant now?	<ol> <li>Yes</li> <li>No</li> <li>88. Don't Know → SKIP TO Q58</li> </ol>
Q56. How many months pregnant are you? [RECORD COMPLETED MONTHS]	Months
Q57. SOURCE OF PREGNANCY LENGTH [PROBE WHETHER SHE ESTIMATED THE PREGNANCY LENGTH ON HER OWN OR WITH THE HELP OF A HEALTH PRACTITIONER (WITH OR WITHOUT AN ULTRASOUND].	<ol> <li>Estimated by respondent only</li> <li>Estimated by respondent and health practitioner, no ultrasound</li> <li>Estimated by respondent and health practitioner, using ultrasound</li> <li>→ CONTINUE TO Q60</li> </ol>
Q58. Have you been pregnant in the past 24 months?	<ol> <li>Yes</li> <li>No</li> <li>88. Don't Know → SKIP TO Q60</li> </ol>
Q59. Did the pregnancy result in the child being born alive? ANSWER IS NO FOR MISCARRIAGE, ABORTION, OR IF CHILD WAS BORN DEAD (STILL BIRTH).	1. Yes 2. No
Q60.a How many total births have you had in your life? [THIS INCLUDES BIRTHS TO CHILDREN WHO WERE BORN ALIVE BUT LATER DIED AND THOSE WHO CURRENTLY LIVE ELSEWHERE].	_ Total Births
Q60.b From those births, how many total children were born?	Total Children

Now let me ask you about each of the children you have given birth to, starting from the youngest child to the oldest.				
CHILD_ID [RECORD ORDER]	Q61. Is (CHILD NAME) a boy or a girl?	Q62. In what month and year was (CHILD NAME) born?	Q66. Is (CHILD NAME) still alive?	Q67. How old was (CHILD NAME) when (he/she) died? [88=Don't Know]
01	1. Boy 2. Girl	Month     Year 88. //	<ol> <li>Yes → SKIP TO NEXT CHILD OR Q68</li> <li>No</li> </ol>	Days    Months    Years
02	1. Boy 2. Girl	Month     Year 88. Don't Know	<ol> <li>Yes → SKIP TO NEXT CHILD OR Q68</li> <li>No</li> </ol>	Days    Months    Years
03	1. Boy 2. Girl	Month     Year 88. Don't Know	<ol> <li>Yes → SKIP TO NEXT CHILD OR Q68</li> <li>No</li> </ol>	Days    Months    Years
04	1. Boy 2. Girl	Month     Year 88. Don't Know	<ol> <li>Yes → SKIP TO NEXT CHILD OR Q68</li> <li>No</li> </ol>	Days    Months    Years
05	1. Boy 2. Girl	Month     Year 88. Don't Know	<ol> <li>Yes → SKIP TO NEXT CHILD OR Q68</li> <li>No</li> </ol>	Days    Months    Years
06	1. Boy 2. Girl	Month     Year 88. Don't Know	<ol> <li>Yes → SKIP TO NEXT CHILD OR Q68</li> <li>No</li> </ol>	Days    Months    Years
07	1. Boy 2. Girl	Month      Year 88. Don't Know	<ol> <li>Yes → SKIP TO NEXT CHILD OR Q68</li> <li>No</li> </ol>	Days    Months    Years
08	1. Boy 2. Girl	Month     Year 88. Don't Know	<ol> <li>Yes → SKIP TO NEXT CHILD OR Q68</li> <li>No</li> </ol>	Days    Months    Years

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VIII. EXPOSURE AND PARTICIPATION			
Q68.a In the <u>last 24 months,</u> have you received:	A. [LOCAL NAME: MULTIP POWDER]? [SHOW CARD]	LE MICRONUTRIENT	1. Yes 2. No
	B. [LOCAL NAME: READY THERAPEUTIC FOOD L [SHOW CARD]	TO USE IKE PLUMPY'NUT]?	1. Yes 2. No
	C. [LOCAL NAME: READY SUPPLEMENTAL FOOD [SHOW CARD]	TO USE ) LIKE PLUMPY'DOZ]?	1. Yes 2. No
	D. Voucher or other form of subsidy to purchase water filter?		<ol> <li>Yes</li> <li>No → SKIP TO E</li> </ol>
	D2. Was it NOURISH or other program? [SHOW CARD] MARK ALL THAT APPLY E. Voucher or other form of subsidy to purchase latrine or materials for latrine? E2. Was it NOURISH or other program? [SHOW CARD] MARK ALL THAT APPLY		<ol> <li>NOURISH</li> <li>Other program</li> <li>88. Don't know</li> </ol>
			<ol> <li>Yes</li> <li>No → SKIP TO F</li> </ol>
			<ol> <li>NOURISH</li> <li>Other program</li> <li>88. Don't know</li> </ol>
	F. Voucher for food basket?	)	1. Yes 2. No → SKIP TO Q68b
	F2. Was it NOURISH or [SHOW CARD] MARK ALL THAT AF	other program? PPLY	<ol> <li>NOURISH</li> <li>Other program</li> <li>88. Don't know</li> </ol>
Q68b. In the <u>last 24 months</u> , have you enrolled in a conditional cash transfer program for health and nutrition?		1. Yes 2. No → SKIP TC	) Q69
Q68c. With which bank	or program?	1. AMK / NOURISH (S	HOW CCT CARD TO CONFIRM)
MARK ALL THAT APPLY		2. WING/ATHEALT	I CENTER
PROBE WHERE THEY ENROLLED OR		4. Other (specify:	)
RECEIVED CASH TRANSFER. IF VILLAGE FAIR SELECT NOURISH		BOILT KIOW	→ CONTINUE TO Q70
Q69. Do you have your own bank account, Wing account, or other money account?		1. Yes 2. No	
Q70. In the last 24 months, have you:			
A. Heard or seen Grow Together campaign messages, or materials       1. Yes       2. No         on TV, radio, or in print?       [SHOW LOGO]			1. Yes 2. No
B. Participated in a "first 1,000 days" community dialog		ogue?	1. Yes 2. No → SKIP TO C
B2. Was it NOURISH or other program? [SHOW CARD] MARK ALL THAT APPLY			<ol> <li>NOURISH</li> <li>Other program</li> <li>88. Don't know</li> </ol>

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C. Participated in a "first 1,000 days" caregiver group	education sessions? 1. Yes 2. No → SKIP TO D
C2. Was it NOURISH or other program? [SHOW CARD] MARK ALL THAT APPLY	<ol> <li>NOURISH</li> <li>Other program</li> <li>88. Don't know</li> </ol>
D. Participated in a "first 1,000 days" village fair?	1. Yes 2. No <b>→ SKIP TO E</b>
D2. Was it NOURISH or other program? [SHOW CARD] MARK ALL THAT APPLY	<ol> <li>NOURISH</li> <li>Other program</li> <li>88. Don't know</li> </ol>
E. Participated in a community activity where VSHG w	veighs young children? 1. Yes 2. No → SKIP TO Q70b
E2. Was it NOURISH or other program? MARK ALL THAT APPLY	<ol> <li>NOURISH</li> <li>Other program</li> <li>88. Don't know</li> </ol>
Q70b. In the <u>last 24 months</u> , have you received home health visits from a village health support group?	<ol> <li>Yes</li> <li>No → SKIP TO Q71</li> </ol>
Q70c. Was this visit(s) for you when you were pregnant, for your child's health, or for both?	<ol> <li>Caregiver during pregnancy</li> <li>Child's health</li> <li>Both</li> <li>88. Don't know</li> </ol>
Q71. In the <u>last 24 months</u> , have you received home garden support by a district agriculture officer?	1. Yes 2. No
Q71b. Does your household grow any food that you consume at home?	1. Yes 2. No → SKIP TO Q72
Q71c. What do you grow throughout the year? MARK ALL GROWN AT DIFFERENT TIMES OF THE YEAR	<ol> <li>Ptee (Amaranth)</li> <li>Moringa</li> <li>Pumpkin</li> <li>Sweet potato</li> <li>Long bean</li> <li>Eggplant</li> <li>Jackfruit</li> <li>Mango</li> <li>Banana</li> <li>Other</li> <li>Bon't Know</li> </ol>
Q72. In the <u>last 24 months</u> , have you participated in Community Led Total Sanitation activities (village meeting mapping open defecation and toilets)? [SHOW IMAGE OF CLTS ACTIVITY]	g on 2. No → SKIP TO Q80
Q73. Did other members of the community encourage yo construct a latrine after participating in the CLTS activities	u to 1. Yes s? 2. No

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Q74. Did you feel pressured to construct a latrine after participating in the CLTS activities?	1. Yes 2. No
Q75. Did you build a latrine as a result of this CLTS activity?	1. Yes → SKIP TO Q77 2. No
Q76. What is the main reason that you did not construct your own latrine? [RECORD TOP REASON ONLY]	<ol> <li>Lack of funds</li> <li>No construction knowledge</li> <li>Tough soil conditions</li> <li>Open defecation is preferred</li> <li>Lack of strength or illness</li> <li>No time</li> <li>Already had a latrine before</li> <li>Other (specify):</li></ol>
Q77. What is the main reason that you constructed a latrine? [RECORD TOP REASON ONLY]	<ol> <li>Privacy</li> <li>Security</li> <li>Health</li> <li>Hygiene</li> <li>Comfort</li> <li>Status</li> <li>Others in my community also built a latrine</li> <li>Someone told me to (specify:)</li> <li>Open defecation is harmful</li> <li>Other (specify:)</li> <li>Bon't know</li> </ol>
Q77b. Did you use a voucher to purchase the latrine or for materials to construct the latrine?	1. Yes 2. No 88. Don't know
Q78. Do you think you are more respected by your community because you have your own latrine?	1. Yes 2. No 88. Don't know
Q79. Who in your household or community inspired you the most to build your own latrine?	<ol> <li>Spouse</li> <li>Children</li> <li>Other family member</li> <li>Neighbor</li> <li>Chief/leader</li> <li>VHSG</li> <li>Other government officer</li> <li>Other community member</li> <li>NGO or other organization (specify:)</li> <li>Other (specify:)</li> </ol>

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IX. HOUSEHOLD WATER AND SANITATION					
Now I would like to ask you some questions about water and sanitation in your household.					
Q80. What is the main source of drinking water for members of your household right now? [MARK ONE ANSWER ONLY] [IF THE FAMILY FETCHES DRINKING WATER FROM MULTIPLE SOURCES, ASK WHAT IS THE SOURCE <u>MOST</u> USED NOW].	<ol> <li>Piped into dwelling</li> <li>Piped to yard or plot</li> <li>Public tap / standpipe</li> <li>Tube well or borehole</li> <li>Protected dug well</li> <li>Unprotected dug well</li> <li>Protected spring</li> <li>Unprotected spring</li> <li>Rainwater</li> <li>Tanker truck</li> <li>Cart with small tank</li> <li>Surface water (river, dam, lake, pond, canal, irrigation channel)</li> <li>Bottled water</li> <li>Other (Specify:)</li> </ol>				
Q81. Where is that drinking water source located?	<ol> <li>In own dwelling</li> <li>In own yard/plot</li> <li>→ SKIP TO Q83</li> <li>Elsewhere</li> </ol>				
Q82. How long does it take to go there, wait, get the drinking water, and come back?	Minutes 888. Don't know				
Q83. Do you do anything to the water to make it safer to drink?	<ol> <li>Yes</li> <li>No</li> <li>88. Don't know → SKIP TO Q86</li> </ol>				
Q84. What do you usually do to make the water Anything else? [RECORD ALL ANSWERS MENTIONED]	safer to drink? <i>[IF YES]</i> Q85. How often do you [METHOD] your water to drink?				
<ol> <li>Boil</li> <li>Add bleach or chlorine</li> <li>Strain through cloth</li> <li>Use water filter (ceramic sand/composite/</li> <li>Solar disinfection</li> <li>Let it stand and settle</li> <li>Buy purified water</li> <li>Other (Specify:)</li> </ol>	1. Always2. Sometimes3. Rarely1. Always2. Sometimes3. Rarely				

Q86. We would like to learn about the places that households use to wash their hands. Can you	1. 2.	Observed, fixed place Observed, mobile		
please show me where members of your household most often wash their hands?	3. 4. 5.	Not observed, not in dwelling/yard/plot Not observed, no permission to see Not observed, other reason	→	SKIP TO Q90

Q87. PRESENCE OF WATER AT THE PLACE FOR HANDWASHING.	<ol> <li>Yes, water is available</li> <li>No, water is not available</li> </ol>
RECORD OBSERVATION	
Q88. PRESENCE OF SOAP, DETERGENT, OR OTHER CLEANSING AGENT AT THE PLACE FOR HANDWASHING.	<ol> <li>Soap or detergent (bar, liquid, powder, paste)</li> <li>Ash, mud, sand</li> <li>None</li> </ol>
	4 Defense annoning an application food
[RECORD ALL ANSWERS MENTIONED]	<ol> <li>Before preparing or cooking food</li> <li>Before eating</li> <li>Before breastfeeding or feeding young child under 28 months</li> <li>After defecating</li> <li>After changing diapers / cleaning child feces</li> <li>After cleaning animal feces</li> <li>After cleaning toilet or potty</li> <li>None of the above</li> </ol>
Q90. What kind of toilet facility do members of your household usually use?	<ul> <li>Flush or Pour Flush Toilet</li> <li>1. Flush or pour flush to piped sewer system</li> <li>2. Flush or pour flush to septic tank</li> <li>3. Flush or pour flush to pit latrine</li> <li>4. Flush our pour flush to somewhere else</li> <li>Pit Latrine</li> <li>5. Ventilated improved pit latrine</li> <li>6. Pit latrine with slab</li> <li>7. Pit latrine without slab / open pit</li> <li>8. Composting toilet</li> <li>9. Bucket toilet</li> <li>10. Hanging toilet / hanging latrine</li> <li>11. No facility / bush / field → SKIP TO Q102</li> <li>12. Use another household's latrine → SKIP TO Q97</li> <li>13. Other (Specify:)</li> </ul>
ASK FOR PERMISSION TO TAKE A PHOTO OF THE TOILET FACILITY USED IN THE HOUSEHOLD.	Photo 1 Upload:
Q91.TAKE A PICTURE OF LATRINE SUPERSTRUCTRE FROM THE OUTSIDE, CAPTURING SEPTIC TANK IF PRESENT	1. Yes 2. No
Q92.TAKE A PICTURE OF THE LATRINE DROP HOLE	Photo 2 Upload: [PHOTO TAKEN] 1. Yes

	2. No				
Q93. OBSERVE WHETHER THERE IS A SUPER- STRUCTURE THAT PROVIDES PRIVACY	<ol> <li>Yes, there is a super-structure provides privacy</li> <li>No super-structure</li> </ol>				
[PRIVACY: DOOR OR CURTAIN THAT CAN BE CLOSED AND A NO-SEE THROUGH SUPERSTRUCTURE]					
RECORD OBSERVATION					
Q94. OBSERVE WHETHER THERE IS A CLEANABLE SLAB (PORCELAIN, PLASTIC, CONCRETE OR STONE)	<ol> <li>Yes, there is a cleanable slab → SKIP TO Q95</li> <li>No cleanable slab</li> </ol>				
RECORD OBSERVATION					
Q94b. OBSERVE WHETHER LATRINE IS SAFE FOR SMALL CHILDREN.	<ol> <li>Yes, latrine is safe for small children</li> <li>Not safe for small children</li> </ol>				
[NOT SAFE IF CHILD CAN FALL IN OR GET INJURED BECAUSE DROP HOLE IS BIG, OR PLATFORM IS UNSTABLE]					
Q95. Do you share your toilet facility with other households?	1. Yes 2. No 88. Don't Know				
Q96. How many other households use this toilet	Households 88. Don't Know				
	→ GO TO Q99				
[IF USES ANOTHER HOUSEHOLD'S LATRINE: Q90=12], ASK: Q97. What kind of toilet facility is it? Q98. How long does it take to get to the toilet	Flush or Pour Flush Toilet         1. Flush or pour flush to piped sewer system         2. Flush or pour flush to septic tank         3. Flush or pour flush to pit latrine         4. Flush our pour flush to somewhere else         Pit Latrine         5. Ventilated improved pit latrine         6. Pit latrine with slab         7. Pit latrine without slab / open pit         8. Composting toilet         9. Bucket toilet         10. Hanging toilet				
facility?					
Q99. Are small children able to use the toilet easily and without assistance from someone else?	1. Yes 2. No				

	88. Don't Know
Q100.a Does the pit or toilet leak, overflow or flood at any time of the year?	1. Yes     2. No     88. Don't Know     → SKIP TO Q101
Q100.b How often does the pit or toilet leak, overflow or flood?	<ol> <li>It happened once</li> <li>Rarely</li> <li>Often</li> <li>Always</li> <li>88. Don't know</li> </ol>
Q101. Thinking of yourself and your household members, excluding small children unable to use the toilet, has anyone defecated in the bush or field in the last 7 days?	<ol> <li>Yes</li> <li>No</li> <li>88. Don't Know → SKIP TO Q103</li> </ol>
Q102. Why didn't you or members of your household use the latrine? <i>IF RESPONDENT SAYS THEY DO NOT HAVE ONE, ASK WHY THEY DO NOT USE ANOTHER HOUSEHOLD'S LATRINE</i> <i>MARK ALL THAT APPLY</i>	<ol> <li>Smell</li> <li>Heat inside latrine</li> <li>Too dirty/no hygiene</li> <li>Fear of animals</li> <li>Taboo</li> <li>Flies</li> <li>Fear of falling inside</li> <li>Fear of infection</li> <li>Harassment</li> <li>Queue of people</li> <li>Too far away</li> <li>Latrine is damaged</li> <li>Full pit</li> <li>Open defecation is preferred</li> <li>Other (specify):</li></ol>
Q103. Do you think there are community members from this village who have defecated in the bush or field in the last 7 days?	1. Yes 2. No 88. Don't Know
Q104. The last time your youngest child passed stools in the past 24 hours, what was done to dispose of the stools?	<ol> <li>Child used toilet or latrine</li> <li>Put / rinsed into toilet or latrine</li> <li>Put / rinsed into drain or ditch</li> <li>Thrown into garbage</li> <li>Buried</li> <li>Left in the open</li> <li>Other (Specify:)</li> </ol>

Can you show me the space where the young children we measured usually play? Q105. OBSERVE WHETHER ANIMALS ARE KEPT AWAY FROM WHERE CHILD PLAYS RECORD OBSERVATION	<ol> <li>Animals are kept separate from play space</li> <li>Animals are not kept separate from play space</li> <li>No animals observed</li> </ol>
Q106. OBSERVE WHETHER THERE IS GARBAGE OR HOUSEHOLD WASTE WHERE CHILD PLAYS	<ol> <li>Yes, Garbage or household waste observed</li> <li>No garbage or household waste observed</li> </ol>
RECORD OBSERVATION	
Q107. OBSERVE WHETHER THERE ARE SHARP OBJECTS THAT CAN CAUSE HARM WHERE CHILD PLAYS	<ol> <li>Yes, Sharp objects that can cause harm are observed</li> <li>No sharp objects observed</li> </ol>
RECORD OBSERVATION	
Q108. OBSERVE WHETHER THERE IS FECES AROUND THE HOUSEHOLD (animal or humans- children)	<ol> <li>Yes, Animal or human/child feces observed</li> <li>No feces observed</li> </ol>
RECORD OBSERVATION	
Q109. Does your household have an ID Poor Card? [REQUEST TO SEE THE CARD]	<ol> <li>Yes, observed card</li> <li>Yes, not observed card</li> <li>Yes, expired card</li> <li>No</li> <li>Bon't know</li> </ol>

	X. HOUSEHOLD CHARACTERISTICS					
We are almost done. I will now a	sk you a few additiona	al questions about you	ır household.			
Q110. Does your household A. Electricity?			1. Yes	2. No		
have:	e: B. A radio?		1. Yes	2. No		
	C. A television?		1. Yes	2. No		
	D. A mobile teleph	one?	1. Yes	2. No		
	E. A non-mobile te	lephone?	1. Yes	2. No		
	F. A refrigerator?		1. Yes	2. No		
	G. A wardrobe?		1. Yes	2. No		
	H. A sewing mach	ine or loom?	1. Yes	2. No		
	I. A CD/DVD play	er?	1. Yes	2. No		
	J. A generator / ba	attery / solar panel?	1. Yes	2. No		
Q111. What type of fuel does your household mainly use for cooking?		<ol> <li>Electricity</li> <li>LPG</li> <li>Biogas</li> <li>Kerosene</li> <li>Coal, lignite</li> <li>Charcoal</li> <li>Charcoal</li> <li>Wood</li> <li>Straw / shrub</li> <li>Agricultural c</li> <li>Animal Dung</li> <li>No food cook</li> <li>Other (Special</li> </ol>	os / grass crop ked in household fy:	)		
Q112. MAIN MATERIALS OF THE FLOORS		<ol> <li>Earth / Sand</li> <li>Dung</li> <li>Wood planks</li> <li>Palm / bambo</li> <li>Parquet or po</li> <li>Vinyl or asph</li> <li>Ceramic tiles</li> <li>Cement</li> <li>Other (Specifier)</li> </ol>	/ Clay oo olished wood alt strips fy:	)		
Q113. MAIN MATERIAL OF THE I	ROOF	<ol> <li>No Roof</li> <li>Bamboo / tha</li> <li>Rustic mat</li> <li>Wood planks</li> <li>Cardboard</li> <li>Plastic sheet</li> <li>Metal</li> </ol>	atch / palm leaf			

	<ul> <li>8. Finished wood</li> <li>9. Calamine / cement fiber</li> <li>10. Ceramic tiles</li> <li>11. Clay tiles</li> <li>12. Cement</li> <li>13. Other (Specify:)</li> </ul>					
Q114. MAIN MATERIAL OF EXTE	RIOR WALLS	1. No walls				
RECORD OBSERVATION	<ol> <li>Palm / bam</li> <li>Dirt</li> <li>Bamboo wi</li> <li>Straw with</li> <li>Stone with</li> <li>Stone with</li> <li>Uncovered</li> <li>Plywood</li> <li>Cardboard</li> <li>Reused wo</li> <li>Reused wo</li> <li>Metal</li> <li>Cement</li> <li>Stone with</li> <li>Hericks</li> <li>Covered ac</li> <li>Wood plant</li> <li>Other (Spe</li> </ol>	iboo / thatch th mud mud adobe iod lime/cement ocks dobe ks / shingles cify:	)			
Q115. How many rooms in this house sleeping?	 88. Do	_  Rooms n't Know				
Q116. Does any member of this	A. Watch?	I	1. Yes	2. No		
household own:	B. Bicyle or cyclo	o?	1. Yes	2. No		
	C. Motorcycle or	motor scooter?	1. Yes	2. No		
	D. Motorcycle ca	rt?	1. Yes	2. No		
	E. Oxcart or hors	secart?	1. Yes	2. No		
	F. Car, truck, tra	actor, or van? 1. Yes 2. No				
	G. Boat with a m	otor?	1. Yes	2. No		
	H. Boat without a	a motor?	1. Yes	2. No		

XI. INSTRUCTIONS FOR STOOL SAMPLE COLLECTION					
That concludes our interview. Thank you for participating in this survey and for your time and attention. As I mentioned earlier, we would like to collect a stool sample from your children ages 1 to 28 months. This will help us to better understand the health of children in this area and to improve programming.					
Q117. For the children aged 1 to 28 months, may we collect a sample of their stool?					
1. YES 2. NO → <b>STOP INTERVIEW</b>					
I will leave this labelled bag with supplies for you to collect your child's stool and our team will return tomorrow to pick it up. When your child is going to defecate, please help your child to defecate into this plastic bowl. Then use the plastic spoon to collect the stool from the bowl and put into the plastic container. Then close the cap tightly and place the filled container in the bag and seal it.					
[ENUMERATOR DEMONSTRATES HOW TO USE THE PLASTIC CONTAINER AND PUT IT IN BAG AND SEAL IT]					
Q118. SCAN BARCODE OF PLASTIC CONTAINER [ENUMERATOR: LABEL THE LID WITH CHILD NUMBER AND EXPLAIN WHICH CONTAINER CORRESPONDS TO WHICH CHILD]					
CHILD 1:   _ _ _  CHILD 2:   _ _ _  CHILD 3:   _ _					
XII. CONTACT INFORMATION FOR STOOL SAMPLE PICK-UP					
Q119. At what time(s) will you likely be home tomorrow so our team can pick up the sealed bag with the stool sample? 1. FROM $\rightarrow$    :    AM / PM 2. TO $\rightarrow$    :    AM / PM					
May I please get your contact information for our team to contact you tomorrow?					
This information will not be shared with anyone outside our team and it will only be used for the purpose of picking-up the stool sample container.					
Q120. Respondent Name:					
Q121. Telephone Number:					
May I please get the contact information from a relative who lives nearby or a neighbor in case we cannot reach you?					
Q122. Name of Relative or Neighbor:					
Q123. Relationship to You:					
Q124. Telephone Number of Relative/Neighbor:					

That concludes our visit. Thank you once again for your time and participation.

### **Introduction**

This document outlines the standard operating procedure used to collect and store stool samples from children who are participating in the NOURISH study. Stool samples will be tested for enteric pathogens, including soil-transmitted helminthes. Care should be taken to prevent cross contamination between samples or contamination from outside sources. Aseptic technique should be followed while collecting and handling samples.

#### **Stool collection protocol**

- A. Materials and Supplies
  - 1. Large Whirl-Pak bags (2 liters)
  - 2. Disposable diapers in sizes suitable for children ages 1 28 months
  - 3. Gloves
  - 4. Permanent marker
  - 5. Plastic child potties
- B. Procedure: DAY ONE child identification and supply drop-off
  - 1. Identify children living in the household who meet study enrollment criteria. A stool sample will be collected from each enrolled child who is eligible.
  - 2. For each enrolled child, label a sterile, 2-liter Whirl-Pak bag with the household ID, child ID, and date.
  - 3. Tell the head of household you will leave the labeled Whirl-Pak bags with him/her overnight, and ask for stool samples from each child to be collected in the designated bag before you return the following day. Ask the caregiver to suggest good times to return.
  - 4. If a child is still wearing diapers, provide the head of household with three clean diapers per child and ask for one used diaper containing feces to be deposited into the designated Whirl-Pak bag once the child has made a bowel movement in it.
  - 5. If a child is no longer wearing diapers, inform the head of household that he/she may need to assist the child with using a potty. The bowel movement should be made in the plastic child potty provided to the head of household (provide 1 child potty per enrolled child if the caregiver reports that the child is no longer wearing diapers). The feces should then be transferred from the bin to the provided Whirl-pak bag.
  - 6. Explain the use of the Whirl-Pak bag to the head of household. Use an example bag to demonstrate how to open the bag and hold it from the tabs. Explain that the bag should remain sealed until the sample is to be deposited. Hand-washing with soap and water is recommended for both the child and caregiver following this process.
  - 7. Leave the instruction sheet with the head of the household. The instruction sheet will outline everything just explained to the head of household.
- C. Procedure: DAY TWO Sample pickup

- 1. Return to households visited the previous day and collect the Whirl-Pak bags containing stool samples.
- 2. If no stool was produced by and enrolled child, see the note below.
- 3. Ensure the sample bag is properly closed and the label is intact before placing it in the cooler with cold-packs.
- 4. Once the sample has been picked up, fill out two copies of the Stool Sample Collection Form. The sample ID # will combine the household ID, child ID and date. See the example below.
  - a. Household ID #: 1103
  - b. Child ID #: 02
  - c. Date: 26/10/2019
  - d. Corresponding Sample ID #: 1103,02,26102019
- 5. Repeat steps 1 5 for all households visited the previous day.
- 6. At the end of the day, transport the sample coolers to the storage facility.

Note: If a sample was not collected from an enrolled child, leave a new set of collection supplies (if necessary), re-label the original Whirl-Pak bag with the current date, and ask the head of household to repeat the stool collection. Return the following day and follow this SOP.

### **Stool Sample Collection Form**

Date	Compound ID	Household ID	Child ID	Stool Sample ID (compound ID#,HH ID#,child ID#,date)	Sample collected by We Consult (yes/no; name of enumerator)	Sample received by MISAU (name of technician, date)
e.g. 26/10/2014	2001	03	02	2001,03,02,26102014	Yes name	Yes name

### Stool Sample Collection Instructions (for household participant)

*Important Note: Only collect a stool sample from the child or children identified by the study team. Collecting a sample from a different child will affect study results.* 

If your child wears diapers:

- Put the clean diaper provided by the study team on your child.
- When your child makes a bowel movement, remove the diaper and place the entire diaper into the provided plastic bag. If more than one child in your household is enrolled in the study, make sure to place the diaper into the correct bag.
- Try not to touch the inside of the bag when depositing the diaper.
- Close the bag by folding the top over 3 times and folding in the yellow tabs.
- Store the bag out of reach of small children until the study team returns to pick it up the next day.

If your child does not wear diapers:

- Accompany your child to the latrine when he or she needs to make a bowel movement.
- Ask the child to make a bowel movement into the provided plastic bin instead of the latrine.
- Carefully transfer the feces from the bin into the provided plastic bag.
- Once the stool has been deposited into the bag, fold the top of the bag over 3 times and fold in the yellow tabs.
- Store the bag out of reach of small children until the study team returns to pick it up the next day.

Thank you for your participation!

#### Standard Operating Procedure: Stool Storage

#### Introduction

This document outlines the standard operating procedure used to receive and store stool samples from children who are participating in the NOURISH study. Care should be taken to prevent cross contamination between samples or contamination from outside sources. Aseptic technique should be followed while handling samples.

<u>All procedures at Georgia Tech and in Cambodia must comply with Brown Lab BMSC guidelines as</u> <u>approved by Georgia Tech EHS.</u>

#### **Stool Sample Storage Protocol**

- A. Materials and Supplies
  - 1. Sterile 2.5 or 5 mL cryovial tubes
  - 2. Sterile 15-mL screw-cap tubes
  - 3. Gloves
  - 4. Fine point permanent marker
  - 5. Sterile spatulas
- B. Procedure
  - 1. Upon receipt, determine if all of the samples listed on the stool sample collection sheet (transferred from field team) are present in the sample cooler. Once you have verified that a sample is present, fill in the corresponding line of the "Sample received" column on the sample collection form. If a sample listed on the form is missing, immediately contact the field team.
  - 2. Samples may be temporarily stored in sample bags at 4°C until processing (within 24 hours of receipt).
  - 3. Use a sterile spatula to add 1 g of stool to each of three cryovial tubes. Disposal, sterile, wooden spatulas or similar are recommended for transferring stool material to storage tubes. If the child produced a liquid stool, see step 5.
  - 4. Add 1 ml of UNEX lysis buffer to TWO of the three tubes. Vortex both tubes for 10 seconds with the mixture of UNEX and stool.
  - 5. If the child did not produce a solid stool, pieces of the diarrhea-soaked diaper material will be stored in cryovials for future analysis. Using a sterile scalpel, cut 1 cm x 1 cm area from the lining of the diaper and place into a cryovial using sterile forceps. Ensure the area you cut out for storage is covered in stool material. If there is enough diarrhea-soaked area remaining in the diaper, repeat this procedure up to two times so that a total of three cyrovials of diaper material are stored. In TWO of the three tubes, add 1 ml of UNEX lysis buffer and vortex for 10 seconds.
  - 6. If using reusable spatulas, scalpels, or forceps to handle samples, these tools need to be sterilized between samples. If available, use ethanol and heat sterilization (via Bunsen

burner or similar) to sterilize metal tools. Ensure tools have cooled before use. If heat sterilization is not possible, sterilize using a 10% bleach solution (contact time of at least 15 minutes) followed by a rinse with 70% ethanol to remove bleach residual. Let dry before use.

- 7. Once the storage tubes have been filled and capped, wipe the outside of each tube with ethanol and a paper towel. Dispose of the used paper towel in the hazardous waste bin.
- 8. Label the clean cryovial tubes with the corresponding sample ID #. Before labeling the tube, check that the sample ID was correctly written on Whirl-Pak bag and Stool Sample Collection form by the field team. The sample ID is a combination of the following information: household ID, child ID, and date (in that order). See the example below to understand how the sample ID is written.
  - a. Household ID #: 1103
  - b. Child ID #: 02
  - c. Date: 26/10/2019

Corresponding Sample ID #: 1103,02,26102019

- 9. Once the sample ID has been verified, use a permanent marker to label the storage tubes with the sample ID. If the sample ID does not match the household ID, child ID and date written on the sample bag, contact the trial manager.
- 10. Place the labeled tubes in a labeled microcentrifuge box for storage. Boxes should be labeled with the contents (e.g. NOURISH stool samples), date of storage, and unique box number.
- 11. Fill out the Stool Sample Storage Form. Record the location (including box and rack number) of the samples in the freezer in the appropriate column.
- 12. Repeat steps 3 11 for each sample. Change gloves if they become contaminated.
- 13. Store boxed samples at -20°C.
- 14. One aliquot of all samples will be shipped to Georgia Tech along with documentation of CDC permit and notes covering the shipment of lysed stool samples. A replicate aliquot will be preserved in Cambodia until analysis is complete.
- 15. Following molecular analysis at Georgia Tech, all samples will be autoclaved and discarded.

## Stool Sample Storage Form

Stool Sample Storage Information (MISAU)					
Stool Sample ID (HH ID#_child ID#_date)	Number of small tubes stored	Stool is liquid (yes, no)	Storage box and rack # and location	Name of lab technician, date stored	
1103,02,26102014	3		Box 1, drawer 3 freezer A	Name, 09/09/2019	

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