SAFETY, ACCEPTABILITY, AND FEASIBILITY OF ENTERADE® IN CHILDREN AT RISK FOR ENVIRONMENTAL ENTERIC DYSFUNCTION IN KAKAMEGA COUNTY, KENYA

Short title: Safety, Acceptability, and Feasibility of enterade® (SAFE)

Study Protocol

Version 1.7
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Study Sponsor: PATH
Funding Source: Department for International Development (DFID)
Location of Research: Kakamega County in Western Kenya
Proposed Project Dates: October 2018–January 2019 (4 months)

Co-Principal Investigator's Signature — **28 August 2018** Date

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Confidentiality Statement

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ABBREVIATIONS AND ACRONYMS

AA-ORS: amino acid–based oral rehydration solution

AE: adverse event

CGH: Kakamega County General Hospital

CRF: case report form

CWC: child welfare clinic

DFID: Department for International Development

DO: direct observation

DSMB: Data and Safety Monitoring Board

EC: ethics committee

EED: environmental enteric dysfunction

ELISA: enzyme-linked immunosorbent assays

EPI: Expanded Program on Immunization

GCP: good clinical practices

GRAS: generally regarded as safe

HCP: health care provider

ICH: International Council on Harmonisation

IDI: in-depth interview

IRB: institutional review board

ISM: independent study monitor

LAZ: Length-for-age z-score

LRS: low-resource settings

MAL-ED: Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development

MOH: Ministry of Health

MUERC: Maseno University Ethics Review Committee

MUAC: mid-upper arm circumference

ORS: oral rehydration solution

PI: principal investigator

PID: Patient Identification Number

PPB: Pharmacy and Poisons Board

RA: research assistant

REC: research ethics committee

SAE: serious adverse event

SOP: standard operating procedure

SUSAR: suspected unexpected serious adverse reaction

Vit. A: vitamin A

WHO: World Health Organization

STUDY OVERVIEW

Title:	Safety, Acceptability, and Feasibility of enterade® in children at risk for environmental enteric dysfunction in Kakamega County, Kenya
Sponsor:	Drug Development, PATH
Investigators:	USA: Michael Arndt (co-PI), Gwen Ambler, Jaclyn Delarosa Kenya: Dr. James Mukabi (co-PI), Peninah Murunga, Alfred Ochola
Ethics committees:	Maseno University Ethics and Research Committee (MUERC) PATH Research and Ethics Committee (REC)
Regulatory authority:	Kenyan Ministry of Health (MOH) Pharmacy and Poisons Board (PPB)
Phase:	I
Study goals:	Determine the safety, acceptability, and pediatric dosing of enterade® solution, an amino acid–based oral rehydration solution (AA-ORS), for potential use in the management of environmental enteric dysfunction (EED) among children aged 12–24 months in Kenya.
Study objectives:	1. Primary: To determine the safety of a 2-week course of AA-ORS among children with length-for-age Z-scores (LAZ) between –1 and –3. 2. Primary: To determine the feasibility and best tolerated dose of AA-ORS among children with LAZ between –3 and –1. 3. Secondary: To determine the perceptions among caregivers on the acceptability of AA-ORS as a potential intervention for EED. 4. Exploratory: To determine the impact of AA-ORS on markers of metabolism, gut dysfunction, systemic inflammation, and micronutrient status among children with LAZ between –3 and – 1.
Study endpoints:	1. Primary: Adverse events and serious adverse events through 21 days of follow-up, and changes to dietary and digestive habits through day 14 of follow-up. 2. Primary: Average daily product volume consumed through 14 days of follow-up, time trends in average daily volume consumed, and factors that influence children's willingness and ability to ingest the product as intended. 3. Secondary: Key factors that influence caregivers' willingness to have the product used by their children, and their perception of their children's response to its consumption. 4. Exploratory: Average plasma/serum concentrations of metabolic, gut dysfunction, systemic inflammation, and micronutrient biomarkers at baseline and day 15 of follow-up.
Study design:	This is a mixed-methods pilot study to assess the safety, acceptability, and feasible dosage of AA-ORS among children with mild to moderate stunting (LAZ between –1 and –3) who are at risk for EED and to assess the acceptability of this fluid supplementation for their caregivers. During the main study, child participants will be

randomized to receive 14 days of AA-ORS or placebo solution daily, dosing of up to 237 ml over a 3-hour period. Study staff and participants will be blinded to the study product allocation. Caregivers will store and return unused study product. Data collection methods to include direct observation of product consumption, adverse event assessments, structured surveys with caregivers, average daily volume consumed, and measurement of plasma/serum markers of metabolism, gut dysfunction, systemic inflammation, and micronutrient status pre- and post-administration of product.

Study population: This study will recruit subjects from a health facility in Western Kenya. The study population is children 12–24 months of age with mild to moderate stunting (–3 < LAZ < –1), who are presenting to health facilities for routine immunizations, healthy child visits, or vitamin A supplementation. Caregivers of enrolled children will also be consented for interviews and direct observation.

Sample size: N = 132 total individuals
AA-ORS: 33 children; 1 dose of up to 237 ml × 14 days
Placebo solution: 33 children; 1 dose of up to 237 ml × 14 days

	Study Arm	
	AA-ORS	Placebo solution
Children	33; receive 1 dose of up to 237 ml x 14 days	33; receive 1 dose of up to 237 ml x 14 days
Caregivers	33; interviews and direct observation	33; interviews and direct observation

Study duration: 4 months

A. RESEARCH STUDY PERSONNEL

Statement of compliance: The trial will be conducted in accordance with the International Council on Harmonisation (ICH) Good Clinical Practices (GCP) E6(R2), the Declaration of Helsinki, and applicable local regulatory requirements. The principal investigator (PI) and site PI will assure that no deviation from or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the relevant ethics committees (ECs) and regulatory body, except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

Dr. Michael Arndt, PhD, MPH.

Roles and Responsibilities: Lead protocol and questionnaire development, conduct site evaluation and study initiation visits, and direct quantitative analyses. Project oversight to complete deliverables in a thorough, thoughtful, and timely manner. Direct quantitative analyses. Responsible for notifying the PATH Research and Ethics Committee of serious adverse events (SAEs) per its reporting requirements.

Dr. James Mukabi, MD.

Roles and Responsibilities: Review protocol and questionnaires; provide scientific and strategic input for study design and implementation. Responsible for overseeing the study nurse and pediatrician, reviewing adverse events (AEs) and SAEs submitted by the pediatrician, and notifying the Maseno University Ethics and Research Committee and local regulatory authority of SAEs per their reporting requirements.

Gwen Ambler, MPH.

Roles and Responsibilities: Lead SAFE protocol and questionnaire development, and direct qualitative analyses. Ensure project is completed within scope, on time, and within budget.

Jaclyn Delarosa, MPH.

Roles and Responsibilities: Contribute to SAFE protocol and questionnaire development; ensure compliance with ethical, regulatory, and scientific review guidelines; and assist with qualitative analyses.

Peninah Murunga, MPH.

Roles and Responsibilities: Contribute to SAFE protocol, CRF, and questionnaire development; oversee qualitative data collection; obtain necessary approvals.

Alfred Ochola, MPH.

Roles and Responsibilities: Oversee day-to-day project activities in Western Kenya.

Dorothy Brown, Kenya Registered Nurse.

Roles and Responsibilities: Conduct weekly check-in phone calls with caregivers to answer any medical questions or provide advice. Responsible for monitoring and evaluating subject safety in consultation with study pediatrician.

Dr. Nick Aduro Kidaha, MBChB, MMed (Paeds).

Roles and Responsibilities: Conduct medical evaluations at enrollment and post-dosing visits. Responsible for medical oversight: monitoring, evaluating, recording, and treating all adverse events and serious adverse events that occur during follow-up.

Research assistant(s), to be determined.

Roles and Responsibilities: Conduct direct observations, caregiver interviews, data entry, and data management.

B. ABSTRACT

Environmental enteric dysfunction (EED) is an intestinal disorder common among people living in low-resource settings (LRS), which in children has been associated with increased risk of growth stunting, reduced cognitive development, and reduced oral vaccine responsiveness. An effective EED therapeutic would offer an opportunity to improve child growth and development in LRS. One promising intervention, enterade® (an amino acid–based oral rehydration solution [AA-ORS]), is a medical food product already sold in the United States. It consists of oral rehydration salts and a proprietary blend of amino acids designed to restore gut function, improve nutrient and electrolyte absorption, and improve barrier integrity. There is evidence that this AA-ORS reduces inflammation and promotes healing of damaged intestinal epithelium in murine models of intestinal damage (irradiated gut), and it may provide benefit to pediatric EED patients. Supplementation of amino acids may lessen or improve intestinal injury related to enteric illnesses commonly experienced in settings of poor hygiene and sanitation infrastructure. We will conduct a randomized, double-blinded, placebo-controlled pilot study in Kakamega County, Kenya, to assess the safety, feasible dosage, and acceptability/tolerability of the product in the target population (children aged 12–24 months) and their caregivers.

C. STUDY PROTOCOL

i. Background and significance of the research study

Environmental Enteric Dysfunction (EED)

Environmental enteric dysfunction (EED) is an intestinal disorder common among people (especially young children) living in low-resource settings (LRS) with a high enteric pathogen burden and poor sanitation and hygiene (1–3). EED is characterized by mucosal inflammation, reduced barrier integrity, and nutrient malabsorption, and it is associated with increased risk of growth stunting, poor cognitive outcomes, and reduced oral vaccine responsiveness in children in low- and middle-income countries (4–8).

Growth stunting (length-for-age z-score [LAZ] < –2) is associated with increased risk of chronic health issues including, but not limited to, cognitive deficits and increased susceptibility to infections (9–14). EED thus directly and indirectly contributes to child mortality and morbidity due to other common childhood diseases such as pneumonia, acute diarrhea, and malaria. Furthermore, deficits in cognitive function can diminish school performance and have lasting impacts on a child's future financial success and economic productivity (12, 14). Finally, women that were stunted as children are more likely to suffer from malnutrition in adulthood and when pregnant, leading to low birthweights and increased likelihood of their children being stunted, thus completing a vicious cycle (12, 13, 15, 16). In fact, children who have a LAZ from less than −1 SD to −2 SD, while technically not considered stunted, have an increased risk of death, especially from diarrhea and pneumonia, compared to those with LAZ > −1 SD (13).

In sub-Saharan Africa, limited published data are available on the prevalence and incidence of EED. However, studies in Tanzania have observed that elevations in 1) antibodies related to microbial translocation and 2) markers related to immunotolerance of gut-based immunogens were associated with growth faltering in young children (17, 18). In the multi-site Etiology, Risk Factors and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development (MAL-ED) birth cohort, LAZ declined most precipitously among children enrolled at the Tanzania site; stunting prevalence rose from 16% at birth to 72% at 24 months of age. Fecal markers of intestinal inflammation and barrier disruption as well as urinary measures of intestinal permeability are markedly higher in children from Tanzania and South Africa in comparison with Brazilian children, who more closely resemble child populations in developed settings (19–21). Additional sub-Saharan African data highlighting negative associations between local and systemic inflammation and pediatric linear growth come from Malawi and Zimbabwe (22, 23). Among children under 5 years of age in Kenya, an estimated 26% have moderate or severe stunting, 11% are underweight, and 4% have wasting (24). Western Kenya has high stunting prevalence; stunting affects an estimated 31% of males and 35% of females under age 5 years (25).

There remain major challenges in establishing a formal case definition for EED; however, a recently published editorial in the *American Journal of Tropical Medicine and Hygiene* noted the following considerations in the development of therapeutics to treat EED (26):

> To advance the EED field, we now propose a definition construct analogous to the Jones criteria for acute rheumatic fever, intended for therapeutic trials, and which can be modified as new data emerge. In building this proposal, we recognize that EED is, except for poor growth, rarely accompanied by clinical signs or symptoms, so our criteria highly depend on laboratory assessment. Accordingly, our definition is based on three domains, which, taken

together, indicate an acquired intestinal inflammatory disorder with substantial clinical impact. Domain 1 includes age, presence of linear growth failure, negative celiac disease testing; domain 2 includes gut histopathology consistent with EED or at least two intestinal deficits assessed by less invasive biomarkers; domain 3 consists of biomarkers nonspecific to enteric dysfunction, but representing consequences of EED.

EED is a multifactorial disease with a complicated etiology. The main goal of an effective EED therapeutic is to lessen or improve intestinal injury related to enteric illnesses commonly experienced in settings of poor hygiene and sanitation infrastructure. Viable therapeutic candidates should have evidence from human and/or animal studies that the product(s) address at least one of the two issues that appear to play a big role in EED: repeated bouts of infection and impaired gut healing. Evidence from one clinical trial suggested that provision of zinc or albendazole (anthelminthic drug) may slow the progression of EED in children based on the L:M ratio (27). However, a subsequent clinical trial did not observe any effect of a combination intervention of albendazole, zinc, and multiple micronutrient supplements on EED progression (28).

In contemplating development of an EED therapeutic with potential for widespread use, one important consideration is that, in contrast to diarrhea where symptoms immediately raise caregiver concern, EED manifests its symptoms slowly and chronically. Therefore, EED therapeutics may be even more difficult to promote than therapeutics for acute diarrhea. Furthermore, it is likely that EED therapeutics would need to be administered over long periods or even chronically to successfully treat the syndrome due to the complexity of reducing ongoing environmental exposures, thus complicating both patient compliance and clinical trial design. Formative, qualitative and quantitative research in the form of patient feasibility and acceptability studies could provide information critical to the development and implementation of effective EED interventions and generate data useful to inform the design of future clinical trials.

Amino Acid–Based Oral Rehydration Solution

As described in the Investigator's Brochure (Appendix 4), enterade® (an amino acid–based oral rehydration solution [AA-ORS]) is a medical food product that consists of oral rehydration salts and a blend of amino acids that drive the uptake of water and electrolytes. The blend of amino acids appears to reduce intestinal damage and promote healing of the intestinal epithelium in irradiated mouse models commonly used to simulate gut damage encountered in inflammatory bowel diseases (29, 30). This AA-ORS contains water, a proprietary blend of amino acids (L-Valine, L-Aspartic Acid, L-Serine, L-Threonine, and L-Tyrosine), sodium chloride, sodium citrate, potassium chloride, gum acacia, magnesium chloride, calcium chloride, and stevia leaf extract (sweetener). AA-ORS may be useful in treating children with EED, as low circulating levels of three of the five amino acids present in AA-ORS (valine, threonine, and serine) have been associated with stunting among children aged 6–59 months in rural Malawi (31). In an experimental irradiated mouse model, mice treated with a solution of amino acids similar to the AA-ORS for this trial (containing lysine, aspartic acid, glycine, isoleucine, threonine, tyrosine, valine, tryptophan, and serine) showed increased electrolyte absorption and decreased paracellular permeability, IL-1β levels, and plasma endotoxin levels in comparison to untreated controls. Treated mice also had increased weight gain and better survival following irradiation compared to untreated controls (29). In another study, a simpler amino acid– based oral rehydration solution (ORS) containing threonine, valine, serine, tyrosine, and tryptophan increased enterocyte proliferation, maturation, and differentiation and improved electrolyte and nutrient absorption in irradiated mice (30).

The safety and efficacy of AA-ORS have been tested in cohorts of adult cancer patients, including an ongoing study by the Dana-Farber Cancer Institute (NCT02919670). This AA-ORS was tested in more than 300 Indonesian adults with diarrhea, resulting in no observed serious adverse events (SAEs) or adverse events (AEs) (32). In a small hydration study among US adults comparing water, ORS, AA-ORS, and a commercial sports drink, AA-ORS and ORS both hydrated significantly better than water (NCT03262597). This AA-ORS has not yet been evaluated in children, save for a small ongoing study in five patients (2–14 years of age) with short bowel syndrome (NCT03105362). No AEs were observed in pediatric or adult studies to date (32). Pending receipt of study funding, this AA-ORS will be tested by icddr,b among children 6–36 months of age in Bangladesh for effectiveness in decreasing the duration and severity of diarrhea. AA-ORS is categorized by the US FDA as a medical food, like Pedialyte®, which is defined in section 5(b)(3) of the Orphan Drug Act (21 U.S.C. 360ee(b)(3)), as:

> 'a food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation.'…The patient should generally see the physician on a recurring basis for, among other things, instructions on the use of the medical food as part of the dietary management of a given disease or condition. (33)

This AA-ORS is not currently marketed or approved for use in Kenya. It would be classified as a "food supplement" according to local guidelines and would fall under the authority of Kenya's Ministry of Health (MOH) regulatory body, the Pharmacy Poisons Board (PPB). The PPB is responsible for approving and overseeing the importation and use of unregistered products to be used in clinical trial settings in Kenya. A license for importation will be obtained from PPB prior to shipment of study product (i.e., AA-ORS and placebo) and any required regulatory approvals will be secured in accordance with PPB guidance before trial initiation.

The active ingredients of the AA-ORS are described in Table 1, below.

	AA-ORS	**Total mg (in 237 ml)**
Aspartic Acid (g/L)	1.06	251.2
Threonine (g/L)	0.95	225.2
Tyrosine (g/L)	0.21	49.8
Valine (g/L)	1.17	277.3
Serine (g/L)	1.05	248.9
Sodium (mEq/L)	42.25	230.3
Potassium (mEq/L)	10	92.4
Citrate (mEq/L)	3.35	50.0
Magnesium (mEq/L)	1.2	3.4
Calcium Chloride (mEq/L)	1.2	15.6

The placebo solution for this study is the same as that being used in the ongoing Dana-Farber Cancer Institute (NCT02919670) study, and contains all the non-amino acid and non-electrolyte ingredients of the AA-ORS including purified water, trisodium citrate, citric acid, stevia, and natural flavors. All the non-amino-acid AA-ORS ingredients are generally regarded as safe (GRAS), a designation used by the US Food and Drug Administration (FDA) to classify components of food that are safe and suitable for extensive use in the United States population. Amino acids are not considered GRAS, but are governed by 21CFR172.320, a food additive regulation that sets out the conditions under which amino acids

used as food additives may safely be added to foods. Section F of this regulation refers to medical foods like this AA-ORS:

> (f) The food additive amino acids added as nutrients to special dietary foods that are intended for use solely under medical supervision to meet nutritional requirements in specific medical conditions and comply with the requirements of part 105 of this chapter are exempt from the limitations in paragraphs (c) and (d) of this section and may be used in such foods at levels not to exceed good manufacturing practices.

For children at age 1-2 years, the World Health Organization (WHO) reports the average daily intake requirement of threonine and valine as 23 mg/kg and 36 mg/kg, respectively (34). At 12 months of age, healthy female children average 9 kg. Such a child would therefore require a minimum of 207 mg of threonine and 324 mg of valine. Estimated requirements for overall daily protein intake in children under 2 years of age range from 1 to 1.2 g/kg, and therefore a 9-kg child needs between 9 and 10.8 g of protein daily (35).

Table 2. Amino acids in breastmilk and amino acid–based oral rehydration solution (AA-ORS) based on expected daily intake for a 10–12 month-old child (36).

Amino Acid	Breastmilk (mg in 800 ml)	AA-ORS (mg in 237 ml)	Total daily mg
Aspartic Acid	682	251.2	933.2
Threonine	364	225.2	589.2
Tyrosine	394	49.8	443.8
Valine	438.4	277.3	715.7
Serine	352.8	248.9	601.7

Most doctors recommend that women breastfeed their baby for at least 1 year (37). While breastmilk is the beverage recommended by the American Academy of Pediatrics guidelines for infants under 1 year of age (38), children aged 12 months and older are consuming many other beverages in considerable volumes. A 2004 survey among several thousand parents in the United States reported that more than two-thirds of children between 12 and 14 months were consuming non-milk beverages in addition to breastmilk (39). The low total caloric content of AA-ORS makes this product unlikely to satiate children and reduce their consumption of breastmilk. After 6 months of age, an increasing proportion of calories in the young child diet come from solid foods.

Oral rehydration solution (ORS) recommended for treatment of dehydration and acute diarrhea has similar osmolality to AA-ORS, and the two share some common ingredients, including potassium and sodium. However, a key difference (aside from its amino acid content) is that this AA-ORS lacks glucose. Glucose is widely held to be a key component in the rehydration mechanism of ORS, although according to some researchers, it has detrimental effects as well (40, 41). Table 3, below, from a poster created by researchers at the University of Florida compares the ingredients of ORS, a sports drink, and AA-ORS. The beverages (including water) and their corresponding osmolalities are as follows: distilled water (~0 mmol/kg), AA-ORS (195 mmol/kg), ORS (270 mmol/kg), and a sports drink (330 mmol/kg). This poster can be found here**.**

Table 3. Comparison of the test beverage composition.

	Caloric Content (kcal/L)	Carbohydrate (g/L)	Sodium (mmol/L)	Potassium (mmol/L)
enterade®	21	0	55	10
ORS	105	25	44	20
Sports drink	237	61	20	3

Should AA-ORS prove to be effective for treating EED alone or in combination with other interventions, additional research will be necessary to optimize the dosing schedule for children of different ages, improve the formulation (e.g., powder sachets rather than bottles of solution), and target/prioritize subgroups most likely to benefit. The amino acid components of AA-ORS may be sufficient to elicit a therapeutic benefit, even when not formulated as an aqueous solution. Such questions would need to be answered in order to optimize the intervention for deployment in LRS.

ii. Aims of proposed research study

We propose to conduct a small pediatric pilot study to determine patient safety, feasible product volume (trends in dosage consumed over 14 days, etc.), and caregiver (i.e., parent or legally acceptable representative) acceptability of AA-ORS in the target population at risk for EED. To assess the product's safety, we will randomize children to receive active product or placebo and compare the rate of adverse events (AEs) in the two groups. We will also assess the extent to which the product might meet the needs of the target population and setting. Results from this study will be used to inform:

a) The design of a randomized controlled trial to test the efficacy of AA-ORS for treating pediatric EED.
b) Future EED product development efforts.
c) Program implementation strategies for EED interventions.

Objectives

PRIMARY OBJECTIVE: To determine the safety of AA-ORS among children with mild to moderate stunting, administered as a daily dose over 2 weeks, ad libitum up to 237 ml in 3 hours.

> *Safety assessment in children will compare adverse event (AE) and serious adverse event (SAE) rates and changes to dietary and digestive habits between children receiving AA-ORS versus an inactive placebo solution containing no amino acids.*

PRIMARY OBJECTIVE: To determine the feasibility and well-tolerated dose of AA-ORS among children with mild to moderate growth stunting administered as a daily dose over 2 weeks, ad libitum up to 237 ml in 3 hours.

> *User preferences among children include average daily volume consumed over 14 days, trends in volume consumed, and factors that influence children's willingness and ability to ingest the product as intended.*

SECONDARY OBJECTIVE: To determine the perceptions among caregivers on the use and acceptability of this product as a potential intervention for EED.

> *Acceptability by caregivers includes factors that influence their willingness to have the product used by their children and their perception of their child's response to its consumption.*

EXPLORATORY OBJECTIVE: To determine the impact of AA-ORS on gut dysfunction, metabolism, gut and systemic inflammation, and micronutrient status.

Impact of AA-ORS on gut dysfunction, systemic inflammation, and micronutrient status will be assessed by comparing non-invasive biomarker levels in blood samples collected following the intervention. These biomarkers will be assessed in both groups at baseline in order to have setting-specific descriptive data with which to power a future EED efficacy trial.

Endpoints

Primary: Adverse events and serious adverse events through 21 days of follow-up, and changes to dietary and digestive habits through day 14 of follow-up.

Primary: Average product volume consumed daily through 14 days of follow-up, time trends in average daily volume consumed, and factors that influence children's willingness and ability to ingest the product as intended.

Secondary: Key factors that influence caregiver willingness to have the product used by their children, and their perception of their child's response to its consumption.

Exploratory: Average plasma/serum concentrations of metabolic, gut dysfunction, systemic inflammation, and micronutrient biomarkers at baseline and day 15 of follow-up.

iii. Study design, population, and study procedures

Study Approach and Study Population

To achieve the objectives listed above, we will closely monitor adverse events (AE) and serious adverse events (SAE) among study participants, conduct direct observations (DOs) and semi-structured in-depth interviews (IDIs) with potential end users (caregivers) in Kenya. IDIs will be undertaken to obtain an in-depth understanding of acceptability among end users (caregivers of pediatric patients 12–24 months of age) after AA-ORS use (post-deployment). The DOs will involve the study team observing initial administration of AA-ORS or placebo solution to a participating child. We will also gather and test blood samples in order to understand enteric dysfunction, systemic inflammation, and micronutrient deficiency in this child population.

The study population is 66 child/caregiver pairs. Eligible children will be 12–24 months of age without diarrheal or febrile illness, within the prior 7 days, who are experiencing mild to moderate stunting (LAZ between –3 and –1). Children with mild to moderate stunting at this age do not fall under the WHO guidelines for exclusive breastfeeding (< 6 months of age), are at increased risk for morbidity and mortality compared to children without stunting (13, 42), and may still be able to regain linear growth. That is, when children are under the age of 24 months, their growth is more plastic. Thus, stunting is more easily reversed than in older children. On an individual level, growth stunting is not always caused by EED, and those suffering from EED may not always have stunting. However, we have taken a practical or proxy case definition for "at risk for EED" based on stunting. This will allow us to determine how AA-ORS is tolerated in a population that at least overlaps considerably with true EED cases. Caregiver/child pairs will be enrolled and randomized into one of two groups receiving a daily dose of either (1) AA-ORS or (2) placebo solution (no amino acids) for 2 weeks, ad libitum over 3 hours up to 237 ml each day.

The study will be double-blinded as both study staff and participants will be blinded to study product allocation. Active and control product bottles will be labeled with identical product information. Each bottle will be also labeled with a randomization number assigned to a particular participant according to the appropriate allocation arm. The randomization list where randomization number and allocation code are provided will be maintained in a secure place by an independent statistician. The participants

will thus be blinded to whether they receive AA-ORS or the control solution. Study staff administering the study product and laboratory staff performing any laboratory testing will also be blinded to the product allocation.

This study will provide critical information about the safety of AA-ORS in this at-risk pediatric population and qualitative and quantitative information on the user-preferred product volume for this age group, palatability, and ease of administration. The study will also describe whether the product can be feasibly consumed for a 2-week period and if there are differences in gut health between AA-ORS and placebo groups based upon diarrhea frequency and plasma biomarkers of systemic inflammation and gut damage/repair at the end of follow-up. However, the study has not been statistically powered or designed to answer such gut health questions. Even though such exploratory comparisons will be underpowered, the baseline and follow-up biomarker data will be useful for informing study design and sample size of future EED clinical trials in this setting.

The questionnaire guides are a mix of structured and semi-structured questions. Structured questions are necessary to standardize responses to questions such as the experience with diarrheal disease treatment. Semi-structured questions allow for flexibility and richness in responses, especially those on perceptions and experiences around AA-ORS use and acceptability. Semi-structured questions are open-ended and allow for probing a participant's answers for in-depth responses. The DO checklist includes a list of questions to assess the administration of AA-ORS and explore ease of use, palatability, and acceptance of the product by the user(s).

Study Site

The study will be conducted in Kakamega County in Western Kenya. This county was selected as containing both rural and urban settings and is suitable for this research due to the high prevalence of malnutrition and PATH Kenya's established relationships with health facilities in the area. In former Nyanza province, the stunting (LAZ < –2) prevalence in children under 5 years ranges from 2.5% in Migori County to 5.14% in Kisumu County. In neighboring Western province, where Kakamega is located, the prevalence is thought to be much higher.

PATH has been involved in the establishment of the APHIA*plus* program in Western Kenya. APHIA*plus* promotes HIV testing and improved access to ORS for children experiencing diarrhea through ORS corners in several hundred health facilities in the region. Participants will be recruited from Kakamega County General Hospital (CGH). This facility draws from a peri-urban catchment area, has been participating in APHIA*plus* programming, and has set up an ORS corner for treatment of pediatric diarrhea. Therefore, health care providers in the facility are quite familiar with the use of ORS.

Key Activities

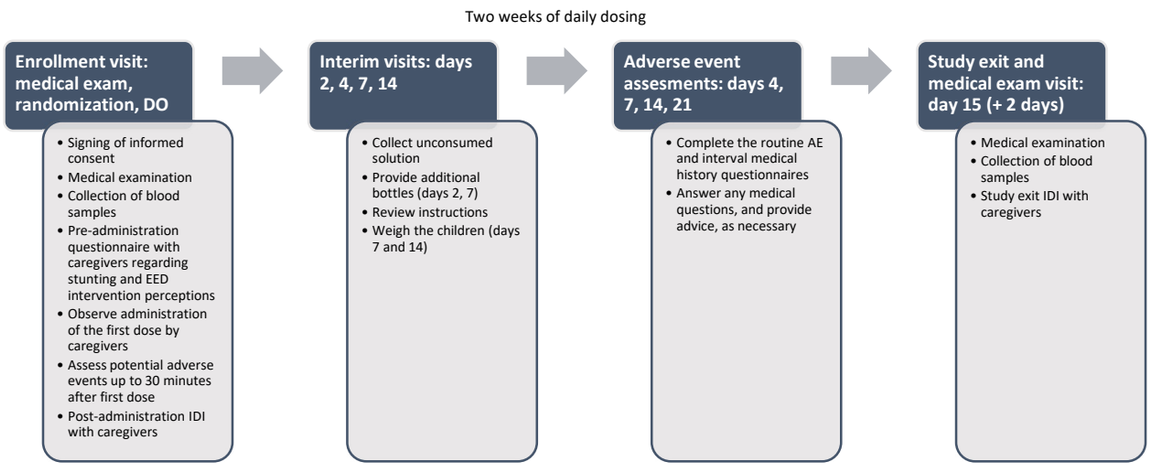
Following the training of research assistants (RAs), piloting study IDI guide(s) and questionnaires, and subsequent IDI guide and questionnaire revision, the key activities will include the following:

1. Screen potential participants at routine immunization visit or vitamin A (Vit. A) supplementation visit after the child is 12 months of age and schedule enrollment visit at least 1 week later. OR: identify potentially eligible children from using the CGH child welfare clinic (CWC) register of patients who received immunizations in the first year of life. Call the caregivers of prospective participants using the telephone number provided, and schedule screening visit with interested caregivers (and their child). Schedule enrollment visit any time following the screening visit.
2. Following informed consent, participants will be randomized to receive active product or placebo using sealed, unmarked envelopes with numbers generated previously by a biostatistician. These

envelopes will contain randomization numbers that are linked to numbers printed on a set of bottles containing either AA-ORS or placebo solution. Once the randomization number has been attached to a patient ID number, that participant will only receive bottles labeled with his/her randomization number.

3. At enrollment, blood samples will be collected (between 1 and 1.5 ml) using venipuncture into heparinized collection tubes. Samples will be centrifuged into plasma or serum within 30 minutes of collection and stored in cryo tubes at –20°C until they can be analyzed.
4. Study RAs will conduct a baseline questionnaire with caregivers before study product use and will perform IDIs with caregivers after using AA-ORS or placebo with their child at enrollment and then after daily study product use for 2 weeks. The RA will explain product directions to caregivers (recommend using a cup and spoon, provided) and give an instruction sheet to take home. RAs will conduct DOs with caregivers and their children, observing the administration of the solution for the first dose provided, for up to 3 hours. Any solution that has not been consumed after 3 hours will be measured, recorded, and discarded. Patients will be monitored in the clinic for 30 minutes following the end of product consumption (after 3 hours, or the 237 ml is consumed, whichever occurs first). A study nurse will be available on site in case the child needs medical attention.
5. Interim visits: RAs will visit the home on days 2, 4, 7, and 14 to collect unconsumed solution, review instructions, provide additional bottles (on days 2 and 7 only), and weigh the children (on days 7 and 14 only).
6. Adverse event check-ins: Periodically during dosing (days 4, 7, 14, and 21), and one week after the end of dosing (day 21), a study nurse will contact the caregiver by phone and check in with the caregiver to assess any potential AE or SAE, complete the routine interval medical history (with feeding and breastfeeding assessment)/stool frequency questionnaire, answer any medical questions, and provide advice, as necessary.
7. On the fifteenth day of follow-up (or within 2 days after), following the 2-week dosing period, patients and their caregivers will return to the clinic for medical evaluation, collection of blood samples, and caregiver IDIs. Samples will be processed and stored for laboratory analyses following the same procedures from the enrollment blood collection.

Study Flow Diagram



Study Products

AA-ORS is an amino acid–based, glucose-free, medical food/beverage that is lightly sweetened with stevia leaf extract. AA-ORS provides select amino acids and electrolytes (sodium and potassium)—nutrients intended to rebuild and protect the gastrointestinal tract and deliver hydration.

The AA-ORS for use in this trial, enterade®, will be commercially produced by Entrinsic Health Solutions, Inc. in accordance with US FDA standards for medical food products and good manufacturing practices. The AA-ORS will be used as supplied, meaning that other than packaging and labeling, there is no further study product preparation required. The corresponding placebo solution will be produced by Entrinsic Health Solutions for clinical trial use only.

In this study, AA-ORS and placebo solution will be formulated in bottles with 237 ml of clear liquid. All bottles will be clearly labeled as for research use only. Labels will meet all national and local requirements. The label must also include the product expiry date, batch number, and manufacture date.

The AA-ORS is stable at room temperature and has a 2-year shelf life when stored unopened in ambient temperatures below 25°C. Long-term (6-month) stability of the AA-ORS has also been demonstrated at 40°C with 65% relative humidity. The AA-ORS is stable for the entire study period, even at higher environmental temperatures that may be encountered in participants' homes during the study. The placebo solution is presumed stable. All study product not already dispensed to participants will be stored securely, only accessible to the study staff.

The study investigators will be required to maintain complete records of all study products received from the sponsor and/or manufacturer and will be responsible for maintaining an accurate record of the randomization codes and inventory and an accountability record for this study. The study investigators will also be responsible for ensuring the security of these documents, maintaining them under lock and key and/or electronic encryption.

At the completion of the study, the study investigators and site PI (or designee) will conduct and document a final reconciliation of all study product shipped, received, dispensed, consumed, and remaining. Any discrepancies identified will be investigated, resolved, and documented before any unused study product is destroyed. After all accounting and reconciliation procedures are complete and approved by the sponsor, all unused study product will be destroyed on site and documented in the master study files.

Study Procedures

All IDI guides and data collection instruments will be pretested prior to data collection for gauging time required and flow of questions. IDI guides will be revised and adapted accordingly prior to data collection. Therefore, research assistant (RA) training will be composed of 1) review of guides and instruction on carrying out recruitment and consenting procedures, enrollment and interim questionnaires, weighing of children, and IDIs; 2) pretesting of IDI guides; and 3) revision/adaptation of guides with investigators, as necessary. RAs will field/pilot test the IDI and other questionnaires with a small number of caregivers in Kakamega, Kenya, before finalizing the instrument. Results from initial pretesting will be used to refine the data collection instruments before starting the study. Specifically, testing will be used to confirm target respondents are able to understand the questions being asked, that questions are understood in the same way by all respondents, and that respondents

are willing and able to answer questions. Table 4 presents an overview of data collection procedures and timing.

Table 4. Table of procedures (N=66 child/caregiver pairs).

	Screening	Enrollment (Day 1) *	Day 2	Day 4	Day 7		Day 14	Day 15	Day 21	Comments
Review of medical records for child eligibility	X									
Obtain consent		X								
Physical exam	X	X						X		
Blood draw (1–2 mL)		X						X		
Enrollment questionnaire (caregiver)		X								
Teaching caregiver to administer study product		X								
Direct observation of feeding		X								
AE assessment 30 mins after dose		X								
In-depth interview (caregiver)		X					X			
Supply product to home/pick up unused product			X	X#	X		X			
Review product administration with caregiver			X		X		X			
Text message to remind caregiver to start and stop study product			X	X	X		X			Twice daily during 2-week daily product administration.
Weight assessment	X				X		X			Done at the household, but may be done at the clinic, if needed.
Check in on adverse events; administer AE/interval medical history questionnaire		X		X	X		X			Done by study nurse in person on day 1, and then by phone all others.
Final recording of SAEs and close out of AEs									X	Done by study nurse, as needed, by phone or in person.
Report SAEs/SUSARs to regulatory entities		X	X	X	X		X	X	X	
*Enrollment will be at least 1 week post routine immunization, healthy child visit, or vitamin A supplementation visit at the study site, if recruited in-person rather than through CGH register review. #Day 4 is for pick-up of unconsumed product (bottles) only.										

Abbreviations: AE, adverse event; SAE, serious adverse event; suspected unexpected serious adverse reaction, SUSAR.

Screening visit:

During the screening visit, potentially eligible participants will be identified by clinic staff through the clinic records and routine information gathered at Expanded Program on Immunization (EPI)/Vit. A supplementation visits. Caregivers and their children will be referred to a study RA by clinic staff so the study RA can inform the caregivers about the study activities. Alternatively, potentially eligible children and their caregivers will come to the clinic after being identified from the CWC register and contacted by study staff (as described in *Recruitment and Consent Procedures*, below). As part of routine care, the child will receive a physical exam by a clinician or nurse. The routine physical examination conducted at EPI and Vit. A supplementation visits includes assessment of weight, weight categories (underweight, severe underweight, overweight, obese), height/length, stunted (stunted, severe stunted), and mid-upper arm circumference (MUAC) (green, yellow, red). If appropriate, the children identified from the CWC register will be given Vit. A supplements and any missing immunizations by clinic staff. If the caregiver is interested in participating in the study, he/she will be given a copy of the consent form and patient information leaflet to take home and review before coming back to the clinic for enrollment. In the case of an illiterate caregiver, the RA will confirm that there is at least one literate adult living in the child's household, and the RA will offer to read the consent form in full with the caregiver before sending the caregiver home. Any review of medical records prior to completion of informed consent will be done solely to identify potential participants for this study. Only data relevant to screening criteria will be recorded. If the caregiver declines enrollment in the study, any medical record data collected by study staff will be shredded and discarded.

Enrollment visit:

Upon return to the clinic (≥ 1 week post screening visit) the RA will review the consent form with the parent or legally acceptable representative and answer any questions. The caregiver will be asked if they are still interested in continuing with participation in the study; if they agree, then the RA will complete the informed consent process and obtain their written signature on the consent form.

Once the consent form is signed and a copy has been given to the participant, the study nurse or clinician will conduct a medical examination, and a trained phlebotomist will draw blood from the child and collect a bloodspot on filter paper (see "Blood specimen collection and processing"). The RA will then proceed with the enrollment questionnaire and direct observation of the oral solution. For all children who join the study and are still breastfeeding, the enrollment questionnaire will include questions to establish a baseline breastfeeding rate (how many times a day). After direct observation of dosing with the solution, an RA will complete an IDI with the caregiver.

Training of caregivers

Caregivers will receive one 237-ml bottle of AA-ORS or placebo at enrollment along with a small cup and spoon to aid administration. The RA will train the caregiver to give the solution to their child and show them how to properly seal the bottle (containing unused solution) following solution provision (Appendix 6). The caregiver will administer the solution to their child in a private room in the clinic, under direct observation by an RA for 3 hours or until the solution has been finished, whichever comes first. Breastfeeding and solid feeding is permitted whenever desired during the 3-hour period.

Direct observation:

The goal of DOs is to directly witness the administration of AA-ORS or placebo solution and instruct caregivers to properly reseal bottles with unused solution. Target observations during the DOs include:

- Perceived palatability of study product by children.
- Perceived acceptable volume of study product by children.
- Ease of administration of study product by caregivers to their child.

Interim visits:
The following day after enrollment, the RA will visit the caregiver in their home to review the instructions and give the caregiver six additional 237-ml bottles. The RA will instruct the caregiver to give doses using the same technique and time limit used in the clinic for the next 20 days, to reseal unconsumed solution in bottles, and store bottles in a safe location (Appendix 6). The RA will help the caregiver to select an appropriate storage location for new and used bottles of AA-ORS/placebo that is out of reach of children and protected from heat and sunlight.

Each day during follow-up, an automated text message (SMS) will be sent to remind caregivers to administer the study product, and a second message (3 hours later) will remind them to mark and reseal bottles using tape and markers provided at enrollment. If automated SMS proves unfeasible, RAs will send SMS reminders to caregivers manually. The RA will visit the home on days 2, 4, 7, and 14 to collect unconsumed solution, review instructions, provide additional bottles (on day 2 and 7), and on days 7 and 14 only weigh the children. All empty or partial bottles will be collected, and unconsumed solution will be measured as part of the longitudinal volume feasibility assessment.

Interval medical history interviews:
Caregivers will receive study nurse contact information and can contact the nurse by phone at any time during the course of the study. Periodically during dosing (days 4, 7, 14, and 21) and 1 week after the end of dosing (day 21), a study nurse will contact the caregiver by phone and check in with the caregiver to assess any potential adverse events or serious adverse events, complete the routine interval medical history/stool frequency questionnaire, ask if the child is still breastfeeding (for children who were still breastfeeding at enrollment), answer any medical questions, and provide advice, as necessary. If breastfeeding has stopped, the caregiver will be asked if the child instituted the stoppage or if the mother decided to wean. All AEs and SAEs reported by the nurse will be reviewed by the study clinician, who will provide medical advice and care as needed.

Post-dosing visit:
Upon return to the clinic (within 3 days of the final dose) the study nurse or clinician will conduct a medical examination, and a trained phlebotomist will draw blood from the child and collect a bloodspot on filter paper (see “Blood specimen collection and processing“). After the medical examination and blood draw, an RA will complete an IDI with the caregiver. For all children who were still breastfeeding at baseline, the IDI will include questions to determine the breastfeeding rate (how many times a day) at the end of the study. The IDI will also assess whether breastfeeding has stopped in the prior week, and if so, if the child instituted the stoppage or if the mother decided to wean.

On day 21, a study nurse will contact the caregiver by phone for a final check-in with the caregiver to assess any potential adverse events 1 week after study product dosing ended.

In-depth interviews

- *Sixty-six IDIs with caregivers, after initial study product use and after prolonged use:*
  The goals of the IDIs are to understand the experiences of the caregivers when administering study product to their child, how they perceive the value of the product, the potential advantages and

disadvantages of study product, and the factors relating to uptake. Topics explored in the IDIs will include:

- Perceived palatability of solutions by children.
- Ease of use, including how easy it was to understand use instructions and administer to the child.
- Behavioral intention to use.
- Perceived acceptability of study product by caregivers of children.

Please see Appendix 2 for IDI guide and DO checklist.

Blood Specimen Collection and Processing

Between 1 and 1.5 ml venous blood will be collected from each child twice—at enrollment and at the post-dosing visit, 15 days after onset of the intervention. A trained phlebotomist will perform venipuncture using a technique appropriate for children who are younger than 2 years old, and a small drop of blood will be blotted from the insertion site and dried on filter paper. Blood samples will be centrifuged into plasma and/or serum within 30 minutes of collection and stored in cryo tubes at less than –20°C until they can be analyzed. Commercial enzyme-linked immunosorbent assays (ELISA) and a Quansys Biosciences Q-Plex™ panel will be used to quantify plasma/serum micronutrients, EED markers (intestinal fatty acid-binding protein [I-FABP], soluble CD14 [sCD14], glucagon-like peptide 2 [GLP-2], insulin-like growth factor 1 [IGF-1], and fibroblast growth factor 21 [FGF21]), and systemic inflammation (alpha-1-acid glycoprotein [AGP] and C-reactive protein [CRP]). Mass-spectroscopy will be used to evaluate metabolic markers from dried bloodspot (e.g., acylcarnitine profile) to ascertain whether there is a tendency for child metabolism to switch to an oxidative state when supplementing a subset of essential amino acids. Detail provided in Table 5, below.

Table 5. Plasma/serum biomarkers assessed in SAFE at enrollment and post-dosing visit.

Biomarker	**Assay name**	**Company**	**µl of plasma required (per well)**
I-FABP	Human I-FABP ELISA	Hycult	25
GLP-2	GLP-2 ELISA Kit	ALPCO	25
IGF-1	Human IGF-I Quantikine ELISA Kit	R&D Systems	20
sCD14	Human CD14 Quantikine ELISA Kit	R&D Systems	10
FGF21	Human FGF-21 Quantikine ELISA Kit	R&D Systems	50
AGP	Q-Plex™ Human Micronutrient (7-Plex)	Quansys	10
CRP			
Ferritin			
sTfR			
RBP4			
Thyroglobulin			
HRP2			
Acylcarnitine	Acylcarnitine profile analysis	Mass spectrometry	40 (80 µl whole blood)
	Total		**320**

Discontinuation of Study Product Use and Withdrawal From the Study

Participants have the right to decline study procedures for any reason and at any time during the study with no penalty. If a participant decides to discontinue study procedures, including AA-ORS or placebo administration, this will be recorded as a study deviation and the reason will be clearly documented.

Participants have the right to withdraw from the study at any time and for any reason, without penalty. If a caregiver does not wish to remain in the study and/or decides to stop administering the solution altogether to his/her child, the participant can choose to withdraw consent and be withdrawn from the study. That decision will be documented, and study staff will attempt to schedule a final post-dosing IDI and medical examination as soon as possible to confirm the welfare of the child.

The principal investigator and site PI may, at their discretion, withdraw a subject from continuing in the study if it is considered to be in the participant's best interest to do so, or if the participant is not willing or able to comply with the study requirements. The reason for withdrawal will be documented. Withdrawn subjects will not be replaced in the study. The data collected before withdrawal will be analyzed.

Withdrawal from further study product administration may be at the discretion of the PI or site PI, in consultation with the study pediatrician if it is in the interest of the subject. In addition, participants will be withdrawn from further study product administration for the following reasons:

- Ineligibility (either arising during the study or retrospectively having been overlooked at enrollment).
- Significant protocol deviation.
- Significant noncompliance with the study product regimen or study requirements.
- Significant weight loss: if a child loses more than 5% of his or her body weight from that measured at enrollment, the child will be withdrawn from the study. The study team will refer the withdrawn child and caregiver to his or her primary health care provider or CGH, as appropriate.
- An AE (including intercurrent illness, diseases, or medical treatment) that requires discontinuation of study product use or results in inability to continue to comply with study procedures (Appendix 2). The study team will refer anyone that develops an acute illness during the study to his or her primary health care provider or CGH, as appropriate.

Sampling Strategy

Estimated sample size: We expect to enroll 132 participants, which includes 66 caregivers, and 66 children (aged 12–24 months); half of these child-caregiver pairs will be randomized to receive AA-ORS (n= 33) and half to receive placebo solution (n= 33). We anticipate that there will be a dropout rate not to exceed 10%. We have incorporated this expected withdrawal rate into the final sample size numbers.

For the primary endpoint of safety in this study, 30 subjects in each arm would result in more than 95% probability of observing at least one AE if the rate of that type of event in the subject population after being exposed to AA-ORS is 10%. To have a 95% probability of being able to detect at least one AE if the rate of that event is truly 5%, the sample size would have to be at least 60 per arm. For more

rare events, the sample size would have to be much larger. Table 6 below shows the probabilities of observing an event with various sample sizes and varying true rates of that event in the population.

Table 6. Probability (P) of observing none, one, or more adverse events (AEs) at different true rates of AEs in the population.

N (per arm)	Rate of AE	P of 0 AEs	P of 1+ AEs	P of 2+ AEs	P of 3+ AEs
20	0.15	3.9%	96.1%	82.4%	59.5%
	0.2	1.2%	98.8%	93.1%	79.4%
30	0.1	4.2%	95.8%	81.6%	58.9%
	0.15	0.8%	99.2%	95.2%	84.9%
40	0.1	1.5%	98.5%	92.0%	77.7%
	0.15	0.2%	99.9%	98.8%	95.1%
50	0.1	0.5%	99.5%	96.6%	88.8%
	0.15	0.03%	99.97%	99.7%	98.6%
60	0.05	4.6%	95.4%	80.8%	58.3%
	0.1	0.2%	99.8%	98.6%	94.7%

Gray highlighted cells are those with a probability of 95% or higher. Blue highlighted cells are those representing a true AE rate of 0.1 or lower.

Increasing the per-arm sample size above 30 (but < 60) does not add much in terms of increasing the probability of detecting an AE with a true rate in the population of 10%. However, 30 subjects per arm has relatively low power for an efficacy endpoint using biomarkers. For example, a sample size of 30 per arm provides approximately 80% power for a t test to detect a 376 ng/ml (21.9% reduction) difference in soluble CD14 (marker of bacterial translocation) between the arms assuming the mean sCD14 is 1,715 ng/ml in the placebo group. These calculations used data from an infant cohort in Zimbabwe (23) (SD = 520 ng/ml).

Recruitment and Consent Procedures

Recruitment of child and caregiver participant pairs:

Children and their caregivers potentially eligible for the study will be identified by clinic staff from the child welfare clinic (CWC), which is the outpatient department where they are administered routine child immunizations (measles and yellow fever at 9 months of age, but sometimes postponed until the second year), healthy child visits, or receive routine Vit. A supplementation (at 12 and 18 months of age). Potential participants will be screened using medical records that include child anthropometry and medical history. Height and weight will be converted into z-scores using an algorithm.

a. Recruitment at clinic visit: During the potential participant's CWC visit, a study RA will introduce the study to the caregiver and ask if they are interested in learning more. Following a short recruitment script (Appendix 5), study staff will inform the caregivers that a research study on a product that may improve pediatric gut health will be tested. If the caregiver is interested in learning more about the study, the caregiver will be provided with a product information form and study consent form to take home and review before returning to the clinic for an enrollment visit. If the caregiver declines enrollment in the study, any medical record data collected will be discarded and shredded.
b. Recruitment from CWC register: Study RAs will identify potentially eligible children from CGH CWC register that lists childrens' names, age, caregiver contact information, exclusive breastfeeding status, and anthropometric measurements from patients receiving immunizations in the first year of life. The hospital has approved using retrospective data from the CWC register for this pre-screening activity. The CWC register is an alternative recruitment

mechanism whereby the caregivers of children who have been seen previously for routine EPI immunizations (such as measles and yellow fever vaccine at age 9 months) will be contacted for potential interest in study participation. The RA will record information into a pre-screening log from the register for children who would currently meet age eligibility requirements, limited to CWC visit date, child age, name, and contact number. RAs will call the caregivers of prospective participants using the telephone number provided and introduce the study to the caregiver and ask if they are interested in learning more. Using a short telephone-specific recruitment script (Appendix 5), study staff will inform the caregivers that a research study on a product that may improve pediatric gut health will be tested. If the caregiver is interested in learning more about the study, they will be invited to set up a screening visit (to be conducted at the CWC) where they will be provided with a product information form and study consent form to take home and review before returning to the clinic for an enrollment visit. If the caregiver is not interested in the study during the phone call and declines to schedule a screening visit, their information in the pre-screening log will be blotted out using dark ink. If the caregiver declines enrollment in the study at the screening visit, any medical record or contact data previously collected will be shredded and discarded or blotted out, as applicable.

Following the visit, those who are still interested and eligible will be scheduled to undergo the informed consent process and enrollment visit at CGH at a time convenient to them, 1–3 weeks after the immunization or Vit. A supplementation visit, or whenever is convenient for those who had been identified from the CWC register (but did not receive immunizations or Vit. A supplementation). The delayed enrollment visit is to avoid conflating potential immunization-related AEs with those related to the study product. Caregivers will receive a reminder call from study staff the day before their scheduled enrollment visit. During the appointment reminder call, caregivers will be advised of changes that would make them ineligible (to avoid an extra trip to the hospital). These changes include diarrhea in household in past week, child acute illness, and child antibiotic treatment within the past 2 weeks. If the child is no longer eligible based on one of these changes, the enrollment visit will be postponed and rescheduled or cancelled, depending on the caregiver's preference. The caregiver will be told that they have the option to decline to participate at the time they are contacted by the study team or any time thereafter.

Children and their caregivers will be recruited as a dual enrollment participant pair. Upon their return to the facility, the RA will escort the caregiver and child to a private exam room where they will review the consent form and answer questions they may have. The RA will confirm that both the child and caregiver still meet both sets of eligibility criteria. The RA will then obtain written informed consent from willing and eligible caregivers prior to the child medical examination, blood draw, IDI, and administration of AA-ORS or placebo solution.

All participants will be informed that the study does not offer any direct benefit to them and the use of this product is currently for exploratory research purposes only. The study does not aim to assess the reliability or compare the outcomes of AA-ORS to the standard of care. The information participants will provide throughout this study is not considered sensitive and will not pose any significant risk to them personally. Participants have the option to decline being interviewed for the study without any negative consequences.

Once informed consent is obtained, participants will be assigned an identification number that will be used to identify the paper forms, solution consumption data, and blood specimens collected from that patient during the study. No personal identifying information will be recorded on any of the data collection tools.

iv. Control or comparison group use

Controls will be children from the same patient population as those receiving AA-ORS. These children will receive a placebo solution containing all the non-amino acid and non-electrolyte components of the AA-ORS and therefore will be able to contribute useful information regarding the feasible dose of the solution. This placebo solution is produced by the manufacturer of the AA-ORS for the purpose of clinical studies. Because these children will not be receiving the amino acids, they will provide the background rate of AEs (and morbidity experience) of children in this population, allowing for the detection of an unusually high event rate in the AA-ORS group, should it arise.

v. Number and age range of research study participants/groups whose data shall be used

Sixty-six children between the ages of 12 and 24 months and their caregivers will be enrolled into the study.

vi. Inclusion criteria for each group of research study participants

Inclusion Criteria

Pediatric and caregiver pairs (must meet inclusion criteria for both categories):

Child:

1. Is between 12 and 24 months of age.
2. LAZ between –1 and –3 SD.
3. At least one week post routine immunization or vitamin A supplementation visit at the study site.
4. Has a parent or legally acceptable representative willing and able to provide informed consent.
5. No plans for travel outside of the community for the duration of the study.

Caregiver of child:

1. Is a parent or legally accepted representative of a child eligible for this study.
2. Is 18 years of age or older.
3. Has a working mobile phone.
4. Is willing and able to provide informed consent.
5. If illiterate—there is at least one literate adult living in the child's household.

vii. Exclusion criteria for each group

Exclusion Criteria

Pediatric and caregiver pairs (must meet none of the exclusion criteria for either category):

Child:

1. Has any sign of acute illness, including but not limited to fever, cough, and diarrhea.
2. Is wasted (weight for length z-score < –2 or mid-upper arm circumference [MUAC] < 12.4 cm) or has pitting edema.
3. Is exclusively breastfed.

4. Is seeking medical attention at the health facility other than for routine, preventative care (e.g., immunization visit, vitamin supplementation).
5. Has suffered within the prior week from illnesses that might impact nutritional status (e.g., severe diarrhea or pneumonia; vomiting; persistent diarrhea; cleft lip or palate; blindness; tuberculosis; jaundice; renal or cardiac disease; cerebral palsy; known metabolic disorders; and chromosomal disorders, including trisomy 21).
6. Medical history of chronic health condition (i.e., HIV, hepatitis B or C, end stage renal disease, severe liver disease—absence of a diagnosis is sufficient).
7. Participating in any other clinical trials.
8. Recent (prior 2 weeks) use of antibiotics or any other medical treatments (including ORT, but not including vaccines or vitamin/mineral supplementation).
9. Cannot give the necessary biological (blood) sample.

Caregiver:

Reports diarrhea in the household in the prior 7 days.

viii. Gender, race, or ethnicity use as variables in participant selection

Not applicable.

ix. Time period in which these data shall be collected and/or stored and time frame for entire study

Data will be collected for this study over a 4-month period, and a permanent master set of data will be kept by PATH for at least 5 years post-completion of the funded project, up to indefinitely. All plasma/serum specimens collected at enrollment and post-dosing visits will be stored in Kenya, analyzed, and destroyed within 5 years. We have included language in the consent form to allow for potential future research with the samples and data related to nutrition or infectious diseases.

x. Monitoring conduct of the study to ensure participants' safety, confidentiality, and data integrity

Study Monitoring

PATH investigators will monitor study activities at the health care facilities where DO and IDIs are conducted. An adverse event case report form (CRF) will be used to record any unanticipated adverse events (Appendix 2). On on-site independent study monitor (ISM) will be contracted to oversee compliance with safety monitoring and stopping rules. The ISM will conduct safety monitoring visits every two weeks during the first month of the study, and at least once per month for each subsequent month of the study. During monitoring visits, the ISM will review safety data for participants, including all AEs and SAEs. The ISM will confirm the severity and relatedness assessment initially determined for all AEs, assess weight loss and breastfeeding practices in accordance with the study stopping rules, and confirm that all appropriate AE follow-up and SAE reporting procedures have been followed.

PATH will also regularly review study records and logs for quality assurance purposes.

Study participant confidentiality will be respected. Site monitoring visits may be conducted to:

- Verify compliance with human subjects and other research regulations and guidelines, including confidentiality procedures, informed consent process, and regulatory documentation.
- Assess adherence to the study protocol and study-specific procedures manual.

- Confirm the quality and accuracy of information collected at the study site and entered into the study database, including the validation of data reported on data collection forms.
- Inspect the study facility and documentation (e.g., informed consent forms, data collection forms) as well as observe the performance of study procedures. If a monitor is present during consent, participants will be asked their permission to accept or decline the monitor observing.

To minimize protocol deviations, study staff will be thoroughly trained on study conduct, safety, and careful data management. In the event of a protocol deviation, the event will be documented in the corresponding protocol deviation CRF and the study team will notify the co-PIs within 24 hours. Protocol deviations will be reported to the appropriate ethics committees in accordance with their reporting requirements.

Study procedures will begin only after the study has received necessary ethical and regulatory approval(s) from relevant parties and written informed consent from the participants.

The Pharmacy and Poisons Board (PPB) may also inspect the clinical trial site and trial sponsor to ensure that the principles of GCP are being met. In order to demonstrate compliance with the protocol and applicable regulations, the PI and site PI will maintain a Clinical Trial Master File containing relevant documentation to allow effective supervision. At the request of a properly authorized officer of PPB, the site PI shall permit such officer to have access to, copy, and verify any records or reports made by the investigator. The site PI will notify the sponsor within 24 hours following contact by a regulatory authority. The sponsor will provide any needed assistance in responding to regulatory audits or correspondence.

Safety Monitoring

The following procedures/evaluations will be conducted as part of the study safety assessment:

- **Medical examination** at enrollment and day 15 of follow-up: assessment of height, weight, MUAC, vital signs (i.e., temperature, pulse, respiration rate), organ systems, breathing, hydration status, fontanelles, and skin irritation/rashes.
- **Counseling procedures** that need to be adhered to during study participation.
- **Assessment of study intervention adherence** via measurement of residual solution will be performed on each bottle (numbered 1–21).
- **Assessment of adverse events** including solicited AEs (i.e., vomiting, diarrhea, fever, constipation, irritability, allergic reactions, etc.) and unsolicited AEs by study nurse initially in person, and subsequently by phone (days 4, 7, 14, 21), and review of any AEs/SAEs by study clinician.
- **Assessment of child weight** by the RA, who will weigh the child at interim visits on day 7 and 14.

To screen children for recent illness or chronic illness (e.g. HIV, hepatitis, etc.), medical charts created as part of regular medical care will be reviewed, as available. Confidentiality of medical records will be protected.

Adverse Events and Serious Adverse Events

All participants will be monitored closely for adverse events during the study at five time points by the study nurse. The nurse will do these assessments 60 minutes after the first administration (in person) and via phone questionnaire on days 4, 7, 14, and 21. All clinical personnel involved in the study will be trained in identifying adverse events and all participants will be educated regarding symptoms that require urgent medical attention. Any adverse events will be managed by the appropriate clinic site, and if necessary, hospitalization. The costs of care directly attributable to participation in the study

will be borne by the study sponsor. The clinical site involved in this study is a Provincial Hospital, which can provide emergency care if necessary.

Definition of Adverse Event (AE)

An adverse event (AE) is any untoward medical occurrence in a participant after administration of the study product and that does not necessarily have a causal relationship with the study product. An AE can therefore be any unfavorable and unintended signs (including abnormal laboratory findings), symptoms, physical examinations, or disease temporally associated with the use of the study product, whether related to the study product or not. This definition includes exacerbations of pre-existing conditions. Stable pre-existing conditions that do not change in nature or severity during the study are not considered AEs; however, these should be reported as part of the medical history.

Solicited AEs are pre-specified adverse events that are actively monitored by study staff during the full 21 days of follow-up. Personal communication with the study product manufacturer indicated that no adverse events have been found to be associated with the AA-ORS in adult studies or in the small study (n=5) featuring children with short bowel syndrome.

For this study, solicited AEs will be assessed by study nurse 30 minutes after the first administration (in person), and then by phone on days 4, 7, 14, and 21.

The following specific solicited adverse events will be monitored for this trial:

- *Vomiting.*
- *Diarrhea.*
- *Fever.*
- *Constipation.*
- *Lethargy.*
- *Irritability/restlessness.*
- *Allergic reactions:* sudden swelling of the face or throat, itching, and rash.
- *Other reactions:* fits (convulsions/seizures); gas, abdominal cramps, or stomach pain; weight loss.

Changes in the severity of an AE (e.g., diarrhea) will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Stopping rule: RAs will weigh children in the household on a weekly basis (end of weeks 1 and 2). If a child loses more than 5% of his or her body weight from that measured at enrollment, he/she will be withdrawn from the study. The study team will refer the withdrawn child and caregiver to his or her primary health care provider or CGH, as appropriate. In addition, there will be a review of the first 10 enrolled breastfeeding participants by the investigators and ISM. If two of the first 10 participants who are breastfeeding at enrollment stop breastfeeding during the 2-week course of the study, the study will halt the enrollment of children who are currently breastfeeding. If there is evidence of a negative impact on participants' breastfeeding, PATH REC will be notified.

Unsolicited AEs are any significant events not specified as a solicited AE by study personnel and can be reported spontaneously by the participant, observed by the study personnel during study visits, or identified during review of medical records or source documents.

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

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

Serious Adverse Event (SAE)

A serious adverse event (SAE) is any adverse event that results in any of the following outcomes:

1. Death.
2. Is life-threatening (life-threatening means that the study participant was, in the opinion of the site PI or sponsor, at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe).
3. Requires inpatient hospitalization or prolongation of existing hospitalization.
4. Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Is an important medical event that may not result in one of the above outcomes but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious adverse event.

Suspected Unexpected Serious Adverse Reactions (SUSARs)

A suspected unexpected serious adverse reaction (SUSAR) is a serious adverse reaction that is not identified in practice, severity, or frequency by the reference safety information. A SUSAR must meet the criteria for an SAE and also be determined to be related to the study product.

Reporting Period and Parameter

Safety events are reported from the time of the first study product administration through completion of the study 21 days later. Specifically, solicited AEs will be collected up to 30 minutes after the first administration and on days 4, 7, 14, and 21. Both solicited and unsolicited AEs and SAEs will be assessed through 7 days after the end of the study product dosing. The final AE and SAE assessment will be done via a phone call on day 21 (or within 3 days thereafter):

1. The caregiver will be contacted via telephone and identity confirmed.
2. Study nurse will ask about any medical event that would constitute an AE or SAE since the last visit.
3. If an AE or SAE is reported, the nurse should record it on the appropriate CRF and consult the study clinician, who will notify the entities who require notification. The nurse will refer the participant for treatment, if warranted.

After recording the information, the participant will be discharged from the study.

Severity of Adverse Events

The severity of all AEs will be assessed by the study clinician based on the following guidelines.

- **Mild**—Events require minimal or no treatment and do not interfere with the participant's daily activities.

- **Moderate**—Events result in a low level of inconvenience or concern. Moderate events may cause some interference with functioning and may require therapeutic measures.
- **Severe**—Events that prevent a participant's usual daily activity and require systemic drug therapy or other treatment. Of note, the term "severe" does not necessarily equate to "serious."
- **Potentially Life-Threatening**—Events that require emergency room visit or hospitalization. Potentially life-threatening symptoms causing incapacitation with intervention needed to prevent disability or death.

A toxicity table for solicited AEs with guides for assessing severity of the specific event is found in Appendix 8.

Causality of Adverse Event

The study investigator/s will determine the causal relationship between the study product and the AE. The causality assessment is made on the basis of the available information at the time of reporting and can be subsequently changed according to follow-up information. Determining of causality is based on clinical judgment and should take into considerations the following factors:

- Is there a temporal (time-based) relationship between the event and administration of the study product?
- Is there a plausible biological mechanism for the study product to cause the AE?
- Is there a possible alternative etiology for the AE such as concurrent illness or concomitant medications?
- Are there previous reports of similar AEs associated with the study product?

For this study, the investigator/s must classify the causality of the AE according to the categories defined below:

Related: There is a reasonable possibility that the product caused the event. "Reasonable possibility" means that there is evidence to suggest a causal relationship between the study product and the AE.

Not Related: There is not a reasonable possibility that the administration of the study product caused the event.

Follow-up of Adverse Event

All reported AEs should be followed until resolution or stabilization, or until the participant's participation in the study ends. Participants who have an ongoing study product–related SAEs at study completion or at discontinuation from the study will be followed by the PI or his designee until the event is resolved or determined to be irreversible, chronic, or stable by the PI.

The outcome of an adverse event will be assessed as at the time of last observation as per the following categories:

- Recovered/resolved without sequelae.
- Recovered/resolved with sequelae.
- Ongoing at the end of the study.
- Death.
- Unknown. The outcome of the AE is not known.

Reporting of SAEs and SUSARs

STUDY PEDIATRICIAN REPORTING TO INVESTIGATORS

Within 24 hours of the study pediatrician's awareness of an SAE as defined in the protocol, an AE/SAE form must be completed and submitted to the study's PIs by email. The ISM will also be notified.

The initial AE/SAE form should be completed with all information known at the time and should include as much as possible the following:

- Name and contact of the investigator submitting the AE/SAE report.
- Participant ID number.
- Participant age.
- Date participant received study drug, including cohort group.
- Description of the serious adverse event.
- Date of event onset and date of event resolution.
- Investigator's assessment of severity, causality, and expectedness.
- Action taken and current status.
- If available, any diagnostic test reports or hospital records that may help the sponsor to evaluate the SAE.

The site PI will be responsible for notifying the Maseno University Ethics and Research Committee and the PPB, and the PI will be responsible for notifying the PATH research ethics committee (REC) as per the respective ethical or regulatory authority's reporting requirements.

Notification and Review of SAEs

The site PI is responsible for evaluating SAEs submitted by the study pediatrician within 24 hours to convene a safety review if the investigator reported the SAE as fatal or life-threatening and suspected to be related to study drug. Medical officer(s) from PATH serving as technical consultants will provide technical guidance regarding SAE management including classification and reporting.

Sponsor Reporting to Regulatory Agency

The sponsor has authorized the site PI to execute its responsibility for safety reporting to the appropriate regulatory authorities within specified time period of notification.

The site PI will be responsible for reporting to the Kenya MOH PPB, in accordance with applicable regulatory requirements.

- All fatal and life-threatening, unexpected adverse study product reactions (i.e., SUSARs) should be reported within 7 calendar days after first knowledge by the applicant. The initial notification must be followed by as complete a report as possible, within an additional 8 calendar days.
- SUSARs that are not fatal or life-threatening must be reported as soon as possible and not later than 15 calendar days after first knowledge by the applicant.
- PPB must be notified, within 15 calendar days after first knowledge by the applicant, when there is a suggestion of a change in the nature, severity, or frequency of expected adverse drug reactions or when new risk factors are identified. The basis on which these assessments are made should be included.
- Any information that may in any way influence the benefit-risk assessment of a medicine or that would be sufficient to consider changes in the administration of the medicine or in the overall conduct of a clinical trial must be reported to PPB.

- A safety report will be submitted to PPB once a year throughout the clinical trial, or on request by PPB, that takes into account all new available safety information received during the reporting period. The aim of the annual safety report is to describe concisely all new safety information relevant for the clinical trial and to assess the safety conditions of subjects enrolled. The safety report shall include a summary of all SAEs and SUSARs.

Safety Oversight

The site PI and designated study staff will be responsible for safety monitoring of all study participants and for alerting the PI if unexpected concerns arise. This study will also have a Data and Safety Monitoring Board (DSMB), composed of two pediatric gastroenterologists (one as chair) and a biostatistician. The DSMB is responsible for safeguarding the interests of study participants, assessing the safety of study procedures, and for monitoring the overall conduct of the study. The DSMB is an independent group advisory to the study investigators. The responsibilities are outlined in detail in the DSMB Charter, which will be approved by the DSMB prior to the study's start.

DSMB meetings will be held remotely. The purpose of the first meeting held before the study begins recruitment is to review and discuss the Charter, to review the protocol and informed consent form, and to confirm the report contents and format for data reports during interim and final DSMB meetings. Enrollment in the study will not begin until the DSMB's Charter has been accepted by the DSMB and IRB/regulatory approval has been obtained. The DSMB is expected to meet twice more over the course of the study. One meeting will be held to review interim data when approximately half of the participants have been enrolled or two months have elapsed after the beginning of recruitment, whichever is earlier. The final meeting will be held at the end of data collection, once topline data is available. Additional meetings or conference calls will be scheduled as needed. At the interim meeting, the DSMB will also review formal interim analyses of the primary (safety) endpoint.

There are no *a priori* reasons for a particular safety concern or the possibility of serious toxicity with the study product. As a small, single-site study for a short duration, the trial pediatrician will be able to have close supervision and monitoring of all AEs. Additionally, an ISM will be contracted to oversee compliance with safety monitoring and stopping rules (e.g., weight loss ≥ 5%, cessation of breastfeeding by 2 of the first 10 breastfeeding participants).

xi. Data management and analysis plan

Data Management Procedures

IDI data will be analyzed qualitatively, using transcripts and notes to identify key themes and topics.

DOs will be manually coded and analyzed to identify the time taken to drink up to 237 ml of AA-ORS, ease of administration, and visible signs of palatability.

Quantitative clinical data focused on demographics, anthropometrics, AEs, product volume consumed daily, plasma/serum micronutrients, metabolic markers (acylcarnitine profile), EED markers (gut dysfunction and growth hormone resistance), and systemic inflammation will be collected on standardized forms or exported to computerized databases by RAs and laboratory workers. Form data will be double entered into a password-protected Microsoft Access database by RAs blinded to the supplementation assignment (AA-ORS vs. placebo). After entering all data and resolving inconsistencies, the data set will be locked.

All data will be stored on password-protected computers in PATH offices. Only members of the study team will have access to the raw transcripts, CRFs, and consent forms in a locked filing cabinet.

Data Analysis Procedures

DEFINITIONS OF POPULATIONS TO BE ANALYZED

The intention-to-treat population will include all participants who are assigned AA-ORS or placebo regimen and receive at least one dose, regardless of whether he/she completes the full 14-day regimen (or misses dose[s] of study product for a medical reason such as vomiting). The first primary (safety), secondary (caregiver acceptability), and exploratory (biomarker) analyses will be performed using this population.

The per-protocol population will include all participants who are assigned a regimen with no major protocol violations that are determined to potentially interfere with the ad-libitum dosing of the study solution(s) (i.e., early withdrawal or product discontinuation, etc.). This population will serve as the primary analysis population for the volume consumption and consumption factor endpoint. As the primary objective is to accurately estimate the average daily volume consumed in the full sample (AA-ORS + placebo), it is critical that all subjects in the population have completed a full dosing regimen.

STUDY ENDPOINTS TO BE ANALYZED AND ANALYTICAL METHODOLOGY

Primary: AEs and SAEs through 21 days of follow-up, and changes to dietary and digestive habits through day 14 of follow-up.

For AEs and SAEs that occur within 21 days of enrollment, statistical comparisons will be made between the AA-ORS and the placebo solution groups using Fisher's exact test. Changes to feeding habits (normal, less frequent/less volume, more frequent/more volume), diarrhea (1–2, 3–4, 5–7, or 7+ events in 24 hours), and vomiting (none, 1, 2–5, > 6 events in 24 hours, or hospitalization) will be made using chi-square tests.

Primary: Average daily product volume consumed through 14 days of follow-up, time trends in daily volume consumed, and factors that influence children's willingness and ability to ingest the product as intended.

The average daily volume (and statistical distribution) of solution consumed by children over the 2-week period will be calculated. These descriptive data will be pooled from AA-ORS and placebo arms as the solutions are designed to taste the same. Lowess curves will be used to describe trends in volumes consumed, and linear mixed effects models will be used to test for an association between day of follow-up and product volume consumed. We will use linear regression to identify factors (e.g., child weight, length, and age) associated with a child's average daily intake.

Secondary: Key factors that influence caregivers' willingness to have the product used by their children, and their perception of their children's response to its consumption.

These qualitative analyses will use ATLAS.ti software to identify and group key themes and topics from IDI notes.

Exploratory: Average plasma/serum concentrations of metabolic, gut dysfunction, systemic inflammation, and micronutrient biomarkers at baseline and day 15 of follow-up.

Metabolic, EED, systemic inflammation, and micronutrient marker data collected at baseline will be pooled across intervention groups and used to generate descriptive statistics for the study population. The same descriptive statistics will be generated for day 15 of follow-up. Paired student's t test will be used to compare these continuous parameters between baseline and day 15 of follow-up.

Unpaired student's t test will be used to compare these continuous parameters between the two study groups at day 22. A difference with P < 0.05 will be considered statistically significant. However, there is limited statistical power to make such comparisons between study groups for biomarker outcomes.

ISSUES WITH MISSING DATA AND MULTIPLICITY

We will analyze whether there is differential missingness or loss to follow-up by supplementation arm for the primary endpoints. No imputation of missing outcome data is planned. We will adjust the significance level for multiple comparisons by using the Benjamini-Hochberg method to control for the false discovery rate for related hypotheses.

Quality Assurance

The study will be conducted in full compliance with the protocol and ICH GCP to provide public assurance that the rights, safety, and well-being of trial participants are protected and that the clinical trial data are credible. To ensure quality and standardization, the site will develop standard operating procedures (SOPs) for key protocol procedures and conduct the study guided by the study *Manual of Procedures* or other written guidelines. The site will also develop routine operational checks to verify that critical protocol requirements and procedures are executed correctly and completely at the time the work is being performed. Prior to the initiation of the study, the sponsor will conduct training on the protocol, including applicable SOPs, for study staff.

Clinical and laboratory data for each participant will be abstracted from study CRFs into standardized project files. These data will be entered directly into computers at a centralized site. Information will be cross-checked for accuracy on a biweekly basis. All data, both hard- and soft-copy formats, will be stored in locked cabinets initially at CGH and will be transported to the PATH office in Kisumu after data entry with limited access by project staff only.

Participants will be identified using a unique Patient Identification Number (PID). The code linking the PID to individual identifying information will be kept in a separate secure location by the project coordinator. Clinical staff at CGH will not be able to access identifying information linked to the PID, except to match laboratory results reported by the lab to individual patients.

xii. Study limitations and the expected results

Study Limitations

This is an exploratory study with a small sample size that is not adequately powered for statistical comparisons of the EED outcomes (non-invasive biomarkers). As a result, the findings of this study may not be widely generalizable. This limitation will be mitigated during presentation of the study findings, which will avoid drawing conclusions beyond the scope of the study methods. The expected results from the study are intended to be useful for establishing a safety profile and reasonable pediatric dose and providing study design considerations (e.g., child EED biomarker data) for future research on the efficacy of AA-ORS and the deployment of similar EED interventions in LRS.

xiii. Itemized budget and budget justification

Budget

Category	**Amount (USD)**
PERSONNEL	[redacted]

TRAVEL	[redacted]
EQUIPMENT & SUPPLIES	[redacted]
WORKSHOPS & TRAINING	[redacted]
PARTICIPANT SUPPORT COSTS	[redacted]
OTHER PROJECT COSTS	[redacted]
Overhead	[redacted]
Total	[redacted]

Budget justification

The largest budget item for this study is personnel costs, totaling USD [redacted], which includes salaries and benefits for the study PIs and co-investigators as well as the research assistants, clinical staff, and lab personnel required to implement a quality study. Travel costs of USD [redacted] include international travel for non-Kenyan investigators to attend study trainings and site visits as well as domestic transportation for study staff to travel to the study site. Equipment and supply expenses include paper and printing costs for study materials, biomarker assays, and acylcarnitine mass-spectrometry, totaling USD [redacted]. Workshops and trainings are budgeted to cost USD [redacted] and include the initial training of all study staff on the protocol and consenting process to be followed for this study. A total of USD [redacted] is budgeted for participant support costs to pay individuals according to the compensation scheme outlined in the informed consent document. Other project costs of USD [redacted] include fees for ethical and regulatory submissions, shipment and procurement of study product and placebo, legal and contracting support, among other miscellaneous expenses.

xiv. Research study data collection forms and other references

Appendix 1 – Informed Consent Form

Appendix 2 – Case Report Forms (CRF)

Appendix 3 – Patient Information Leaflet

Appendix 4 – Investigator's Brochure

Appendix 5 – Recruitment Scripts for Caregivers

Appendix 6 – Patient Instruction Sheet

Appendix 7 – Letter of Support From Robert Bandsma

Appendix 8 – Toxicity Table

D. ETHICAL CONSIDERATIONS

This study will be conducted under the auspices of the following ethics committees (ECs):

- PATH Research and Ethics Committee (REC)
  2201 Westlake Avenue, Suite 200
  Seattle, WA 98121
  USA
  rec@path.org
  +1 206 285 3500
- Maseno University Ethics Review Committee (MUERC)
  Directorate of Research, Publications and Innovations
  Maseno University Main Campus
  Along Kisumu-Busia Road
  P.O. Box, Private Bag
  Maseno, Kenya
  muerc-secretariate@maseno.ac.ke
  +254 57 351 622 Ext. 3050

The PI and site PI will be responsible for obtaining approval from the ECs listed above. These committees will review and approve the protocol, informed consent form, and any recruitment materials (advertising or informational material), including any modifications to these documents prior to or during the study. All changes to the protocol or consent form must be reviewed and approved prior to implementation, except where necessary to eliminate apparent immediate hazard to study participants. The PI and site PI also will be responsible for obtaining continuing review throughout the duration of the study in accordance with existing regulations.

i. Risks to research study participants/groups

Risk Considerations

The proposed study is expected to be of minimal risk as the study AA-ORS is a medical food product regulated by the US FDA. As a result, we do not anticipate any adverse events for this study related to the administration of AA-ORS, but we will include a control arm in order to better assess the safety of this product in children with mild-moderate stunting. Children with mild to moderate stunting are at increased risk for morbidity and mortality, so we anticipate that participants may report diarrhea or respiratory infections at high rates, regardless of receipt of active AA-ORS or placebo. While there is mounting evidence that microbiome immaturity contributes to growth deficits in LRS, any changes to the microbiome composition and diversity from provision of AA-ORS are likely to be transient, based upon previous observations among infants and children provided probiotic-supplemented formula or ready-to-use supplementary food (43, 44).

This study involves blood specimen collection. The collection of these samples involves venipuncture, which may cause discomfort, pain, introduction of infection, bleeding, fainting, or bruising. Precautions will be taken to avoid introduction of infection by disinfecting the site of venipuncture and using sterile equipment. The risk of bleeding and bruising will be minimized by immediate application of pressure after venipuncture. The child will be restrained appropriately by the caregiver to prevent kicking of the phlebotomist. A thorough description of potential risk considerations and mitigation strategies is outlined in Table 7.

Table 7. Known risks and mitigation strategies for SAFE study participants.

Potential risk to participant	**Concern**	**Study design feature intended to minimize risk**	**Rationale for the necessity of exposing participants to risk**
Reduction in breastmilk consumption among those breastfeeding at enrollment.	Consumption of solution (AA-ORS or placebo) could reduce amount of breastmilk consumed by child.	Exclusion of exclusively breastfed children. Caregivers instructed to provide breastmilk as needed during the 3-hour dosing period. Monitoring for decreased frequency of breastfeeding and for unplanned (by caregiver) weaning. Will stop the study if it is clear there is a major reduction in breastfeeding from the study drink.	Ingestion required for product delivery. Overall caloric content of AA-ORS unlikely to satiate children given daily intake requirements.
Use in pediatric populations who are acutely ill.	Children experiencing mild-moderate stunting are at increased risk for morbidity and mortality and may develop intercurrent illnesses during the course of the study.	Will recruit children with mild to moderate stunting (length-for-age z-score between –1 and –3), but not acute illness at enrollment. Safety monitoring procedures in place to identify and treat adverse events.	Children with stunting, with or without acute illness, overlap considerably with the target population for an EED intervention.
No safety data in children.	Prior safety data on the study AA-ORS is from adult populations and may not apply to pediatric subjects.	Will directly observe the first dose of the product in the clinic to identify any acute reaction. Will treat all participants who experience adverse events during follow-up.	Study is needed to establish the safety profile of the product. May also assess whether children's metabolism switches to oxidation.
Spoilage/contamination.	Open AA-ORS bottles could pose a health risk if consumed at a later time, due to contamination.	Mark and reseal bottles using tape and markers provided at enrollment. Research assistant (RA) to visit home on second and fourth day of follow-up to instruct and advise caregiver on safe storage and proper resealing of bottles after maximum of 3 hours.	Need to measure and calculate the average daily volume consumed during follow-up in order to guide dosing recommendations in future clinical trial.

Potential risk to participant	Concern	Study design feature intended to minimize risk	Rationale for the necessity of exposing participants to risk
		RAs to pick up partial and empty bottles 1–2 times per week.	
Volume of solution too great.	237 ml may be too much volume for children.	Children to drink as much or as little of the solution (up to 237 ml) as desired over 3 hours. Gavage will not be done. Breastmilk will be permitted throughout this period.	Need to measure and calculate the average daily volume consumed during follow-up in order to guide dosing recommendations in future clinical trial.
Blood draw in children.	Potential discomfort, bleeding, bruising, and minimal risk of infection.	Precautions will be taken to avoid introduction of infection by disinfecting the site of venipuncture and using sterile equipment. The risk of bleeding and bruising will be minimized by immediate application of pressure after venipuncture. Only trained, experienced phlebotomist will perform venipunctures.	Blood samples are needed in order to measure metabolic profile and biomarkers of gut dysfunction, systemic inflammation, and micronutrient status. Metabolic profile assessment will be an important part of exploratory analysis; it is helpful to determine if the absence of certain essential amino acids leads to increase in oxidative pathways. These biomarker measurements are important in order to have setting-specific descriptive data with which to power a future EED efficacy trial. A comparison of these non-invasive biomarkers following the intervention will also be done.
Loss of confidentiality.	Medical information from a participant's medical record or other	Access to study records will be limited to study staff with electronic passwords (data files) or	Review of medical records will help ensure that only eligible

Potential risk to participant	Concern	Study design feature intended to minimize risk	Rationale for the necessity of exposing participants to risk
	study documents are shared inappropriately.	lock and key (physical records). Study staff will only review medical records for eligibility assessment and will not make copies or remove such records from the hospital premises. If the caregiver declines enrollment in the study, any medical record data collected will be discarded.	participants are enrolled in the study. Detailed study records are critical for data analysis and reporting results accurately.

The information participants will provide in the context of this study is not considered sensitive and will not pose any significant risk to them personally. There is a minimal risk of loss of confidentiality. All study staff will be trained in the protection of human subjects, including maintaining participant confidentiality. Study procedures will begin only after the appropriate ethics committees grant approval.

As the sponsor of this study, PATH will secure clinical trials insurance, which will cover the costs associated with any study-related injuries.

Benefits

No direct benefit is anticipated to participants for their inclusion and participation in this study. Information collected during this study may benefit the participants' community in the future for potential EED interventions administered in an aqueous solution, including but not limited to the study AA-ORS.

Rights of the Study Participants

Provisions will be made to protect the participants in this study. Confidentiality, anonymity, and storage of data and specimens will be explained to all participants or their caregivers (i.e., parent or legally acceptable representative). The participants will be informed that they may withdraw from this study at any time with no negative repercussions for themselves or the child participant. All data collection tools and biological samples will be identified only with the individual study participant number and study title. It will be made clear that only the study team will have access to the data that emerges from this study and the data will be only used for research purposes.

ii. Access to protected health information required by proposed research study

The study will have access to protected health information. The use of patient medical records will be necessary to help ensure that only eligible participants are enrolled in the study (e.g., confirm the child does not have chronic health conditions such as HIV, hepatitis B, or hepatitis C).

iii. Sources of data, specimens, and records to be used in the proposed research study

Data Collection Procedures

Data collection tools (e.g., IDI guides and questionnaires) will be administered by study staff in the language of the caregiver's preference. Participants will be allowed to ask questions in order to obtain clarification on study procedures.

Clinical information

Adverse events will be assessed via clinician-directed physical examination (screening, enrollment visit, and day 15 visit) and signs and symptoms reported by participants (days 1, 4, 7, and 14). Data collected at interim visits will use a structured questionnaire to record interval medical history. All clinical information collected as part of this study will be stored on password-protected computers in PATH offices.

IDIs:

Interviews will be conducted by an RA trained in qualitative methods. During the IDIs, the interviewer will guide the flow of the discussion. Any other member of the IDI team present will take note of topics that generate more animated discussions, participants' attitude, body language, and interactions among the interviewer and interviewee. Notes will be documented in an unstructured format and saved as part of the participant study record. Discussions will be guided by a semi-structured data collection guide (Appendix 2), with responses hand-written contemporaneously by the interviewer.

The interview guides will be a mix of structured and semi-structured questions. Structured questions will be necessary for standardizing responses to particular questions. Semi-structured questions will allow for flexibility and richness in responses, especially those on perceptions of facilitators and barriers for AA-ORS uptake. Semi-structured questions are open-ended and will allow for probing a participant's answers for in-depth responses.

Post-dosing IDIs with caregivers will be conducted after use of study product with their child in a private setting (Appendix 2). Caregiver interviews will last between 45 to 60 minutes at enrollment and post-dosing visits.

DO of product administration:

DO will be conducted with caregivers and their child during the very first study product administration at the enrollment visit. AA-ORS or placebo solution will be provided to participants by study staff. The DO will last up to three hours, depending on how quickly the child drinks the product. The researcher will take notes contemporaneously during the DO.

Blood samples:

All samples collected as part of this study will be stored in freezers at Kakamega CGH. Samples may be shipped to Kilifi and/or Nairobi for analysis at laboratories operated by Kenya Medical Research Institute (KEMRI).

iv. Maintaining confidentiality of research study data and protecting the participant privacy

All participants will be assigned unique identification numbers in place of their names to maintain confidentiality. All data will be stored on password-protected computers in PATH offices. Only members of the study team will have access to the raw transcripts and consent forms in a locked filing cabinet.

v. Storage of data and specimens being collected

All data will be stored on password-protected computers in PATH offices in Kenya and the United States. All plasma/serum specimens will be labeled with alphanumeric patient and sample identification numbers and stored in a freezer at Kakamega CGH until shipment. Specimens will be prepared for shipment on dry ice in compliance with recognized standards for air transport and will be transported to KEMRI labs in Nairobi and/or Kilifi, Kenya by a third-party shipper with experience with biological specimens. Once the samples have arrived at the KEMRI laboratory, they will be unloaded into a freezer (temperature ≤20° C), before being assessed using the immunoassays described in Table 5. Dried bloodspots will be shipped to KEMRI or another third-party laboratory in Kenya with the necessary mass spectrometry equipment to run metabolomic analyses. All plasma/serum specimens collected will analyzed for the study, and remaining specimens will be destroyed within 5 years. We have included language in the consent form to allow for potential future research with the samples and data related to nutrition or infectious diseases.

vi. Duration of data storage, ensuring data security, and custodian and staff with data access

A permanent master set of data will be kept by PATH at least 5 years post-completion of the funded project, but may be stored indefinitely. All data will be stored on password-protected computers in PATH offices. Only members of the study team will have access to the raw transcripts and consent forms in a locked filing cabinet. De-identified patient data will be shared with the manufacturer of the product.

vii. Plan for controlling access to stored research study data

All data will be stored on password-protected computers in PATH offices. De-identified patient data will be shared in an electronic format with the manufacturers of the AA-ORS. Explicit permission from PATH is required before the manufacturers can license the de-identified study data to a third party.

viii. Viewing of identifiable research study data by persons unlisted as applicant or study team member

Research assistants, study nurse, and study pediatrician will be contracted to conduct the IDIs and other study procedures as outlined above. These individuals shall collect and or view research study data with identifiers during enrollment and data collection activities in order to carry out visits and project activities. The RAs will not have access to the identifiable data once it has been entered into an electronic format and data cleaning and analysis is complete.

ix. Signing of agreement(s) by research study team members with access to confidential data collected

All study staff will have signed agreements protecting confidentiality of research data prior to conducting any study activities.

x. Potential benefits of research study to community and/or society

Benefits

No direct benefit is anticipated to participants for their inclusion and participation in this study. Information collected during this study may benefit the participants' community in the future for potential EED interventions.

xi. Consent issues: how consent will be obtained

Consent Process

Informed consent is the process of ensuring that potential participants fully understand what will and may happen to them while participating in a research study. An information sheet describing the study product (Appendix 3) will be provided to participants before the consent process begins. All consent materials, including any non-English translations, will be approved by the PATH REC and MUERC prior to use.

In the region where this study is conducted, Luhya is the predominant ethnic group. However, there is no single Luhya language and there are over 10 different Luhya dialects spoken and mutually understood. Kiswahili is commonly spoken and understood as well, so documents translated into the local language will be translated into Kiswahili. Study staff will be conversant in local dialects in addition to Kiswahili to help ensure adequate comprehension of the consent materials.

All study staff will be trained and certified in the protection of human subjects. In obtaining and documenting informed consent, the study staff will comply with applicable local and domestic regulatory requirements and will adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki.

Before an individual is enrolled in this study, it is the site investigators' responsibility to ensure that informed consent is obtained from the participant after adequate explanation of the aims, methods, and potential risks and benefits of the study. The consent form will be reviewed and discussed with potential participants in a private space where others cannot easily overhear the conversation. The informed consent process will give individuals all the relevant information they need to decide whether to participate in this study. Potential research participants will be permitted to ask questions and to exchange information freely with the study team prior to providing written consent. We will not restrict enrollment to literate individuals, as we intend to recruit patients with caregivers from a variety of education levels. However, the requirement for caregivers to own a cellphone is closely associated with basic literacy. In the case of an illiterate potential participant, an independent witness must be present for the entire consenting process and will have to sign and date the informed consent form. In addition, the illiterate participant will also put a thumbprint on the consent form. Consent will be documented by having the participant sign and date the appropriate consent form. The study staff obtaining consent will also sign and date the consent form. A signed and dated copy of the consent form will be given to the participant.

Cost/Volunteer Benefits

There is no financial cost to the participants in this study. Caregivers in this study will receive a nominal compensation for their time and involvement. Compensation values and distribution mechanism will be documented in the informed consent form, and the value for the enrollment/consent visit will be greater than that of the day 15 visit based upon the longer duration of the initial visit. PATH will provide appropriate treatment or reimbursement for the treatment of injuries directly attributable to participation in this study.

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F. APPENDIX 8 – TOXICITY TABLE

Solicited symptoms grading scale[1]

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially Life-Threatening
CLINICAL				
Clinical adverse event NOT identified elsewhere in the grading table	Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death
Dermatologic				
Pruritus [Itching] (without skin lesions)	Itching causing no or minimal interference with usual social & functional activities	Itching causing greater than minimal interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities	NA
Rash	Localized rash	Diffuse rash OR Target lesions	Diffuse rash AND Vesicles or limited number of bullae or superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Stevens-Johnson syndrome OR Toxic epidermal necrolysis

[1] Adapted from the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, version 2.1, March 2017, of the United States National Institutes of Health. This table is available at https://rsc.tech-res.com/clinical-research-sites/safety-reporting/daids-grading-tables.

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially Life-Threatening
Gastrointestinal				
Bloating or Distension	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Constipation	NA	Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated	Life-threatening consequences (e.g., obstruction)
Diarrhea *< 1 year of age*	Liquid stools (more unformed than usual) but usual number of stools	Liquid stools with increased number of stools OR Mild dehydration	Liquid stools with moderate dehydration	Life-threatening consequences (e.g., liquid stools resulting in severe dehydration, hypotensive shock)
Vomiting	Transient or intermittent AND No or minimal interference with oral intake	Frequent episodes with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Neurologic				
Seizures *< 18 years of age (includes new or pre-existing febrile seizures)*	Seizure lasting < 5 minutes with < 24 hours postictal state	Seizure lasting 5 to < 20 minutes with < 24 hours postictal state	Seizure lasting ≥ 20 minutes OR > 24 hours postictal state	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)
Systemic				

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially Life-Threatening
Acute Allergic Reaction	Localized urticarial (wheals) with no medical intervention indicated	Localized urticarial with intervention indicated OR Mild angioedema with no intervention indicated	Generalized urticarial OR Angioedema with intervention indicated OR Symptoms of mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR Laryngeal edema
Lethargy	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating symptom of lethargy causing inability to perform basic self-care functions
Fever	38.0 °C to < 38.6 °C	≥ 38.6 °C to < 39.3°C	≥ 39.3 °C to < 40.0°C	≥ 40.0 °C
Weight Loss	NA	5% to < 9% loss in body weight from baseline	≥ 9% to < 20% loss in body weight from baseline	≥ 20% loss in body weight from baseline OR Aggressive intervention indicated (e.g., tube feeding, total parenteral nutrition)