



# BMJ Open Optimisation, validation and field applicability of a $^{13}\text{C}$ -sucrose breath test to assess intestinal function in environmental enteropathy among children in resource poor settings: study protocol for a prospective study in Bangladesh, India, Kenya, Jamaica, Peru and Zambia

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## ABSTRACT

**Introduction** Environmental enteropathy (EE) is suspected to be a cause of growth faltering in children with sustained exposure to enteric pathogens, typically in resource-limited settings. A major hindrance to EE research is the lack of sensitive, non-invasive biomarkers. Current biomarkers measure intestinal permeability and inflammation, but not the functional capacity of the gut. Australian researchers have demonstrated proof of concept for an EE breath test based on using naturally  $^{13}\text{C}$ -enriched sucrose, derived from maize, to assay intestinal sucrase activity, a digestive enzyme that is impaired in villus blunting. Here, we describe a coordinated research project to optimise, validate and evaluate the usability of a breath test protocol based on highly enriched  $^{13}\text{C}$ -sucrose to quantify physiological dysfunction in EE in relevant target populations.

**Methods and analysis** We use the  $^{13}\text{C}$ -sucrose breath test ( $^{13}\text{C}$ -SBT) to evaluate intestinal sucrase activity in two phases. First, an optimisation and validation phase will (1) confirm that a  $^{13}\text{C}$ -SBT using highly enriched sucrose tracers reports similar information to the naturally enriched  $^{13}\text{C}$ -SBT; (2) examine the dose–response relationship of the test to an intestinal sucrase inhibitor; (3) validate the  $^{13}\text{C}$ -SBT in paediatric coeliac disease (4) validate the highly enriched  $^{13}\text{C}$ -SBT against EE defined by biopsy in adults and (5) validate the  $^{13}\text{C}$ -SBT against EE defined by the urinary lactulose:rahanose ratio (LR) among children in Peru. Second, a cross-sectional study will be conducted in six resource-limited countries (Bangladesh, India, Jamaica, Kenya, Peru and Zambia) to test the usability of the optimised  $^{13}\text{C}$ -SBT to assess EE among 600 children aged 12–15 months old.

**Ethics and dissemination** Ethical approval will be obtained from each participating study site. By working as a consortium, the test, if shown to be informative of

## Strengths and limitations of this study

- A validated non-invasive  $^{13}\text{C}$ -sucrose breath test ( $^{13}\text{C}$ -SBT) would overcome a major current limitation of environmental enteropathy (EE) research by providing an assay that explicitly measures the function of the intestinal epithelium to digest and absorb nutrients.
- The test is supported by early proof-of-concept data from a study that used a naturally enriched  $^{13}\text{C}$ -SBT to characterise intestinal function in children with possible EE. However, this test is limited by low signal to noise in the breath  $^{13}\text{CO}_2$  signal.
- This coordinated research project design includes sequential validation and feasibility studies in both adult and paediatric populations.
- The large network permits validation in multiple geographic sites, in Southeast Asia (India, Bangladesh), Africa (Zambia, Kenya), Latin America (Peru) and the Caribbean (Jamaica).
- If the  $^{13}\text{C}$ -SBT provides a functional readout related to EE, it would facilitate the validation and use of test substrates using a similar approach for the assessment of EE, with broad field applicability.

EE, will demonstrate strong evidence for utility across diverse, low-income and middle-income country paediatric populations.

**Trial registration number** NCT04109352; Pre-results.

## INTRODUCTION

Retarded linear growth, resulting in stunting (length-for-age or height-for-age below 2 Z-scores of WHO growth standards), affects

23% of children under 5 years, most of whom live in low-income and middle-income countries.<sup>1</sup> Stunting is associated with increased child morbidity and mortality,<sup>2</sup> poorer cognitive development,<sup>3</sup> school performance<sup>4-6</sup> and lower adult wages,<sup>7</sup> and, for girls, poorer maternal health outcomes.<sup>8</sup>

The aetiology of stunting is multifactorial, including generational and prenatal factors.<sup>9 10</sup> Postnatal exposures also play a major role. Most postnatal growth faltering occurs around 6–18 months of age,<sup>11</sup> an age when the energy and nutrient requirements for rapid growth are high. For children living in communities without access to improved water and sanitation, this period is also associated with intense exposure to enteric pathogens, while protection from breast feeding and maternal antibodies begin to wane. Although driven by underlying environmental, social and familial factors, inadequate diet and exposure to pathogens are the major, proximal causes of infant growth faltering.<sup>10</sup> However, nutritional interventions lead to only small to moderate improvements in growth,<sup>12</sup> and two recent, large studies to reduce enteric infection found no impact of water and sanitation interventions on child growth, or evidence of synergy between water, sanitation and hygiene (WASH) interventions and nutritional interventions.<sup>13-15</sup> These findings reinforce the need to better understand the biological basis of stunting.

Since the 1960s, it has been noted that adults and children living without access to improved water and sanitation have altered small intestinal morphology,<sup>16</sup> including diffuse, upper small bowel villous atrophy accompanied by evidence of barrier disruption and inflammation.<sup>17 18</sup> This condition is linked to chronic bacterial translocation leading to systemic inflammation, and T-cell-mediated hyperstimulation of the mucosal immune system, negatively impacting nutrient absorption and utilisation.<sup>19 20</sup> This increases the risk of nutrient deficiency, which may in turn further impair gut immune responses.<sup>10</sup> It is theorised that this condition, successively termed, ‘tropical enteropathy’, ‘environmental enteropathy (EE)’ and most recently, ‘environmental enteric dysfunction’,<sup>21</sup> is a major contributing cause of the failure of both nutritional, and water and sanitation-oriented interventions, to significantly improve child growth.

Despite the potential significance of EE to child nutrition and health, there is a lack of simple diagnostic techniques to identify or classify the condition.<sup>22</sup> A primary challenge has been that morphological confirmation of EE requires intestinal biopsy. In the absence of this, EE is studied using non-invasive biomarkers of gut function. The most common approaches to determining EE use the dual-sugar lactulose: mannitol or LR ratio,<sup>23</sup> or composite scores based on multiple biomarkers intended to capture multiple domains of gut dysfunction.<sup>24 25</sup> However, dual-sugar tests, although non-invasive, are time-consuming to administer, vary greatly in protocol details, and concerns have been raised about interplatform consistency.<sup>26</sup> The relationship of alternative biomarkers to EE, and to each

other, is also not well understood.<sup>27</sup> Candidate biomarkers to define EE have frequently been ‘borrowed’ from the field of paediatric gastroenterology, and specifically, from the study of coeliac disease, which produces an enteropathy regarded as having histopathological and immune similarities to EE.<sup>28</sup> However, in many cases, there is a lack of descriptive data bridging biomarker performance between children with severe paediatric gastrointestinal disease, well-child controls in high-income countries and children from high-risk EE settings. This diminishes the interpretability of these tests. For example, faecal biomarkers of intestinal inflammation, myeloperoxidase and alpha-1-antitrypsin, have been proposed for EE, but reference values for healthy, well-growing children remain limited.<sup>25</sup> Despite these limitations, the shift in nomenclature from ‘EE’ to ‘environmental enteric dysfunction’, first proposed by Keusch *et al*<sup>21</sup> and now adopted by a significant proportion of the research community, emerged from the viewpoint that, given the infeasibility of biopsy-confirmed ‘enteropathy’, diagnosis should be based instead on ‘functional’ biomarkers, as well as on functional consequences for child growth and development.

Most existing EE biomarkers reflect processes of intestinal or systemic inflammation, such as bacterial translocation and intestinal repair, and do not directly characterise functional deficits in the gut, such as deficits in macronutrient or micronutrient absorption. The lactulose: mannitol ratio is regarded as indirect marker of dysfunction, but this is based on reported associations with the D-xylose test, a measure of carbohydrate absorption that is challenging to administer and has been reported only infrequently in the EE literature.<sup>29 30</sup> Zinc metabolism can be measured though carefully performed, dual stable isotope studies.<sup>19</sup> Few other such tests are currently available.

Stable isotope-based tests have the potential to overcome this limitation by assessing host function across multiple domains of EE. Labelled substrate tracers can be designed to target specific domains of intestinal epithelial activity.<sup>31</sup> Substrate tracers labelled with <sup>13</sup>C are particularly promising for this purpose, as <sup>13</sup>CO<sub>2</sub> expired in the breath can be non-invasively sampled to obtain a quantitative measure of substrate hydrolysis and absorption.

Intestinal sucrase-isomaltase (SI) activity has been identified as a potentially useful domain to target in a functional test of EE. SI is a small intestinal brush border enzyme that catalyses the hydrolysis of carbohydrates including starch, isomaltase and sucrose.<sup>32</sup> It has a gradient expression along the small intestine, with highest expression in the duodenum and jejunum, and shows higher expression in the villi compared with the crypts. SI levels are reduced with mucosal injury but are relatively stable by race and age. Outside of a few higher-risk populations (eg, indigenous Greenlanders,<sup>33</sup> inherited deficiencies are uncommon.<sup>34 35</sup> SI activity is diminished in villus blunting,<sup>36</sup> thus representing a possible surrogate marker of small intestinal function and integrity.<sup>37</sup> Inflammatory

pathologies of the gut, namely autoimmune disorders, inflammatory bowel disease and intestinal inflammation caused by HIV and giardiasis, have also been shown to cause SI deficiency.<sup>38</sup> A recent transcriptomic study also described SI activity as potentially altered by EE.<sup>39</sup>

Breath tests that characterise intestinal SI activity through the ingestion of <sup>13</sup>C labelled sucrose have been employed in animal models and in childhood cancer chemotherapy as a biomarker of enteropathy.<sup>37 40 41</sup> A <sup>13</sup>C-labelled sucrose breath test (<sup>13</sup>C-SBT) based on naturally <sup>13</sup>C enriched sucrose from maize, was also used by Ritchie *et al* to assess aboriginal Australian children from enteropathy settings.<sup>34</sup> Threefold differences were observed between aboriginal and higher-socioeconomic status (SES) Australians children, and twofold differences among children with and without diarrhoea. However, this test was limited by the necessity for a large dose to produce an exhaled breath <sup>13</sup>CO<sub>2</sub> signal above basal <sup>13</sup>C abundance, making it unsuitable for routine use in very young children. Furthermore, between-population differences in the consumption of naturally <sup>13</sup>C-enriched C<sub>4</sub> crops, such as maize and sugar cane, reduce the utility of this test as a diagnostic for EE or to compare the prevalence of EE between populations.

Inspired by this naturally enriched test, we recently developed a <sup>13</sup>C-SBT based on highly enriched sucrose tracers. This test overcomes limitations of the previous test by dramatically reducing the quantity of substrate

required, as well elevating exhaled <sup>13</sup>CO<sub>2</sub> substantially above the baseline value that might be expected due to diet, thereby improving the signal to noise ratio. To assess whether enriched <sup>13</sup>C-SBT performance is altered among individuals with EE, we will conduct six integrated, complementary studies in two phases.

## METHODS AND ANALYSIS

Our coordinated study design is summarised in [table 1](#).

### Phase 1

In the first phase of the study, our overall objective is to optimise and validate a protocol for a non-invasive stable isotope test based on an enriched sucrose substrate (<sup>13</sup>C-SBT), among adults and children. To accomplish this, we will complete five coordinated studies. We will first optimise the <sup>13</sup>C-SBT protocol and second, validate the <sup>13</sup>C-SBT in successive adult and paediatric populations. We will then establish analytical validity (technical test performance), clinical validity (the test's ability to accurately and reliably identify a disorder of interest) and field usability (assessment of the test in the actual context where it would be used).<sup>42 43</sup>

The objective of the first study is to establish that the highly enriched sucrose tracers for an <sup>13</sup>C-SBT report similar information in comparison to the original naturally enriched <sup>13</sup>C-SBT. We will conduct a cross-over study

**Table 1** Features of the coordinated research projects that make up the study protocol

	Study goal	Study design	Primary study outcome	Population	Site
Phase 1					
Study 1	Optimisation	Cross-over	n/a	Healthy adults (N=20)	Glasgow, UK
Study 2	Validation	Cross-over	Intestinal sucrose inhibition (acarbose dose-response)	Healthy adults (N=20)	
Study 3	Validation	Case-control	Coeliac disease	Children with coeliac (N=20) Children with non-coeliac GI disorders (N=20) Healthy child controls (N=20)	Adelaide, Australia
Study 4	Validation	Case-control	Villous atrophy Intestinal sucrose activity	Adults from an EE setting (N=20) Healthy adult controls (n=20)	Lusaka, Zambia
Study 5	Validation	Cross-sectional	Urinary lactulose: rhamnose ratio Plasma kynurenine: tryptophan ratio	Infants from an EE setting (N=30)	Iquitos, Peru
Phase 2					
Study 6	Field utility and validation	Cross-sectional	Urinary lactulose: rhamnose ratio Length-for-age Z-score	Infants from EE settings (N=540) Infants from higher-SES settings (N=60)	Dhaka, Bangladesh Bangalore, India Kingston, Jamaica Kakamega, Kenya Iquitos, Peru Ndola, Zambia

EE, environmental enteropathy; n/a, not available; SES, socioeconomic status.

of 20 adults using three commercially available sucrose tracers ( $^{13}\text{C}_6$  fructose;  $^{13}\text{C}_6$  glucose and  $^{13}\text{C}_{12}$  sucrose). We will also determine whether the addition of unlabelled carrier sucrose is necessary to replicate the original 'flooding dose' approach reported by Ritchie *et al.*<sup>34</sup> To assess gut permeability, participants will also receive coadministration of 5 g lactulose, 1 g rhamnose, 0.5 g xylose, 0.2 g 3-O-methyl-D-glucose and 5 g sucralose dissolved in water.

The objective of the second study is to characterise the dose response of the  $^{13}\text{C}$ -SBT in response to three different doses of the intestinal sucrose inhibitor acarbose. A randomised cross-over trial of 20 adults will be conducted. Breath  $^{13}\text{CO}_2$  will be collected serially for 4–6 hours and  $^{13}\text{CO}_2$  recovery compared across treatments. Both studies 1 and 2 will be conducted in Glasgow, UK (University of Glasgow).

While the ideal 'gold standard' would be to breath test and biopsy children who are identified as having EE, from a logistical perspective, this study design is infeasible in the resource constrained environments where EE is prevalent. Therefore, the objective of the third study is to examine whether the  $^{13}\text{C}$ -SBT, optimised according to the protocol established in study 1, varies between children with clinically diagnosed coeliac disease presenting with gastrointestinal symptoms to outpatient clinics (n=20) versus healthy coeliac controls (n=20) and healthy non-coeliac controls (n=20) (Adelaide, Australia, Flinders University). In this study, active disease is defined as positive serology for specific IgA antibodies in patients on a gluten-containing diet, or in patients undertaking a gluten challenge, followed by endoscopic evaluation and histological examination of duodenal biopsy for characteristic features of coeliac disease (villus atrophy, crypt hyperplasia and mucosal inflammation), with a Marsh III classification considered coeliac positive. The degree of villous atrophy will be correlated to the patients  $^{13}\text{C}$ -SBT result. Additionally, tissue biopsies, collected from inflamed and normal sections of small bowel, will be assayed *ex vivo* for SI activity and correlated with the  $^{13}\text{C}$ -SBT. The non-coeliac control group includes children presenting for endoscopy for non-coeliac disease, including abdominal pain and reflux disease, and do not present with any small intestinal pathology.

The objective of the fourth study is to validate the  $^{13}\text{C}$ -SBT against intestinal sucrose activity and villus atrophy among adults with and without EE. Both villus atrophy and intestinal sucrose activity are measured using biopsy. A case-control study design (n=20 cases from a high-risk enteropathy setting and n=20 controls) will be used. (Lusaka, Zambia, University of Zambia)

The objective of the fifth study is to correlate the  $^{13}\text{C}$ -SBT with an established, non-invasive biomarkers of EE (urinary LR ratio) and one secondary biomarker of EE (plasma kyrenine:tryptophan ratio) among children under 2 years of age from a high-risk enteropathy setting (n=30). (Iquitos, Peru, Asociación Benefica Proyectos de Informática, Salud, Medicina, y Agricultura (A.B.

PRISMA) and the University of Virginia). A substudy will remeasure the  $^{13}\text{C}$ -SBT at 7 days to document test reproducibility.

## Phase 2

In the second phase, we will conduct a multisite study in six resource-limited countries to determine the field usability of the optimised and validated  $^{13}\text{C}$ -SBT in diagnosing EE in children aged 12–15 months. The specific primary objectives are to assess the relationship between the  $^{13}\text{C}$ -SBT and the LR ratio among children 12–15 months of age, and to assess the relationship between the  $^{13}\text{C}$ -SBT and child stunting. Our secondary objectives are to assess the relationship between the  $^{13}\text{C}$ -SBT and secondary biomarkers of EE. We will also conduct exploratory analyses to characterise the relationship between the  $^{13}\text{C}$ -SBT and child sex, SES, dietary diversity and household food security.

## Study sites

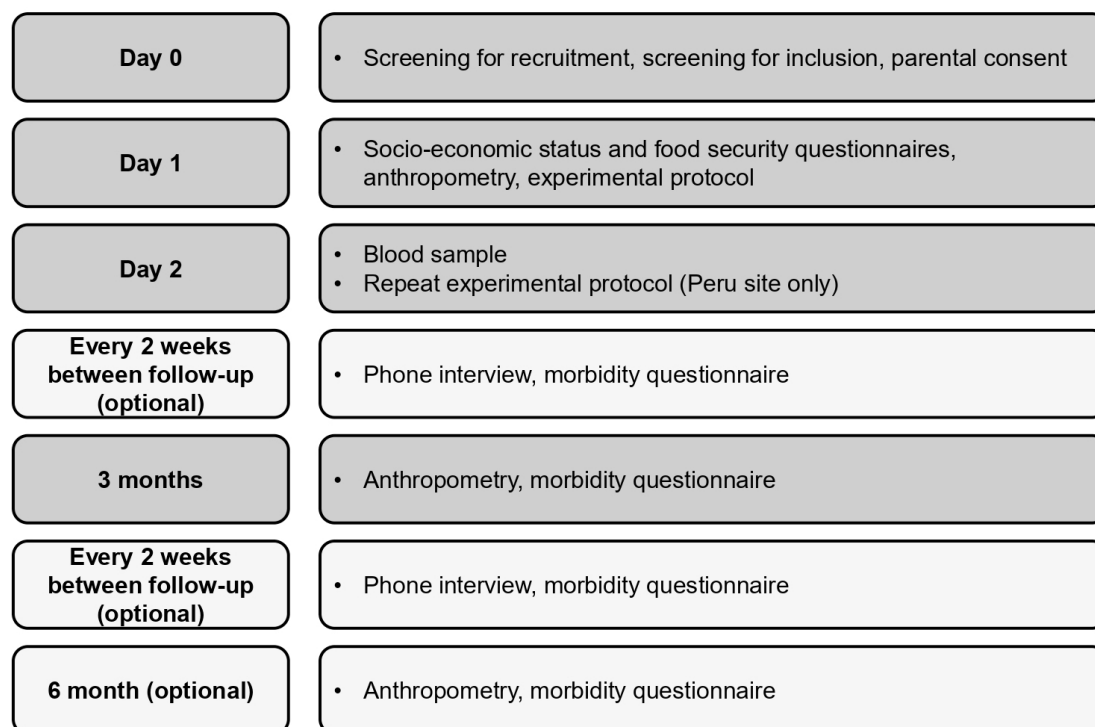
This six study sites are: Dhaka, Bangladesh (the International Centre for Diarrhoeal Disease Research); Bangalore, India (St. John's Research Institute, St. John's National Academy of Health Sciences); Kingston, Jamaica (The Tropical Metabolism Research Unit of the Caribbean Institute for Health Research, University of West Indies); Kakamega, Kenya (Masinde Muliro University of Science and Technology); Iquitos, Peru<sup>44</sup> (Asociación Benefica PRISMA and the University of Virginia) and Ndola, Zambia (Tropical Disease Research Centre). These sites represent a range of epidemiological contexts which enhances the cross-context applicability of study results.

## Coordinated study design

Each site will enrol 100 infants between 12 and 15 months of age. This range was selected because it is within the window of infant growth faltering that implies clinical or public health relevance but is also old enough to reduce the influence of breastfeeding on LR performance and to permit a several hour fast during initial assessment of the test.<sup>45 46</sup> At each site, 90 children will be recruited from areas deemed high risks for EE, due to a lack of improved water and sanitation infrastructure or because the known prevalence of stunting is relatively elevated. Ten relatively high SES infants from a nearby community will also be enrolled. All children will be recruited and enrolled through convenience sampling, either at the community level (if the study site has previously censused the community) or through child clinic visits. Exclusion criteria include the presence of Severe Acute Malnutrition (weight for height z-score  $\leq -3$  SD), HIV positive status, any chronic illness medical or surgical contributing to growth retardation, or weight-for-height z-score more than +2SD.

Study procedures for each participating child are outlined in figure 1 and described in detail in online supplemental appendix 1. In brief, the  $^{13}\text{C}$ -SBT will be





**Figure 1** Flow diagram for phase 2 coordinated study protocol shown here is the timeline of participant activities. Darker grey boxes represent core study activities, while light grey boxes indicate activities that some, but not all, study sites will undertake. Primary and secondary study aims are based on core activities.

assessed in each child at one time point, as well as a 2-hour urinary LR test, an assessment of weight, length and body composition using the deuterium dilution technique (either saliva or urine), and a fasting plasma sample for the assessment of additional EE biomarkers. After 3 months, the height and weight measurement will be repeated. Each site will use the same harmonised study protocols for all data collection. Information about household SES, household food security (using the household food insecurity access scale), and child dietary diversity will also be asked of each caretaker using standardised instruments designed and validated for use across low-income and-middle income countries.<sup>47–49</sup> Information about child morbidity and the consumption of C<sub>4</sub> foods will also be collected using standardised questions. In certain sites with high rates of cell phone coverage, ancillary morbidity data will also be collected by phone every 2 weeks, although this will not be used in the primary or secondary analysis. Key study data are summarised in online supplemental appendix 2, and study forms are provided in online supplemental appendix 3.

All data will be digitised by site via double-data entry and will be managed in accordance with institutional norms and local ethical committee approvals. Each site will confidentially maintain personal information about enrolled participants as necessary for study administration. Deidentified data will be shared with the University of Michigan where centralised data consistency checks will be performed, and inconsistencies will be communicated back to each site for resolution. After this process is complete, pooled analyses will be performed.

### Outcomes and case definitions

<sup>13</sup>C breath tests can be summarised in several ways.<sup>50</sup> Based on expert opinion, cumulative per cent of dose recovered at 90 min post administration (cPDR90), and time to 50% recovery (T50), will be taken as our primary measures of the <sup>13</sup>C-SBT. Other metrics for summarising the <sup>13</sup>C-SBT test, at both specific time points and overall, will also be considered.

**LR ratio:** To assess the relationship between the <sup>13</sup>C-SBT and EE, our case definition for EE, and primary outcome measure, will be based on the LR ratio, which is the most widely accepted, non-invasive test of EE. Because LR ratios vary by analytical platform<sup>26</sup> and may also be influenced by test administration procedures, cut-offs for ‘EE’ will be defined based on the empirical distribution of the data. To establish cut-offs for LR, the distribution of this variable among high-SES children (pooled across sites) will be examined (eg, LR ratios above the 90th percentile for upper SES children will be regarded as ‘elevated’). If insufficient numbers of higher-SES children are recruited, or if a subset of higher-SES children cannot be recruited across all sites, cut-off values for LR will be determined based on internal study percentiles (eg, below vs above the median) or on cut-offs from the literature.<sup>51</sup> If the outcomes of the phase 1 studies support evidence for a LR cut-off based on the extent of villous atrophy, this will also be considered.

**Anthropometry:** We will use WHO growth standards to define length for age Z-score (LAZ), weight for age Z-score (WAZ) and weight for length Z-score. Stunting,

underweight and wasting will be defined based on WHO growth standards ( $\leq -2$  Z-scores length for age, weight for age and weight for length).<sup>52</sup>

Our primary outcome measures are

1. Comparison of the  $^{13}\text{C}$ -SBT to the LR ratio, to the percent lactulose recovery, and to the percent rhamnose recovery.
2. Characterise the relationship between the  $^{13}\text{C}$ -SBT and child anthropometry (LAZ and WAZ).
3. Characterise the relationship between  $^{13}\text{C}$ -SBT and childhood linear growth (change in LAZ) over 3 months and over 6 months.

Secondary outcome measures

1. Assess the relationship between the  $^{13}\text{C}$ -SBT and faecal myeloperoxidase concentration.
2. Assess the relationship between the  $^{13}\text{C}$ -SBT and serum fatty acid binding protein concentration.
3. Assess the relationship between the  $^{13}\text{C}$ -SBT and the kynurenine tryptophan ratio.
4. Assess the relationship between the  $^{13}\text{C}$ -SBT and faecal alpha-antitrypsin concentration.

Other prespecified outcome measures are

1. Reproducibility of the  $^{13}\text{C}$ -SBT.

We will assess the coefficient of variation and correlation coefficient between repeated cumulative percent of dose recovered at 90 min postadministration on separate SBT tests administered 1 week apart done on the same child (Peru site only).

2. Assess the relationship between epidemiological factors and the  $^{13}\text{C}$ -SBT.

We will determine if significant associations exist between  $^{13}\text{C}$ -SBT measured as cPDR90/T50 and the Water Assets Maternal Education and Income (WAMI index). The WAMI index is a previously validated composite index of environmental variables to create an index that expresses the socioeconomic and physical environment in diverse geographical contexts.<sup>47</sup>

### Sample size

The sample size for the phase 2 study was predetermined (N=10 upper SES and 90 lower SES children per site), power calculations were conducted. Calculations were based on previously reported Pearson's correlations of 0.67 (95% CI 0.42 to 0.82) between the naturally enriched  $^{13}\text{C}$ -SBT and LR.<sup>34</sup> The current test, using highly enriched sucrose, is expected to be more sensitive than the original test, so sample size estimates are regarded as conservative. Assuming the Pearson's correlation between the enriched  $^{13}\text{C}$ -SBT and LR is similar, 90 children per site is sufficient to estimate the correlation between the two tests with an SD of 0.58 (95% CI 0.55 to 0.79).<sup>53</sup> The minimal detectable correlation within each site, would be 0.31.<sup>54</sup>

Between sites, we estimate statistical power to detect meaningful differences between children with and without EE, based on a cut-off of the LR ratio. The proportion of children who will be classified as having EE relative to this these cut-offs is unknown, so the estimated detectable difference was calculated across a range

of values (10%–50%). We estimate that differences in the  $^{13}\text{C}$ -SBT on the order of 0.60 standard deviations (50% prevalence of EE) to 0.99 SD (10% prevalence of EE) will be detectable with 80% power. For comparison, Ritchie observed differences in the  $^{13}\text{C}$ -SBT cumulative percentage of dose recovered at 90 min between healthy Aboriginal and non-Aboriginal children on the order of  $\sim 0.84$  SD, and differences between Aboriginal children with and without acute diarrhoea on the order of  $\sim 0.92$  SD.

Power calculations were also performed to assess the relationship between the  $^{13}\text{C}$ -SBT and child stunting based on the known prevalence of stunting in each site (table 2).

### Data analysis plan

All analyses will be stratified by site and then pooled. Analysis stratified by site will be limited to bivariable comparisons, and pooled data will be used to construct multivariable models, using either fixed or random effects to account for site.

We will examine the relationship between the  $^{13}\text{C}$ -SBT and LR both continuously, and as dichotomous variables. Continuous analyses will include calculation of both site-specific and pooled correlation coefficients (to provide direct comparison to Ritchie<sup>34</sup> and regression models will be developed where the dependent variables will be log-transformed LR, and the independent variable will be cPDR90 and T50. For dichotomous analyses, Receiver Operating Characteristic (ROC) curves will be used to calculate the sensitivity and specificity of be cPDR90 and T50 cut-offs to predict relatively elevated LR test results. We will also examine the association between the  $^{13}\text{C}$ -SBT and lactulose and rhamnose excretion individually.

To characterise the relationship between the  $^{13}\text{C}$ -SBT test and child anthropometry, we will compare be cPDR90 and T50 values for the  $^{13}\text{C}$ -SBT and concurrently measured LAZ and WAZ. We will also consider fat mass and fat-free mass. Nutritional status will be analysed both continuously and will dichotomised (ie, into stunted and non-stunted). T-tests will be used to compare be cPDR90 and T50 between the stunted and non-stunted groups within sites, and simple and multivariable linear regression models including random effects for country membership, where the dependent variables will be LAZ, WAZ and the independent variable will be  $^{13}\text{C}$ -SBT. In addition, we will consider adjustment for factors such as the age, sex, breastfeeding status and recent illness history of the child.

Our first secondary objective is to assess the relationship between the  $^{13}\text{C}$ -SBT and secondary biomarkers of EE. This activity will be contingent on the availability of these biomarker results from a sufficient number of infants across the study sites. Following the prior approaches,<sup>27 55</sup> the relationship between EE biomarkers will be explored and EE scores will be generated via principal components analysis, partial least squares regression, or other variable reduction techniques, and comparisons between

**Table 2** Power calculations are based on the primary comparison of stunted to non-stunted children

Country	Estimated prevalence of stunting, %	Sample size	Detectable difference in <sup>13</sup> C-SBT between stunted and non-stunted (per SD)
Bangladesh	36	90	0.62 SDs
India	27	90	0.67 SDs
Jamaica	20	90	0.74 SDs
Kenya	26	90	0.67 SDs
Peru	38	30	0.61 SDs
Zambia	40	90	0.61 SDs
Pooled- no design effect	30.4	480	0.28 SDs
Pooled – design effect=0.9	30.4	432*	0.30 SDs
Pooled- design effect=0.7	30.4	336*	0.34 SDs
Pooled- design effect=0.5	30.4	240*	0.40 SDs

**Table 2** shown here are estimated detectable differences in the <sup>13</sup>C-SBT between stunted and non-stunted children, based on the estimated prevalence of stunting in specifically proposed study communities (estimates of stunting prevalence provided by study community). The percentage of variability in the <sup>13</sup>C-SBT based on site is unknown, so a range of design effects (1.0–0.5) are provided.

\*Asterisks refer to the overall sample size adjusted for the design effect.

<sup>13</sup>C-SBT, <sup>13</sup>C-sucrose breath test.

be cPDR90 and T50 and these scores will be examined similarly to LR.

Following previous approaches, we will examine the association between the <sup>13</sup>C-SBT and subsequent change in WAZ, and LAZ,<sup>25 56 57</sup> which enhances the comparability of our results to those of other studies. We will again consider adjustment for factors that may influence child growth trajectory such as the age, gender, breastfeeding status, and recent illness history of the children in pooled models only.

Finally, we will conduct exploratory analyses. To assess the reproducibility of the <sup>13</sup>C-SBT we will examine the coefficient of variation and correlation coefficient between repeated tests from the same child (Peru site only). We will also examine the relationship between the <sup>13</sup>C-SBT and child sex, SES, dietary diversity and household food security. Regression models will be developed where the dependent variable will be the result of the <sup>13</sup>C-SBT test (transformed if necessary) and the independent variables will include factors that may be associated with the infant gut function, including breastfeeding, dietary diversity, age, sex, food security, history of recent illness and SES scores.<sup>47</sup> If between-site differences in <sup>13</sup>C-SBT are observed, the models will include site-level random intercepts.

### Patient and public involvement

No patient involvement.

### Ethics and dissemination

Each study protocol has been approved (Zambia, Australia, Peru, Bangladesh, India, UK, Zambia, Kenya) or is pending approval (Jamaica) by the institutional review board or boards relevant to that study site. Written

informed consent will be obtained from the participant themselves, and/or the legal guardian of each participant, by members of each local study team. In both the phase 1 and the coordinated phase 2 studies, each study site will use a unique consent form, reflective of both of core study activities and any site-specific activities also being performed.

We will publish and disseminate our results once the project is complete. By conducting the field usability phase of our study across six countries, our test, if shown to be informative of EE, will demonstrate strong evidence for utility across diverse, low-income and middle-income country paediatric populations.

### DISCUSSION

We propose to optimise, validate and assess the field usability of a <sup>13</sup>C-SBT to evaluate EE. The research team brings together experts in stable isotopes, biochemistry, gastroenterology, human nutrition and epidemiology, as well as field teams with extensive expertise conducting human research in resource-limited settings. This breadth of expertise is necessary to overcome previous limitations of EE research. Proposed EE biomarkers have often been carried over from studies of severe gastrointestinal disorders, such as coeliac disease, into enteropathy settings, with unclear biological interpretation and without validation against the 'gold-standard' definition of EE. In other instances, analytical variability has hindered cross-study or cross-context comparisons of test performance.<sup>26</sup> Our staged validation approach and multisite design are intended to overcome these limitations and may serve as a template for future EE biomarker studies.



The protocol is relatively intensive, with the phase 2 study requiring 4-hour breath collections and 2-hour urine collections from 600 infants. This decision to emphasise the comprehensive testing of a relatively smaller number of children was deliberate. If the study results support the utility of the  $^{13}\text{C}$ -SBT, future work would aim to shorten and streamline our protocol for future clinical and epidemiological research.

At present, there is a true deficiency in the number of non-invasive tests to measure intestinal function. The critical evaluation of this test will add to collective knowledge if findings affirm, refute or partially affirm the utility of the test. This study will ascertain whether a stable isotope breath test can report information on the functional activity of an important small intestinal enzyme, or not. This will serve as an important paradigm on the potential utility of other functional breath tests which could be used to characterise EE. Looking ahead,  $^{13}\text{C}$  breath tests have potential utility for better understanding multiple other enzymes and factors of nutrient uptake in addition to SI activity. The digestion of proteins and uptake of one or more amino acids and/or peptides through the development of probes for peptidases would be a logical next step, as would the integration of zinc assays to probe multiple nutrient uptake capacities concurrently. The overarching vision of the coordinated research project is the eventual development of a breath test that, like the urea breath test for *Helicobacter pylori*, can be deployed to rapidly identify children with potential EE. Although the  $^{13}\text{C}$ -SBT we describe here is non-invasive and feasible in low-resource settings, the analysis of these samples currently cannot be performed at the point of care. However, with advancing breath analysis technology, these tests are becoming less expensive and more field-deployable, opening these approaches to a wider user base and especially to researchers and clinicians in low-income and middle-income countries.

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## Appendix 1: Unified Phase 2 Study Protocol

**Phase 2 study protocol:** The  $^{13}\text{C}$ -SBT, LR test, and  $\text{D}_2\text{O}$  dilution to assess body composition will be completed during a single visit with each participant. The study team will screen for recent diarrhea, antibiotic usage, and anti-inflammatory usage in the past month (anti-inflammatories are known to induce transient intestinal permeability<sup>1</sup> as well as enterocyte injury). If any of these are reported by the parents, the appointment will be scheduled one month from the date of the diarrheal episode or antibiotic/anti-inflammatory use.

Guardians will be asked to fast their child for one hour prior to the appointment. Time will be given for the child to settle and adjust to their surroundings, including time for the child to play with, and become familiar with, the breath sampling equipment. Two baseline breath collection measurements will be completed, using a cannula apparatus for breath collection, consisting of a piece of PVC tubing taped just underneath the child's nostril and controlled with a 3-way tap, or, if the field team prefers it, a face mask and breath collection bag. Exhaled breath will then be transferred to Labco Exetainers (evacuated tubes Labco Order No 428W or 439W, Burlington, North Carolina, USA) either by positive displacement (syringe) or using evacuated exetainer (bag). A baseline urine sample will be collected, and, for sites using deuterium dilution technique with analysis of saliva samples to assess body composition<sup>2</sup> (Bangladesh, India, Kenya, and Zambia), a baseline saliva sample will be collected.

To avoid  $^{13}\text{C}_{12}$ -sucrose loss, the  $^{13}\text{C}_{12}$ -sucrose solution and the LR solution will be administered separately. First, the 40 mg/mL  $^{13}\text{C}_{12}$ -sucrose solution will be administered at a dosage of 10  $\mu\text{L}$  per kg body weight (spiked in 1 mL of drinking water). For example, for a child weighing 10 kg, the dosage will be 100  $\mu\text{L}$  spiked in 1 mL of drinking water. This will be followed by a 2 mL chaser of drinking water to rinse the vial.

Secondly, a 9 mL of drinking water spiked with 1 g lactulose and 0.2 g rhamnose will be administered, and again a 2 mL chaser of drinking water will be used to rinse the vial. The total volume (1 mL sucrose solution + 2 mL chaser + 9 mL LR solution + 2 mL chaser) is 14 mL.

If the child spits out, vomits, or fails to swallow all the sugar solution, the test will be canceled and re-scheduled. After the child has consumed the sugars, a standard dose of deuterium oxide ( $\text{D}_2\text{O}$ ) based on IAEA protocols will also be administered to the child. As soon as both the sugar solutions and the  $\text{D}_2\text{O}$  have been administered the child will be encouraged to drink water. After 90 minutes, the child will be given a standardized meal. The choice of standardized meal will be site-specific but may include egg, legumes, or rice and will not include any sugary foods or dairy products.

**Breath sample collection:** Using a stopwatch, breath samples will be collected every 15 minutes for the first 90 minutes following the administration of the sugar solution, and then every 30 minutes until 240 minutes (4 hours) have passed.

**Urine sample collection:** All urine passed in the 30-120 minutes of the test will be collected and combined for analysis, and any urine passed in the 120-300 minutes of the test will be collected and combined separately. The volume of all voids will be recorded. Both 2- and 5-hour LR tests are common in the literature<sup>3-5</sup>, with some evidence that 2-hour urine collections better capture small intestinal, rather than colonic, absorption<sup>5</sup>. Here, both measures will be collected to enhance comparability with prior reports. 5-hour recoveries of lactulose and rhamnose will be calculated as the weighted average of the 30-120 and 120-300-minute samples. Samples will either be



stored with chlorhexidine or will be collected without preservative and stored immediately at -80. We will examine whether chlorhexidine results in interference during LC-MS/MS analysis.

**Saliva sample collection:** For sites measuring D<sub>2</sub>O via saliva, the sample will be collected between 180 and 210 minutes of the test.

**Plasma sample collection:** After the breath/urine collection is complete, a second visit will be scheduled with study families to collect a plasma sample. Families will be asked to fast the child for 6 hours prior to the blood draw, and 2mL of blood will be collected in K2 EDTA. Following collection, the sample will be centrifuged and stored at -80C pending analysis.

**Anthropometry and questionnaire data:** The length, weight, and head circumference of each child will be measured, as will the height and weight of their mothers. Standard questionnaires to capture key socio-economic<sup>6</sup> and demographic information, child dietary diversity (including recent consumption of C4 foods (e.g. maize, sugar cane, and sorghum or millet), and household food insecurity<sup>7</sup>, will be administered. Three months following the initial test, a third appointment will be made with the family to re-measure anthropometry, and to administer a questionnaire asking about morbidity over the past three months. Key data are summarized in **Appendix 2**.

**Additional site activities:** In addition to coordinated study activities, some activities will only be undertaken by one, or a sub-group, of study sites. In one site (Peru), the <sup>13</sup>C-SBT test will be repeated after 2 days on 40 children, to assess test reproducibility. Several secondary EE biomarker assays of interest were also identified, to be assayed by sites resource permitting. These include, in order of priority, plasma fatty acid binding protein (FABP); plasma LPS binding protein (LPS-BP); and stool alpha-1-antitrypsin (AAT) and stool myeloperoxidase (MPO).

**Laboratory analyses:** Breath samples from four sites (Peru, Jamaica, Kenya, and Zambia) will be sent to Dr. Roger Yazbek at the South Australian Breath Analysis Research Laboratories (SABAR Lab) where they will be analyzed via ABCA Isotope Ratio Mass Spectrometry. To minimize of inter-platform variability in dual-sugar testing<sup>8</sup>, urine samples for lactulose and rhamnose will be performed either on a single platform, on two or more standardized platforms (to be determined). Breath sample analysis for Bangladesh and India will be analyzed at Saint John's Research Institute, Bangalore.

**Deuterium dilution testing:** Based on available instrumentation, one site (India) will assess body composition analysis through D<sub>2</sub>O analysis of urine, and four sites (Peru, Jamaica, Bangladesh, Kenya, and Zambia) will use saliva. Deuterium equilibrates faster in saliva compared to urine<sup>9</sup> and deuterium dilution analysis in saliva requires a higher dose and different instrumentation (Fourier Infrared Spectroscopy versus Isotope Ratio Mass Spectrometer) compared to urine sampling. However, the collection of urine or saliva for total body water analysis has been standardized and validated, with comparable performance demonstrated in infants younger than those we propose to measure in this study<sup>10</sup>. Deuterium enrichment of urine will be assessed using a Delta V advantage Isotope Ratio Mass Spectrometer (Thermo Fisher scientific Inc)<sup>11</sup>. Saliva samples will be measured for their deuterium enrichment in duplicate by Fourier Transformed Infrared Spectrophotometry (4500t FTIR, Agilent Technologies, CA, USA).

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- 11 International Atomic Energy Agency. IAEA human health series, No.3. Assessment of Body Composition and Total Energy Expenditure in Humans Using Stable Isotope Techniques. Vienna, 2009.

## Appendix 2: Key Study Data

Key variable	Detail
Child age	Months
Child sex	Male; female
<b>Anthropometry:</b>	
Birthweight	kg
Baseline weight	kg
Baseline length	cm
FM	Fat mass, measured by deuterium dilution
FFM	Fat free mass, measured by deuterium dilution
Weight at 3-month follow-up	kg
Length at 3-month follow up	cm
Maternal weight	kg
Maternal height	cm
<b>Questionnaire data:</b>	
Household food security	Using the household food insecurity access scale (HFIAS) <sup>48</sup>
Household socio-economic status	Using the water and sanitation, eight selected assets, maternal education, and household income (WAMI) score <sup>47</sup>
Child dietary diversity score	Adapted to capture consumption of C4 foods
Recent child morbidity	Diarrhea in past month
<b>EE Biomarkers:</b>	
LR	Urinary lactulose: rhamnose ratio
FABP	Plasma fatty-acid binding protein
KT	Plasma kynurenine: tryptophan ratio
MPO	Fecal myeloperoxidase
AAT	Fecal alpha-1-antitrypsin



Child ID Code:

S	B	T	-																
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Urine and Breath Collection Form <b>Version 1.3</b>			
	Question	Code	Response
01	Study researcher/Nurse/Fieldworker ID		<input type="text"/> <input type="text"/>
02	Today's date (DD/MM/YY)		<input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/> / <input type="text"/> <input type="text"/>
03	Time of arrival to Study Clinic	Time (24 h Scale; HH:MM)	<input type="text"/> <input type="text"/> : <input type="text"/> <input type="text"/>
<b>Screening questions</b>			
04	Diarrhea in past month	Yes = 01 No = 00	<input type="text"/> <input type="text"/>
05	Antibiotics in past month	Yes = 01 No = 00	<input type="text"/> <input type="text"/>
06	Anti-inflammatories in past month (ibuprofen, naproxen, aspirin, methenozol) (paracetamol is OK)	Yes = 01 No = 00	<input type="text"/> <input type="text"/>
If any of these questions (03, 04, 05) was yes, please re-schedule the test one month from the date of the diarrhea or antibiotic/NSAID use			
07	Standard meal given (rice: legume mix, egg) or breastmilk	Tick	Idly /Kichadi/ Hard-boiled egg
08	Time of completion of standard meal	Time (24 h Scale; HH:MM)	<input type="text"/> <input type="text"/> : <input type="text"/> <input type="text"/>

BASELINE BREATH COLLECTION		
Breath sample tube number	Time of completion	Comments (note crying, difficult sample collection, etc.)
Baseline	<input type="text"/> <input type="text"/> : <input type="text"/> <input type="text"/>	

BASELINE URINE COLLECTION (LR and D <sub>2</sub> O)			
Urinary collection Time*	Collection Volume (mL)	Time of Collection	Comments (please note any spillage)
Baseline		<input type="text"/> <input type="text"/> : <input type="text"/> <input type="text"/>	

\*Use baseline urine for LR and Body composition (D<sub>2</sub>O)

DO NOT ADMINISTER SUGAR SOLUTION UNTIL THE CHILD HAS FULLY VOIDED THEIR BLADDER AND THE TIME IS 1 HOUR FROM THE LAST MEAL		
Vial label of <sup>13</sup> C <sub>12</sub> -sucrose solution		
Time the <sup>13</sup> C <sub>12</sub> -sucrose was first consumed:		<input type="text"/> <input type="text"/> : <input type="text"/> <input type="text"/>
Total volume (mL) of sucrose solution consumed		<input type="text"/> <input type="text"/>
Total volume (mL) of chaser consumed		<input type="text"/> <input type="text"/>
Time the LR solution was consumed:		Start time: __ : __      End time: __ : __
Total volume (mL) of LR solution consumed		<input type="text"/> <input type="text"/>
Total volume (mL) of chaser consumed		<input type="text"/> <input type="text"/>

Child ID Code:

S	B	T	-												
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How many minutes did it take the child to consume the LR solution in full?		01-20 minutes	<input type="text"/>			
Was the LR solution consumed in full? <i>Weight of pre-weighed tissue =</i> <i>Weight of soaked tissue =</i>		Yes = 01 No = 00	<input type="text"/>			
<i>If the child spits out, vomits, or fails to swallow all the sugar solution, the test cannot be completed. Please stop and re-schedule the test for another day.</i>						
Total volume (mL) of deuterium water consumed			<input type="text"/>			
Total volume (mL) of chaser consumed			<input type="text"/>			
Was the deuterium dose consumed in full? <i>Weight of pre-weighed tissue =</i> <i>Weight of soaked tissue =</i>		Yes = 01 No = 00	<input type="text"/>			
<i>A pre-weighed napkin should be placed under the neck to collect any spill from the dose. This should be weighed immediately after or placed in a small airtight container to avoid evaporation if immediate weighing is not possible.</i>						
<i>Start collection: Encourage the child to drink water throughout the test.</i>						
<b>Breath sample tube time</b>	<b>Exact collection time</b> <i>(use a stopwatch in minutes)</i>	<b>Comments</b> (child was crying Y/N)	<b>Urinary collection Time</b>	<b>Collection Volume (mL)</b>	<b>Exact collection time</b> <i>(using a stopwatch in minutes)</i>	<b>Comments</b> (please note any spillage)
15 minutes			<i>Do not collect urine for the first 30 minutes after the child consumes the sugar solution</i>			
30 minutes						
45 minutes			30 to 90 minutes			
60 minutes						
75 minutes						
90 minutes						
<i>At this time, please give the child the standardized meal</i>						
Standard meal given (rice: legume mix, egg) or breastmilk			Tick	Idly /Kichadi/ Hard-boiled egg/ milk without sugar/ milk + beet sugar		
Time of consumption of standard meal			Time (24 h Scale; HH:MM)	<input type="text"/> : <input type="text"/>		
120 minutes			90 to 120 minutes			
150 minutes			120 to 300 minutes			
180 minutes						
Urine						
210 minutes						
240 minutes						
Total:						

Child ID Code:

S	B	T	-												
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09	How many loose stools were passed during the breath/urine collection?	Range (00-15, NA)	<input type="text"/> <input type="text"/>
10	Did any breastfeeding occur in the first 90 minutes of the test? (if the mother needed to comfort the child)	Yes = 01 No = 00	<input type="text"/> <input type="text"/>
11	Did the child consume any non-breastmilk liquids or foods during the first 90 minutes of the test? (excluding water, which should be encouraged throughout the test)	Yes = 01 No = 00	<input type="text"/> <input type="text"/>
12	Weight of sipper with water at start of experiment (g)		<input type="text"/> <input type="text"/> <input type="text"/>
13	Weight of sipper with water at end of experiment (g)		<input type="text"/> <input type="text"/> <input type="text"/>
Anthropometry (take measurement in triplicates)			
1	Height (cm)	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>
2	Weight (kg)	<input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/> <input type="text"/> <input type="text"/>
3	Head circumference (cm)	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>
4	MUAC (cm)	<input type="text"/> <input type="text"/> . <input type="text"/>	<input type="text"/> <input type="text"/> . <input type="text"/>



Child ID Code:

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Breath Sample Reception in Laboratory <b>Version 1.0</b>																									
	Question	Code	Response																						
01	Study researcher/ Nurse/ Fieldworker ID		<table border="1"><tr><td></td><td></td><td></td></tr></table>																						
02	Today's date (DD/MMM/YY)		<table border="1"><tr><td></td><td></td></tr></table> / <table border="1"><tr><td></td><td></td></tr></table> / <table border="1"><tr><td></td><td></td></tr></table>																						
03	Sample ID:																								
04	Time of arrival of sample at laboratory	Time (24 Hr Scale; HH:MM)	<table border="1"><tr><td></td><td></td></tr></table> : <table border="1"><tr><td></td><td></td></tr></table>																						
05	Please confirm that each sample is complete:	<table border="0"> <tr> <td><input type="checkbox"/> 15-minute sample A</td> <td><input type="checkbox"/> 15-minute sample B</td> </tr> <tr> <td><input type="checkbox"/> 30-minute sample A</td> <td><input type="checkbox"/> 30-minute sample B</td> </tr> <tr> <td><input type="checkbox"/> 45-minute sample A</td> <td><input type="checkbox"/> 45-minute sample B</td> </tr> <tr> <td><input type="checkbox"/> 60-minute sample A</td> <td><input type="checkbox"/> 60-minute sample B</td> </tr> <tr> <td><input type="checkbox"/> 75-minute sample A</td> <td><input type="checkbox"/> 75-minute sample B</td> </tr> <tr> <td><input type="checkbox"/> 90-minute sample A</td> <td><input type="checkbox"/> 90-minute sample B</td> </tr> <tr> <td><input type="checkbox"/> 120-minute sample A</td> <td><input type="checkbox"/> 120-minute sample B</td> </tr> <tr> <td><input type="checkbox"/> 150-minute sample A</td> <td><input type="checkbox"/> 150-minute sample B</td> </tr> <tr> <td><input type="checkbox"/> 180-minute sample A</td> <td><input type="checkbox"/> 180-minute sample B</td> </tr> <tr> <td><input type="checkbox"/> 210-minute sample A</td> <td><input type="checkbox"/> 210-minute sample B</td> </tr> <tr> <td><input type="checkbox"/> 240-minute sample A</td> <td><input type="checkbox"/> 240-minute sample B</td> </tr> </table>		<input type="checkbox"/> 15-minute sample A	<input type="checkbox"/> 15-minute sample B	<input type="checkbox"/> 30-minute sample A	<input type="checkbox"/> 30-minute sample B	<input type="checkbox"/> 45-minute sample A	<input type="checkbox"/> 45-minute sample B	<input type="checkbox"/> 60-minute sample A	<input type="checkbox"/> 60-minute sample B	<input type="checkbox"/> 75-minute sample A	<input type="checkbox"/> 75-minute sample B	<input type="checkbox"/> 90-minute sample A	<input type="checkbox"/> 90-minute sample B	<input type="checkbox"/> 120-minute sample A	<input type="checkbox"/> 120-minute sample B	<input type="checkbox"/> 150-minute sample A	<input type="checkbox"/> 150-minute sample B	<input type="checkbox"/> 180-minute sample A	<input type="checkbox"/> 180-minute sample B	<input type="checkbox"/> 210-minute sample A	<input type="checkbox"/> 210-minute sample B	<input type="checkbox"/> 240-minute sample A	<input type="checkbox"/> 240-minute sample B
<input type="checkbox"/> 15-minute sample A	<input type="checkbox"/> 15-minute sample B																								
<input type="checkbox"/> 30-minute sample A	<input type="checkbox"/> 30-minute sample B																								
<input type="checkbox"/> 45-minute sample A	<input type="checkbox"/> 45-minute sample B																								
<input type="checkbox"/> 60-minute sample A	<input type="checkbox"/> 60-minute sample B																								
<input type="checkbox"/> 75-minute sample A	<input type="checkbox"/> 75-minute sample B																								
<input type="checkbox"/> 90-minute sample A	<input type="checkbox"/> 90-minute sample B																								
<input type="checkbox"/> 120-minute sample A	<input type="checkbox"/> 120-minute sample B																								
<input type="checkbox"/> 150-minute sample A	<input type="checkbox"/> 150-minute sample B																								
<input type="checkbox"/> 180-minute sample A	<input type="checkbox"/> 180-minute sample B																								
<input type="checkbox"/> 210-minute sample A	<input type="checkbox"/> 210-minute sample B																								
<input type="checkbox"/> 240-minute sample A	<input type="checkbox"/> 240-minute sample B																								
06	Notes:																								

*Breath samples should be labeled as follows:* Study code + site code + participant code + timepoint + replicate identifier (A or B) e.g. SBT-ZAM1-P1-T60-A

Child ID Code:

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Urine Reception in Laboratory <b>Version 1.0</b>									
	Question	Code	Response						
01	Study researcher/ Nurse/ Fieldworker ID		<table border="1"><tr><td></td><td></td><td></td></tr></table>						
02	Today's date (DD/MMM/YY)		<table border="1"><tr><td></td><td></td></tr></table> / <table border="1"><tr><td></td><td></td></tr></table> / <table border="1"><tr><td></td><td></td></tr></table>						
03	Sample ID:								
04	Time of arrival of sample at laboratory	Time (24 Hr Scale; HH:MM)	<table border="1"><tr><td></td><td></td></tr></table> : <table border="1"><tr><td></td><td></td></tr></table>						
05	Number of aliquots of urine collected from 30 - 90 minutes (should be 4 aliquots of 250 µL)	01-04	<table border="1"><tr><td></td><td></td></tr></table>						
06	Number of aliquots of urine collected from 90 minutes to 120 minutes (should be 2 aliquots of 250 µL)	01-02	<table border="1"><tr><td></td><td></td></tr></table>						
07	Number of aliquots of urine collected from 120 minutes to 300 minutes (should be 2 aliquots of 250 µL) <i>If applicable</i>	01-02	<table border="1"><tr><td></td><td></td></tr></table>						
08	Notes:								

*Urine samples should be labeled as follows:* Study code + site code + participant code + timepoint (in minutes, from start time) + replicate identifier (A, B, C, D)

e.g. 30-90-minute urine: SBT-ZAM1-P001-T30-A

90-120-minute urine: SBT-ZAM1-P001-T90-A

120-300-minute urine: SBT-ZAM1-P001-T120-A

Child ID Code:

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Blood Sample Collection <b>Version 1.0</b> (6 hours postprandial)									
	Question	Code	Response						
01	Study researcher/ Nurse/ Fieldworker ID		<table border="1"><tr><td></td><td></td><td></td></tr></table>						
02	Today's date (DD/MMM/YY)		<table border="1"><tr><td></td><td></td></tr></table> / <table border="1"><tr><td></td><td></td></tr></table> / <table border="1"><tr><td></td><td></td></tr></table>						
03	Before starting the test, when was the last time the child ate (either breastmilk or solid foods) (post prandial for how many hours)	Time (24 Hr Scale; HH:MM)	<table border="1"><tr><td></td><td></td></tr></table> : <table border="1"><tr><td></td><td></td></tr></table>						
If the child has not fasted overnight, please reschedule the blood draw:									
04	Time of blood collection:	Time (24 Hr Scale; HH:MM)	<table border="1"><tr><td></td><td></td></tr></table> : <table border="1"><tr><td></td><td></td></tr></table>						
05	Tube with <b>up to 2mL</b> blood collected?	Yes=01 No=00	<table border="1"><tr><td></td><td></td></tr></table>						
06	Notes:								

*Blood samples should be labeled as follows:* Study code + site code + participant code + timepoint + replicate identifier (A or B) e.g. SBT-ZAM1-P1-T60-A

Child ID Code:

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Blood Sample Reception <b>Version 1.0</b>											
	Question	Code	Response								
01	Study researcher/ Nurse/ Fieldworker ID		<table border="1"><tr><td></td><td></td><td></td></tr></table>								
02	Date of blood collection (DD/MMM/YY)		<table border="1"><tr><td></td><td></td><td>/</td><td></td><td></td><td>/</td><td></td><td></td></tr></table>			/			/		
		/			/						
03	Date of blood arrival in laboratory		<table border="1"><tr><td></td><td></td><td>/</td><td></td><td></td><td>/</td><td></td><td></td></tr></table>			/			/		
		/			/						
04	Time of arrival	Time (24 Hr Scale; HH:MM)	<table border="1"><tr><td></td><td></td><td>:</td><td></td><td></td></tr></table>			:					
		:									
05	Tube 1 with <b>up to 2mL</b> blood received?	Yes=01 No=00	<table border="1"><tr><td></td><td></td></tr></table>								
06	Evidence of hemolysis?	No=00 Slight=01 Severe=02	<table border="1"><tr><td></td><td></td></tr></table>								
07	Sample ID:										
08	Date of Centrifugation:		<table border="1"><tr><td></td><td></td><td>/</td><td></td><td></td><td>/</td><td></td><td></td></tr></table>			/			/		
		/			/						
09	Time of Centrifugation:	Time (24 Hr Scale; HH:MM)	<table border="1"><tr><td></td><td></td><td>:</td><td></td><td></td></tr></table>			:					
		:									
10	Number of 500µL Plasma Aliquots:	00-03	<table border="1"><tr><td></td><td></td></tr></table>								
06	Notes:										

*Blood samples should be labeled as follows:* Study code + site code + participant code + timepoint + replicate identifier (A or B) e.g. SBT-ZAM1-P1-T60-A



Child ID Code:

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Enrollment Form <b>Version 1.1</b>											
	Question	Code	Response								
01	Study researcher/Nurse/Fieldworker ID		<table border="1"><tr><td></td><td></td><td></td></tr></table>								
02	Today's date (DD/MMM/YY)		<table border="1"><tr><td></td><td></td><td>/</td><td></td><td></td><td>/</td><td></td><td></td></tr></table>			/			/		
		/			/						
<b>A. Child information and anthropometry</b>											
01	Date of birth (DD/MMM/YY)		<table border="1"><tr><td></td><td></td><td>/</td><td></td><td></td><td>/</td><td></td><td></td></tr></table>			/			/		
		/			/						
02	Sex of child	Male=01 Female=02	<table border="1"><tr><td></td><td></td></tr></table>								
03	Birthweight (kg) * (from birth record, if available)		<table border="1"><tr><td></td><td></td><td>.</td><td></td><td></td></tr></table>			.					
		.									
04	Current weight (kg) *		<table border="1"><tr><td></td><td></td><td>.</td><td></td><td></td></tr></table>			.					
		.									
05	Current length (cm)		<table border="1"><tr><td></td><td></td><td>.</td><td></td></tr></table>			.					
		.									
06	Current head circumference (cm)		<table border="1"><tr><td></td><td></td><td>.</td><td></td></tr></table>			.					
		.									
<b>B. Maternal anthropometry</b>											
01	Mother's date of birth		<table border="1"><tr><td></td><td></td><td>/</td><td></td><td></td><td>/</td><td></td><td></td></tr></table>			/			/		
		/			/						
02	Is the mother currently pregnant?	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>								
03	Mother's Weight (kg)		<table border="1"><tr><td></td><td></td><td></td><td>.</td><td></td><td></td></tr></table>				.				
			.								
04	Mother's Height (cm)		<table border="1"><tr><td></td><td></td><td></td><td>.</td><td></td></tr></table>				.				
			.								
<b>C. Socio-economic Information (WAMI)</b>											
<i>Please explain to the mother that these questions are standardized questions used around the world, so some questions may be more applicable to them than others.</i>											
01	Was this child chosen for participation in the study because they come from a low-SES community, or a high SES community?	Low SES= 01 High SES = 02	<table border="1"><tr><td></td><td></td></tr></table>								
02	What is the main source of drinking water for members of your household?	Piped into dwelling = 01 Piped to yard/plot = 02 Public tap/stand pipe= 03 Tube well or borehole = 04 Protected well = 05 Unprotected well = 06 Surface water (river/ dam/ lake/pond/ stream/canal/irrigation canal) = 07 Other = 08	<table border="1"><tr><td></td><td></td></tr></table>								
02a	If other, describe:										
03	What is the main source of water used by your household for other purposes such as cooking and hand-washing?	Piped into dwelling = 01 Piped to yard/plot = 02 Public tap/stand pipe= 03 Tube well or borehole = 04 Protected well = 05 Unprotected well = 06 Surface water (river/ dam/ lake/pond/ stream/canal/irrigation canal) = 07	<table border="1"><tr><td></td><td></td></tr></table>								

Child ID Code:

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03a	If other, describe:	Other = 08									
04	What kind of toilet facility do members of your household usually use?	No facility/bush/field or bucket toilet = 01 Pit latrine without flush = 02 Flush to piped sewer system = 03 Flush to septic tank = 04 Flush to pit latrine = 05 Flush to somewhere else = 06 Other = 07	<table border="1"><tr><td></td><td></td></tr></table>								
04a	If other, describe:										
05	Do you have a separate room which is used as a kitchen?	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>								
06	Does any member of your household have a bank account?	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>								
07	Does your household have a mattress?	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>								
08	Does your household have a refrigerator?	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>								
09	Does your household have a television?	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>								
10	Does your household have a table?	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>								
11	Does your household have a chair or bench?	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>								
12	How many rooms are there in your house?	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>								
13	How many people usually sleep in this household?	01-30 (people)	<table border="1"><tr><td></td><td></td></tr></table>								
14	Have you (the mother of the study child) ever attended school? <i>If no, skip to question 18.</i>	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>								
15	How many years of schooling have you completed?	00-20	<table border="1"><tr><td></td><td></td></tr></table>								
16	<i>If younger than 25 years old:</i> Are you currently attending school or college?	Yes = 01; No = 00	<table border="1"><tr><td></td><td></td></tr></table>								
17	What is the average monthly income for the entire household?		<table border="1"><tr><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr></table>								
18	Currency	I = Indian rupees; J = Jamaica dollars; K = Zambian kwacha; P = Peruvian soles; S = Kenyan Shilling; T = taka	<table border="1"><tr><td></td></tr></table>								

**D. Child's dietary diversity**

01	Are you breastfeeding <CHILD>? If NO, then skip to Q.6	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
01a	Last night, how many times did you breastfeed <CHILD> from sunset to sunrise?	00-99	<table border="1"><tr><td></td><td></td></tr></table>		
01b	Yesterday, during the day, how many times did you breastfeed <CHILD>?	00-99	<table border="1"><tr><td></td><td></td></tr></table>		
02	Do you give <CHILD> infant formula? If NO, then skip to Q.9	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		

Child ID Code:

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02a	Last night, how many times did you feed <CHILD> formula from sunset to sunrise?	00-99	<table border="1"><tr><td></td><td></td></tr></table>		
02b	Yesterday, during the day, how many times did you feed <CHILD> formula?	00-99	<table border="1"><tr><td></td><td></td></tr></table>		
03	Do you give <CHILD> other milks, such as tinned, powdered or fresh animal milk? If NO, then skip to Q.12	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
03a	Last night, how many times did you feed <CHILD> animal milks from sunset to sunrise?	00-99	<table border="1"><tr><td></td><td></td></tr></table>		
03b	Yesterday, during the day, how many times did you feed <CHILD> animal milk?	00-99	<table border="1"><tr><td></td><td></td></tr></table>		
<i>Yesterday, during the day or last night, did &lt;CHILD&gt; have:</i>					
04	Plain water	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
05	Tea, coffee <local examples>?	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
06	Fruit or vegetable juices?	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
07	Any other liquids, such as sugar water, thin soup or broth, carbonated drinks <local examples>	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
08	Is <CHILD> eating any semi-solid, mashed or solid foods? If NO, go to Q24	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
<i>Thinking about yesterday, during the day and at night, did &lt;CHILD&gt; have any of these foods, even if they were in combination with other foods?</i>					
09	Maize?	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
10	Sorghum?	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
11	Millet (any kind)?	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
12	Sugar cane or cane-derived sugar	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
13	Rice, porridge, bread, noodles or other foods made from grains? (do not include foods made from maize, sorghum, or millet) Mention <local examples>	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
14	White potatoes, white yams, manioc, or other foods made from roots? Mention <local examples>	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		

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15	Carrots, squash, or sweet potatoes that are yellow or orange inside? Mention <local examples>	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
16	Any dark green leafy vegetables such as spinach? Mention <local examples>	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
17	Foods made with beans, lentils, peas, corn, ground nuts? Mention <local examples>	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
18	Ripe mangoes, papayas, or other sweet yellow/orange or red fruit? Mention <local examples>	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
19	Any other fruits or vegetables such as banana, apple, oranges, tomatoes, avocado? Mention <local examples> (not including sugar cane)	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
20	Liver, kidney, heart or other organ meats? Mention <local examples>	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
21	Any meat, such as chicken, beef, lamb, goat, duck (others)? Mention <local examples>	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
22	Eggs? Mention <local examples>	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
23	Fresh or dried fish or shellfish? Mention <local examples>	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
24	Cheese, yogurt or other dairy products? Mention <local examples>	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
25	Any sugary foods such as pastries, cakes or biscuits? Mention <local examples>	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
26	Any commercially available foods for infants or young children? Mention <local examples>	Yes = 01 No = 00	<table border="1"><tr><td></td><td></td></tr></table>		
27	Yesterday, counting meals and snacks, how many times did you feed <CHILD>?	00-99	<table border="1"><tr><td></td><td></td></tr></table>		
28	How would you describe your child's appetite? Would you say it is:	01=poor, 02=fair, 03=good, 04=very good	<table border="1"><tr><td></td><td></td></tr></table>		

**E. Food Security (HFIAS)**

01	In the past four weeks, did you worry that your household would not have enough food?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	<table border="1"><tr><td></td><td></td></tr></table>		
02	In the past four weeks, were you or any household member not able to eat the kinds of foods you preferred because of a lack of resources?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	<table border="1"><tr><td></td><td></td></tr></table>		
03	In the past four weeks, did you or any household member have to	No = 00 Rarely = 01 Sometimes = 02 Often = 03	<table border="1"><tr><td></td><td></td></tr></table>		

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	eat a limited variety of foods due to a lack of resources?								
04	In the past four weeks, did you or any household member have to eat some foods that you really did not want to eat because of a lack of resources to obtain other types of food?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	<table border="1"><tr><td></td><td></td></tr></table>						
05	In the past four weeks, did you or any household member have to eat a smaller meal than you felt you needed because there was not enough food?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	<table border="1"><tr><td></td><td></td></tr></table>						
06	In the past four weeks, did you or any other household member have to eat fewer meals in a day because there was not enough food?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	<table border="1"><tr><td></td><td></td></tr></table>						
07	In the past four weeks, was there ever no food to eat of any kind in your household because of lack of resources to get food?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	<table border="1"><tr><td></td><td></td></tr></table>						
08	In the past four weeks, did you or any household member go to sleep at night hungry because there was not enough food?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	<table border="1"><tr><td></td><td></td></tr></table>						
09	In the past four weeks, did you or any household member go a whole day and night without eating anything because there was not enough food?	No = 00 Rarely = 01 Sometimes = 02 Often = 03	<table border="1"><tr><td></td><td></td></tr></table>						
<b>F. Child Morbidity</b>									
01	Does the child have diarrhea today?	Yes = 01 No = 00 Doesn't know = 88	<table border="1"><tr><td></td><td></td></tr></table>						
02	Over the past 1 week (including today), has your child had diarrhea?	Yes = 01 No = 00 Doesn't know = 88	<table border="1"><tr><td></td><td></td></tr></table>						
03	If yes to 02, for how many days?	01-07	<table border="1"><tr><td></td><td></td></tr></table>						
04	Over the past 4 weeks (including today), has your child had diarrhea?	Yes = 01 No = 00 Doesn't know = 88	<table border="1"><tr><td></td><td></td></tr></table>						
05	If yes to 04, how many separate episodes?	01-20 Doesn't know = 88	<table border="1"><tr><td></td><td></td></tr></table>						
06	How many days per episode?  <i>Note: Episodes must be separated by at least 2 days without diarrhea</i>	01-20 Doesn't know = 88	a. First episode <table border="1"><tr><td></td><td></td></tr></table> days b. Second episode <table border="1"><tr><td></td><td></td></tr></table> days c. Third episode <table border="1"><tr><td></td><td></td></tr></table> days						



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07	In how many episodes was blood/pus/mucus seen?  <i>(The total number of episodes)</i>	01-20 Doesn't know = 88	<table border="1"><tr><td></td><td></td></tr></table>						
CHRONIC DIARRHEA (Change in consistency of stools with passing of loose or watery stools lasting for MORE THAN 14 days)									
08	Over the past 4 weeks (including today), has your child had diarrhea for MORE THAN 14 days?	Yes = 01 No = 00 Doesn't know = 88	<table border="1"><tr><td></td><td></td></tr></table>						
09	Were there any hospitalizations in the last 4 weeks?  <i>If no, skip to Q 2.12. If yes, record each hospitalization separately</i>	Yes = 01 No = 00 Doesn't know = 88	<table border="1"><tr><td></td><td></td></tr></table>						
09a	Date of first admission		<table border="1"><tr><td></td><td></td></tr></table> / <table border="1"><tr><td></td><td></td></tr></table> / <table border="1"><tr><td></td><td></td></tr></table>						
09b	Diagnosis:								
09c	Date of second admission		<table border="1"><tr><td></td><td></td></tr></table> / <table border="1"><tr><td></td><td></td></tr></table> / <table border="1"><tr><td></td><td></td></tr></table>						
09d	Diagnosis:								
History of worm infestation									
10	Have you observed worms in your child's stools	Yes = 01 No = 00 Doesn't know = 88	<table border="1"><tr><td></td><td></td></tr></table>						
11	Has your child been treated for worm infestations in the last 6 months?	Yes = 01 No = 00 Doesn't know = 88	<table border="1"><tr><td></td><td></td></tr></table>						
12	If yes, what is the medication taken? (ask for empty syrup bottle/ prescription for medicine details)								
13	Is your child on regular deworming medication?	Yes = 01 No = 00 Doesn't know = 88							

	Morbidities	Does child have symptom today? <sup>a</sup>		If yes, how many days in past week including today? (1-7) <sup>b</sup>	Has child had symptom in past 1 month? <sup>c</sup>		If yes, how many episodes in the past month? (1-28) <sup>d</sup>	Has child had symptom in past 3 months, if yes how many episodes? <sup>e</sup>			
14	Common cold	<input type="checkbox"/> Yes	<input type="checkbox"/> No	<table border="1"><tr><td></td></tr></table>		<input type="checkbox"/> Yes	<input type="checkbox"/> No		<table border="1"><tr><td></td><td></td></tr></table>		
15	Cough	<input type="checkbox"/> Yes	<input type="checkbox"/> No	<table border="1"><tr><td></td></tr></table>		<input type="checkbox"/> Yes	<input type="checkbox"/> No		<table border="1"><tr><td></td><td></td></tr></table>		

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16	Difficulty in breathing	<input type="checkbox"/> Yes	<input type="checkbox"/> No	<input type="checkbox"/>	<input type="checkbox"/> Yes	<input type="checkbox"/> No		<input type="checkbox"/> <input type="checkbox"/>
17	Fever	<input type="checkbox"/> Yes	<input type="checkbox"/> No	<input type="checkbox"/>	<input type="checkbox"/> Yes	<input type="checkbox"/> No		<input type="checkbox"/> <input type="checkbox"/>
18	Pus draining from ears	<input type="checkbox"/> Yes	<input type="checkbox"/> No	<input type="checkbox"/>	<input type="checkbox"/> Yes	<input type="checkbox"/> No		<input type="checkbox"/> <input type="checkbox"/>
19	Vomiting	<input type="checkbox"/> Yes	<input type="checkbox"/> No	<input type="checkbox"/>	<input type="checkbox"/> Yes	<input type="checkbox"/> No		<input type="checkbox"/> <input type="checkbox"/>
20	Rashes	<input type="checkbox"/> Yes	<input type="checkbox"/> No	<input type="checkbox"/>	<input type="checkbox"/> Yes	<input type="checkbox"/> No		<input type="checkbox"/> <input type="checkbox"/>
21	Convulsions	<input type="checkbox"/> Yes	<input type="checkbox"/> No	<input type="checkbox"/>	<input type="checkbox"/> Yes	<input type="checkbox"/> No		<input type="checkbox"/> <input type="checkbox"/>

Child ID Code: 

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3 and 6-month Follow-up <b>Version 1.0</b>			
	Question	Code	Response
01	Study researcher/Nurse/Fieldworker ID		<div><div></div><div></div><div></div></div>
02	Today's date (DD/MMM/YY)		<div><div><div></div><div></div></div> / <div><div></div><div></div></div> / <div><div></div><div></div></div></div>
03	Current weight (kg)		<div><div><div></div><div></div></div> . <div><div></div><div></div></div></div>
04	Current length (cm)		<div><div><div></div><div></div></div> . <div><div></div></div></div>
05	Current head circumference (cm)		<div><div><div></div><div></div></div> . <div><div></div></div></div>