

1. Purpose

Provide a brief explanation of the proposed research, including specific study hypothesis, objectives, and rationale.

The goal of the WASH Benefits study is to generate rigorous evidence about the impacts of sanitation, water quality, and handwashing (WASH) and nutrition interventions on child health and development in the first years of life. The primary hypotheses of the study are:

- H1: Water, sanitation, handwashing, nutrition and their combination improve child health and development.*
- H2: When delivered in combination, water, sanitation and handwashing interventions reduce child diarrhea more than when delivered individually.*
- H3: Combined nutrient supplementation and WASH interventions improve child growth and development more than nutrient supplementation alone.*

The study objectives are to:

1) Rigorously measure health benefits arising from low-cost approaches including local promoters and subsidies for simple technologies (e.g. latrine improvements or potties for children, chlorine dispensers, and handwashing stations) and evaluate the degree to which, in resource-constrained settings, there is added health benefit to delivering multiple interventions concurrently (sanitation services, drinking water, and handwashing promotion)

Most of the burden of diarrheal disease is thought to be preventable with improvements in sanitation, water quality, and hygiene. However, in rural areas of low-income countries it is often prohibitively expensive to provide residents with networked sanitation and water treatment that provide microbiologically and chemically safe water and consistently separate feces from the environment. We have almost no evidence that allows direct comparison of the health benefits or cost-effectiveness of improvements in sanitation, water quality, and hygiene, nor on how the benefits of these interventions aggregate when provided in combination. Such evidence is critical for guiding the allocation of public and donor funds to achieve the maximum health impact given limited resources. In the absence of credible evidence, little change from the *status quo* can be expected, even if the impact of current practices is unknown. A rigorous evaluation of these interventions that documents changes in outcomes associated with long-run economic success, such as early childhood growth, could have at least two important influences on policy. First, such evidence could help to maximize the value of existing resources by shifting expenditures to the most cost-effective interventions. In addition, such evidence could help generate more resources for these sectors by resolving uncertainty regarding the efficacy of water, sanitation, and hygiene interventions and identifying simple technologies and approaches to behavior change that cost-effectively improve health and could be replicated at scale.

2) Measure the impact of lipid-based nutrient supplementation (LNS) alone and in combination with sanitation, water and hygiene interventions on child growth and development.

For children whose food intake is insufficient, LNS helps reduce gross energy shortfalls and provide essential micronutrients. The energy and micronutrients that LNS provides are likely to improve nutritional indicators, length-for-age and child development, particularly among children that are at highest risk for severe stunting. It is possible that improved nutrition alone can reduce the negative effects of infection on growth and development due to the improved ability of

better-nourished children to fight off enteric infections and exhibit catch-up growth during the convalescent period.

A combined LNS+WASH intervention could have greater impacts on growth and development than LNS alone. The reasons for this are two-fold. First, the likely reciprocal relationship between enteric infection and malnutrition in young children suggests that the provision of joint interventions that interrupt both components of the “vicious cycle” may have effects that exceed interventions that interrupt just one component. Indeed, if there is a reciprocal relationship between enteric infection and malnutrition, the feedback loop toward decline could, in principal, be reversed and leveraged to enhance growth and development. There is a second plausible scenario that could result in larger combined effects: if the interventions are deployed together, the available energy for growth and development could be enhanced by improved utilization of the additional nutrition provided by LNS that would have not been available to the child without complementary infection control. Such improved utilization could be achieved by reductions in acute diarrheal disease and the chronic symptoms that characterize environmental enteropathy.

3) Measure the impact of nutritional supplements and household environmental interventions on environmental enteropathy biomarkers, and more clearly elucidate this potential pathway between environmental interventions and child growth and development.

If improvements in sanitation, water quality, and hand hygiene could reduce the severity of intestinal malabsorption from environmental enteropathy (EE) either by preventing its acquisition or by reversing the pathology, this would represent an important contribution to global public health. EE is an inflammatory disorder of the small intestine that results in reduced nutrient absorption and increased gut permeability to pathogens (and thus increased immune system stimulation). The scientific literature to date suggests that EE is most likely caused by poor sanitation, water quality and hygiene in low income countries, but there are no studies that demonstrate a specific association between EE and environmental conditions separated from other exposures in a low income environment. The WASH Benefits study is uniquely positioned to gather randomized evidence about the impact of household environmental interventions on EE in young children. Furthermore, the study will also be positioned to gather rigorous evidence about the independent and combined impact of supplemental nutrition and WASH interventions on EE biomarkers.

4) Measure the impact of WASH and nutrition interventions on intestinal parasitic infection prevalence and intensity. Measure the association between parasitic infection and other measures of enteric health, including acute diarrhea and environmental enteropathy biomarkers.

Observational studies suggest that environmental interventions can reduce parasite infection if sanitation and hygiene conditions improve in a large share of the population. Pre-school aged children spend much of their time in the immediate home environment. It is with this in mind that the WASH Benefits study focuses on household environmental interventions. There is a large body of evidence that documents household level clustering and within-household transmission of *Cryptosporidium*, *Giardia*, and *E. histolytica*, as well as soil-transmitted helminths (*Ascaris*, *Trichuris*, and hookworm). The observed patterns suggest that intestinal parasites may be a useful marker of enteric pathogen transmission more broadly. The simultaneous measurement of parasitic infections, caregiver-reported diarrhea, and environmental enteropathy biomarkers will allow us to explore this secondary hypothesis.

5) Estimate the cost-effectiveness of water, sanitation, handwashing, and nutritional interventions, delivered at scale alone and in combination, incorporating potential spill-over benefits from households enrolled in the study to the rest of the community.

2. Background (include citations and attach bibliography in Attachments section)

An estimated 2.2 million children under the age of 5 years die from diarrheal disease each year (WHO 2008). Children who survive multiple episodes of diarrhea and enteric infections commonly develop environmental enteropathy, an inflammatory disorder of the intestines that compromises nutrient absorption (Haghighi 1997). Repeated episodes of diarrhea and chronic environmental enteropathy in early childhood reduce growth and cognitive function, and impair school performance (Alderman 2006, Checkley 2008, Lornitz 2006, Niehaus 2002, Petri 2008). This in turn can reduce income later in life (Boissiere 1985). Thus, repeated episodes of childhood diarrhea and enteric infection may exact a long-run toll, perpetuating a cycle of poverty and ill health.

Water, Sanitation, and Handwashing Interventions

Most of the burden of diarrheal disease is thought to be preventable with improvements in sanitation, water quality, and hygiene (Ezzati 2003). However, in rural areas of low-income countries it is often prohibitively expensive to provide residents with networked sanitation and water treatment that provide microbiologically and chemically safe water and consistently separate feces from the environment.

This has led to a movement towards alternative non-networked solutions including improved sanitation efforts, efforts to increase water treatment by households, and programs to increase handwashing with soap. Observational studies suggest that reducing open defecation is potentially important in reducing the transmission of both diarrhea and trachoma (Esrey 1996, Esrey 1991). There is evidence that water treatment, in particular with dilute chlorine solution, can reduce self-reported diarrhea (Arnold 2007, Clasen 2007). There is also strong evidence that handwashing with soap can dramatically reduce self-reported diarrhea as well as other diseases (Ejemot 2008, Rabie 2006). Some researchers however have called for evidence on more objective measures such as physical growth and cognition, rather than reports by family members (Schmidt 2009). We have almost no evidence that allows direct comparison of the health benefits or cost-effectiveness of improvements in sanitation, water quality, and hygiene, nor on how the benefits of these interventions aggregate when provided in combination.

Improved Sanitation

Improved sanitation effectively separates human excreta from human contact and the environment. The most common sanitation technologies in developing countries, particularly in rural areas, are various forms of private latrines. Observational studies suggest that households that receive improved sanitation experience 24% less diarrhea than households without sanitary facilities (Daniels 1990, Barreto 2007). However, there has never been a randomized controlled trial (RCT) to confirm these observational findings.

Full subsidies for appropriately designed latrines have resulted in high levels of coverage and regular use. For example, in the Gambia improved pit latrines were provided free of charge to 666 households in 32 villages. After 25 to 47 months each household was revisited; 77% of the

provided latrines were still in use and 97% of latrines owners said they would make a new latrine when their current one was full (Simms 2005). In an evaluation of one of the Carter Center's subsidized latrine provision programs that included a random sample of 200 households across 50 villages in Niger, 86% of latrines were in regular use and 70% were clean after one year during unannounced visits (Diallo 2007). In southern India, a recent observational study found that despite high levels of latrine coverage (57%) following a community mobilization campaign, 40% of households with toilets continued to defecate in the open and there was no improvement in child diarrhea or growth – suggesting that health impacts are not guaranteed by high coverage and are likely context dependent (Arnold 2010).

Handwashing Promotion

There is significant evidence from randomized controlled trials that households receiving intense encouragement to regularly wash their hands with soap have less self-reported diarrhea and respiratory disease than households who continue their normal hand hygiene practices (Ejemot 2008, Rabie 2006). However, the existing evidence comes from trials which used very intensive interventions to encourage handwashing interventions that would not be practical to implement at scale. A key issue is the difficulty in knowing how to encourage greater take up of handwashing at reasonable cost.

One key barrier to handwashing is the difficulty and high use of water required in filling a basin, washing hands in the basin, and emptying the basin. In an observational evaluation in rural Bangladesh, households that had soap or water at their most convenient place to wash hands were twice as likely to wash their hands with soap after fecal contact than households that lacked these essential supplies (Luby 2009). Of course causality is not clear in the absence of a randomized controlled trial. A variety of simple low-cost handwashing stations have been developed which provide a place to wash hands and a source of flowing water. These can be as simple as plastic containers which one can tilt to create a stream of water by pulling rope with one's feet, or plastic containers with plugged with sticks, which release a trickle of water when the stick is removed. A well-placed handwashing station provides a visual cue to spur handwashing and greatly increases the convenience of handwashing. There is considerable evidence across the social sciences that convenience is a key factor in promoting behavior change. In fact, one important lesson from the literature on behavioral change is that making something easy can be more effective at inducing change than education or promotional messaging (Kaplan 1986, Sallis 2008, Kremer 2009). Additionally, if they are placed outside of the latrines they are at least partially in public view, and there is evidence at least in a western context that people are much more likely to wash hands after using a toilet if they believe they believe they may be observed (Pederson 1986, Ram et al 2010).

Water Treatment

There is little evidence that providing water supplies that meet the engineering definition of "improved" lead to health or social benefits for the population. In contrast, evidence from settings where diarrhea is a leading cause of death shows that improving the microbiological quality of drinking water markedly reduces reported diarrhea (Clasen 2006, Fewtrell 2005, Arnold 2007). Randomized controlled trials conducted using various household-based point-of-use water treatment technologies have demonstrated that households that consume regularly treated water report substantially less diarrhea than households using untreated water. However, self-reported diarrhea, which is the outcome measure for the majority of these studies, may be subject to measurement error potentially correlated with treatment. For this reason, long-term objective outcomes such as those planned for the proposed study (i.e. anthropometric measurements, cognition, and environmental enteropathy) may prove more

convincing from a policy perspective and may be more likely to motivate action from policy makers (WHO & UNICEF Progress on drinking water & sanitation 2008)

Chlorination is the household water treatment solution of choice in many contexts given its safety and cost-effectiveness. It has been used in piped water systems around the world for almost a century. In many places where such infrastructure is absent or imperfectly maintained, dilute chlorine solution is marketed as a consumer good used in the home. Although chlorinating household drinking water reduces reported diarrhea by 20-40%, take-up has been low under the current social marketing model. Members of the study team for this proposal have developed and piloted a chlorine dispenser in rural Kenya, which is a simple device that is installed at communal water sources to enable water treatment at the point of collection. When the dispenser is provided along with a local promoter to encourage its use, we find that take-up is on the order of 60-70% in communities with access to a dispenser, as compared to 5-10% in communities with access only to the traditional model of chlorine distribution. Moreover, in contrast to other strategies that we tested, chlorine use appears to be stable or even rising over time, likely because the dispenser technology makes water treatment cheap and easy for users, and harnesses positive peer effects: the public nature of the dispenser allows community members to implicitly and explicitly remind each other to treat their water.

Our approach to household water treatment will be to provide a community-level chlorine dispenser for disinfecting water at the point of collection, supplemented by bottled chlorine for use in the home when the study compound members collect water from sources without dispensers, including rainwater collection. In Kenya, the study will use dilute chlorine (marketed locally under the brand name WaterGuard)) for the water quality intervention.

Nutrition Intervention

There is abundant evidence that the prenatal period and the first two years of life are a critical window for intervention in growth and development: infection and poor nutrition during this window can negatively impact an individual's long-term cognitive development and lifetime physiologic trajectory (Checkley 2003, Berkman 2002, Black 2008, Guerrant 1999, Niehaus 2002, Tarleton 2006, Bhutta 2008, Crimmins 2006, Grantham-McGregor 2007, Victora 2010). Nutritional interventions during the first years of life improve schooling and income in adolescents and adults up to 35 years later (Victora 2008, Hoddinott 2008). Yet, a systematic review of the impacts of complementary feeding and supplementation interventions reports that even the most successful of these interventions increase length-for-age Z-scores by 0.69 SDs, which is approximately 1/3 of the mean growth deficit for African and Southeast Asian populations (the mean intervention effect is 0.28 SDs) (Dewey 2008).

One hypothesis for why nutritional supplementation appears to be necessary but not sufficient to eliminate growth shortfalls is that chronic infection and colonization of the gut by fecal bacteria impedes nutrient absorption and creates low-level immune system stimulation, a condition called environmental enteropathy (Lunn 2000). Environmental enteropathy is characterized by damage to mucosa in the wall of small intestine that decreases its surface area for nutrient absorption and increases its permeability to antigenic molecules that stimulate immune system defenses (Lunn 2000, Campbell 2003). Biomarkers for intestinal permeability and immune system stimulation have been more strongly associated with growth shortfalls than acute diarrhea (Campbell 2003). The mucosal damage that characterizes environmental enteropathy is caused by the body's inflammatory response to the ingestion of fecal bacteria,

and when people move to lower-bacteria environments the condition resolves (Haghighi 1997). Recently, nutritionists have hypothesized that reducing a child's fecal bacteria exposure during the first years of life through improved sanitation, handwashing or water treatment may improve gut function and subsequent growth (Humphrey 2009).

For children whose food intake is insufficient, Lipid-based Nutritional Supplementation (LNS) helps reduce gross energy shortfalls and provide essential micronutrients. The energy and micronutrients that LNS provides are likely to improve nutritional indicators, length-for-age and child development (Adu-Afarwuah 2008, Adu-Afarwuah 2007, Dewey 2008, Walker 2007, Rosales 2009, Bryan 2004), particularly among children that are at highest risk for severe stunting (Phuka 2008, Phuka 2009). It is possible that improved nutrition alone can reduce the negative effects of infection on growth and development due to the improved ability of better-nourished children to fight off enteric infections and exhibit catch-up growth during the convalescence period (Guerrant 1992, Guerrant 2008).

The specific LNS we propose to use is a next generation version of Nutributter; members of our team (Drs. Dewey and Stewart at UC Davis) have been involved in the development of the supplement and are currently deploying it in ongoing randomized, controlled trials in Bangladesh, Burkina Faso, Ghana and Malawi as part of the iLiNS project and related studies (iLiNS.org). Nutributter and related LNS interventions have demonstrated efficacy for improving child growth and development when provided daily after age six months (Adu-Afarwuah 2008, Adu-Afarwuah 2007). We propose a combined energy / micronutrient supplement because micronutrient supplementation alone is unlikely to have a large impact on linear growth (Ramakrishnan 2009). LNS is administered daily using 10 gram sachets that can be mixed into existing meals (e.g., porridge); a child eats two sachets per day. LNS is intended to supplement – and not replace – breastfeeding and locally available complementary foods, by providing 108 kcal/day and including a broad suite of essential fatty acids and micronutrients at dosages appropriate for children in this age group. It has an 18-month shelf life, does not spoil at high temperatures and costs as little as \$0.10 per day. Its compliance has been in excess of 88% in controlled trials (Adu-Afarwuah 2008), in part due to the ease of incorporating it into existing feeding routines. Breastfeeding is highly prevalent in both populations, and so we have focused on supplements that would not replace this essential source of nutrition (Black 2008). The Kenyan government is committed to promote, protect and support exclusive breastfeeding for the first six months of life and continued breastfeeding for two years and beyond through creating an enabling environment at the household level (GOK, 2012).

Our collaborators at UC Davis have a series of ongoing randomized trials evaluating the impact of LNS supplementation provided to pregnant and lactating women and/or their infants in Ghana, Malawi, and Burkina Faso through the International Lipid Based Nutrient Supplementation Project (www.ilins.org). The objectives of the project include the development of low-cost, acceptable LNS formulations using locally available ingredients and evaluation of the efficacy of reduced cost formulations of LNS for infants, young children and pregnant women. Acceptability trials have been conducted in all the three of the countries with positive results. Importantly, the iLiNS project has already demonstrated that LNS is acceptable among young children in similar cultures to the rural Kenyan population.

There is only one factory in France, run by Nutriset, with the capacity to manufacture the LNS proposed for this trial. In the event that the specified LNS is not immediately available for distribution at the time the nutrition intervention is rolled out, we will plan to provide Nutributter, an off-the-shelf product that is very similar in nutrient content to our research

formulation of LNS, and is available through a second factory in the United States (Edesia) that has not experienced the contamination problem. We have provided a comparison table in an attachment. We feel that when provided for a short time as a stop-gap measure, Nutributter is similar enough to our research LNS to be comparable. We will only switch to Nutributter if we discover that the problems within the Nutriset factory persist or unexpected new problems arise.

For the first six months of life we will promote exclusive breastfeeding in line with the WHO/UNICEF and Government of Kenya's guidelines. After 6 months, we promote locally available nutrient-rich complementary foods in combination with the LNS that is provided.

Pilot Work (2010-12-2601)

The WASH Benefits pilot work has allowed the study teams to refine the sanitation, handwashing, nutrition and water quality interventions and identify hardware and behavior change packages that result in high levels of uptake.

Based on the results of the pilot work, the following water, sanitation, hygiene, and nutrition interventions will be implemented:

| Intervention class | Examples of front-running candidate interventions |
|--------------------|--|
| Sanitation | Sanitation promotion, child potties, sani-scoop hoes to remove feces from household environments, latrine upgrades and subsidized construction |
| Handwashing | Promotion of handwashing with soap at critical times, tippy-tap handwashing stations, soapy water at handwashing locations |
| Water quality | Water treatment promotion, chlorine dispensers at water sources, supplemental bottled chlorine for in-home use |
| Nutrition | Appropriate nutrition for pregnant mothers, promotion of exclusive breastfeeding for children 0-6 months, Lipid-based Nutritional Supplementation (LNS) for children 6-24 months, promotion of appropriate complementary foods and continued breastfeeding |

Biomarkers for Environmental Enteropathy

Environmental enteropathy, an inflammatory disorder of the intestines that compromises nutrient absorption, is associated with child malnutrition and poor development (Haghighi 1997, Humphrey 2009, McKay 2010). Environmental enteropathy is one of the main hypothesized pathways for the impact of our interventions on growth and development. Measurement of environmental enteropathy symptoms will provide important information about the mechanism for intervention impacts (or lack of impact) in this study. The gold standard measurement for environmental enteropathy is an intestinal biopsy, which would not be feasible in this study. Altered intestinal permeability is an indicator of environmental enteropathy, measured using a dual-sugar permeability test in which the lactulose: mannitol urinary excretion ratio is measured (Lunn 2000, Campbell 2003). The child is given a combination of the two sugars, lactulose and mannitol. Mannitol diffuses through a transcellular pathway and is used to assess the absorptive capacity and mucosal surface area of the enterocytes. Lactulose is typically minimally absorbed

via the paracellular tight junctions and thus, it is used to assess epithelial integrity. A normal intestinal epithelium absorbs nearly all mannitol, but almost no lactulose. A damaged epithelium absorbs mannitol less efficiently and more lactulose. By measuring the lactulose : mannitol ratio in the urine passed over the subsequent 3-5 hours, the intestinal absorptive efficiency can be calculated and the severity of environmental enteropathy inferred.

Earlier studies demonstrated that environmental enteropathy as assessed by altered intestinal absorption and permeability, is widespread in low income tropical countries where fecal contamination of water, food, and the environment are common in contrast to rarely being seen among normal residents of high income temperate countries (Haghighi 2003). Environmental enteropathy is acquired early in childhood. Stillborn children in tropical countries have normal intestinal small intestinal cellular structure (Haghighi 1979). During the first three months of life mannitol/lactulose absorption is normal in children in The Gambia compared to children in the UK, however after three months intestinal absorption among Gambian children progressively decreases during the first year of life (Lunn 1991). Recent studies in rural Bangladesh confirm intestinal malabsorption consistent with environmental enteropathy is present in children 3 – 24 months of age; the degree of impairment in absorption increased in children between 3 and 12 months of age (Goto 2009a, Goto 2009b). The intestinal absorption and pathology of migrants who move from highly contaminated low income tropical countries to developed temperate countries normalizes within 3 to 5 years (Gerson 1971).

If improvements in sanitation, water quality, hand hygiene and nutrition could reduce the severity of intestinal malabsorption from environmental enteropathy either by preventing its acquisition by reversing the pathology, this would represent an important contribution to global public health, and would be a useful outcome assessment for the larger planned intervention study. Nutritionists have recently argued that environmental enteropathy is most likely caused by poor sanitation and hygiene in low income countries (Humphrey 2009, McKay 2010). Yet, there are no studies that demonstrate a specific association between environmental enteropathy and poor sanitation separated from other exposures in a low income country environment, although one study from Rhodesia 30 years ago noted an association between intestinal absorption and socioeconomic status (Thomas 1976).

A growing body of evidence suggests that future adult disease risk is shaped by “developmental origins” (Barker 2007). Multiple in utero and early life exposures to psychosocial stress (e.g., family stress, maternal depression) and biological stress (e.g., infections, environmental enteropathy, micronutrient deficiencies) may increase allostatic load (the cumulative biological damage from chronic stress) and increase susceptibility to disease later in life (Shonkoff, Boyce et al. 2009, Entringer, Buss et al. 2010, Tomiyama, O'Donovan et al. 2012). The attrition of telomeres, the repetitive DNA sequences protecting the tips of chromosomes, may serve as a biomarker of cumulative lifetime stress or play a causal role in the etiology of various diseases, or both (Entringer, Epel et al. 2011). During the sensitive period of early postnatal life, cellular replication occurs at a rapid rate as the immune system, brain, and other systems develop (Zeichner, Palumbo et al. 1999). Since telomeres are a key determinant of tissue development and shorten at a dramatically faster rate in infancy compared to in adulthood, it is imperative to focus on early childhood factors that may accelerate telomere attrition (Frenck, Blackburn et al. 1998, Zeichner, Palumbo et al. 1999). Although telomere attrition within the context of various diseases in adult populations has been widely studied (Calado and Young 2009, Lin, Epel et al. 2012), little is known about the pregnancy and early life risk factors associated with telomere attrition in infants from low-income countries. Complex pathways connect early life insults – micronutrient deficiencies, environmental enteropathy, and family stress – to adverse child health outcomes, and accumulating evidence implicates telomere

attrition, allostatic load, inflammation, and growth factors as potentially important underlying mechanisms linking these environmental stressors and disease susceptibility. This trial will be the first to evaluate the impact of water, sanitation, hygiene, and nutrition interventions on telomere length in infants living in rural, low-income countries.

Breastmilk is known to have both nutritive and non-nutritive components that may contribute to gut permeability (Weizman, 2013; Bode et al., 2012). Based on maternal dietary intake, concentrations of water soluble vitamins and fatty acids fluctuate in breastmilk, both of which play important physiologic roles in the growth and development of children (Allen, 2012; Alvarado et al., 2005; Huffman et al., 2011). Having a profile of breastmilk components, such as fatty acid profiles, concentration of B vitamins and lactoferrin could help elucidate mechanisms related to child growth and development during the 0-6 month age window, when only breastmilk is recommended.

Pilot Environmental Enteropathy Work in Bangladesh (CPHS Protocol # 2010-11-2536)

In our Bangladesh environmental enteropathy pilot study, we selected 119 children from the existing SHEWA-B cohort who lived in different levels of household environmental cleanliness based on sanitation, water quality and handwashing indicators. The children were between age 8 and 48 mo in May 2010 and lived in 83 different rural villages across Bangladesh.

The 66 children from households with improved household hygiene lived in homes with good sanitation (flush/septic/piped sewerage or a pit latrine with slab and water seal), good water quality (median *E. coli* < 10 CFU/100 ml in up to 8 samples collected over 24 mo), and favorable handwashing conditions (a dedicated location to wash hands stocked with soap and water). In contrast, the 53 children who lived in homes with poor household hygiene lacked adequate sanitation (open defecation, open pit latrines, slabs with broken water seals, toilets that flush to “somewhere else” or hanging toilets), had poor water quality (median *E. coli* ≥ 10 CFU/100 ml), and had unfavorable handwashing conditions (no dedicated location to wash hands, or a dedicated location that lacked either water or soap). The definitions of improved hygienic conditions were chosen to reflect indicators that we hope to improve through intervention in the WASH Benefits study.

Children in the two environments differed greatly in their growth: after statistical adjustment for potentially confounding differences, children in households with improved hygienic conditions had 0.54 SDs (95%CI 0.06, 1.01) higher HAZ than children in households with poor hygienic conditions (unadjusted difference = 0.91 SDs). Importantly, the children also differed in biomarkers for environmental enteropathy. After statistical adjustment, children living in improved hygienic households had lactulose: mannitol (L:M) ratios that were –0.32 SDs lower than children living in poor hygienic conditions (95% CI –0.72, 0.08). Children in improved hygienic households also had lower Immunoglobulin G endotoxin core antibody (IgG EndoCAb) titers (–0.23 SDs, 95% CI: –0.63, 0.17) than children living in poor hygienic conditions. After adjusting for age and sex, the L:M ratio was also strongly associated with HAZ in the population: a 1-unit increase in the log L:M was associated with a –0.36 SDs reduction in HAZ (95% CI –0.64, –0.07).

These pilot results support our original rationale to conduct the main WASH Benefits study. However, because household environmental conditions in the pilot were not randomized, it remains possible that differences observed between the children in growth and EE biomarkers

result from unmeasured or unquantifiable differences between groups that we cannot control for without an experiment. A randomized trial that delivers high impact household environmental interventions (i.e., interventions with good uptake and high efficacy at reducing pathogen transmission to young children) in large populations as we have in our Kenya and Bangladesh cohorts would provide more conclusive evidence.

3. Collaborative Research (Intentionally Left Blank – to be filled in online)

4. Qualifications of Study Personnel (Intentionally Left Blank – to be filled in online)

5. Subject Population

a) Describe proposed subject population, stating age range, gender, race, ethnicity, language and literacy.

The intervention trial will be implemented in rural communities in Bungoma, Kakamega, and Vihiga Counties in Western Kenya. The subject population will be young children and their mothers/guardians living in Western Province, Kenya, in communities that meet the following study criteria:

- The water source located in a rural area
- The majority of village members collect their water from communal sources
- The majority of the community members relies on unimproved sanitation facilities
- There are at least 2 eligible target children in the village whose family owns their home and who have no plans to move away in the next 12 months
- The village cannot already have a chlorine dispenser installed
- The respondent can speak either English, Kiswahili or Luhya

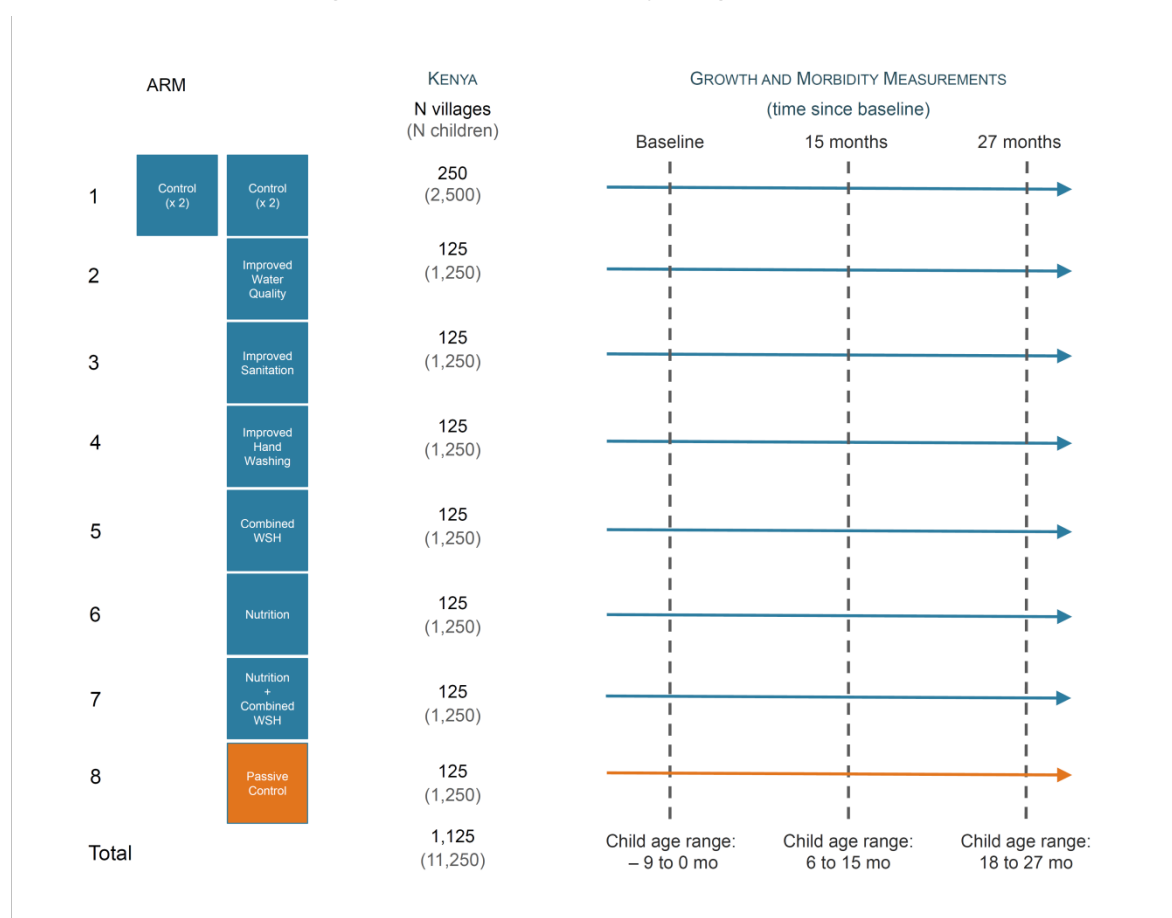
Because of efficiencies of scale in intervention delivery, villages with larger numbers of pregnant women in their second or third trimester will be prioritized for enrollment. Two or more neighboring villages may be combined to create one cluster as long as the cluster of villages has at least 8 eligible pregnant women and shares a border. After the survey visit the village/cluster will have at least 6 eligible surveyed women to be enrolled.

Target children will be unborn children of pregnant women identified by report of their pregnancy length and reported expected date of delivery (EDD) at enrollment or infants up to 3 months old, although we will only enroll the latter if we are falling short of our targets with children in utero. Target children will age to between 18 and 30 months over the course of the study. Older (3-15 years) siblings (or neighbors) of the target children, and children < 36 months of age living in the same compound, will be included in certain aspects of the study as well. For example, the study will measure caregiver-reported diarrhea in an additional child < 36 months at enrollment who lives in enrolled compounds. The subject population will include both males and females, and no one will be excluded based on their race, ethnicity or literacy.

- b) **State total number of subjects planned for the study and how many must be recruited to obtain this sample size. Explain how number of subjects needed to answer the research question was determined.**

Figure 1 (attached) provides an overview of the WASH Benefits study design (the Bangladesh study is described in a separate protocol). The interventions will require about 3 months from the baseline survey to deliver. The follow-up rounds are planned for 12 and 24 months after intervention delivery.

Figure 1. WASH Benefits study design overview.



In Kenya, we plan to enroll 125 clusters (consisting of 1 or more administrative villages) per intervention arm, a double-sized control arm, and a single-sized passive control arm, with approximately 10 children per cluster. Clusters of villages will be formed based on geographic location and number of pregnant women identified in each village. The Kenya study includes a passive control arm because there is concern among study investigators that the simple act of promoter visits (independent of any WASH or nutritional intervention) could lead to improved child health (Zwane et al. 2011). The passive control arm will not include any promotion activities at all, while the active control arm (double-sized), will include routine visits to measure mid-upper-arm circumference in the study children. This active control visit is standard across all treatment arms and therefore will allow us to assess the impact of the promoter visits. We will enroll as many eligible children per cluster as the field team is able to process, up to all of the eligible children in that village that are known to us at the time of the baseline survey. The

control arms will include 3750 children and the 6 intervention arms will include a total of 7,500 children.

The total number of children planned for the study is 11,250, but because of the uncertainty of pregnancy outcome and variation in village populations we may end up with somewhat more or less than this precise target.

We plan to enroll a total of 22,113 children and 32,250 adults in this study, for a total of 57,363 people. The breakdown of the different enrollees contributing to these totals is as follows:

- 11,250 target children (measured at mid- and end-line)
- 2,250 non-target children from whom we take parasite samples at baseline (20% of our households have a child in the parasite-eligible age range)
- 7000 non-target children from whom we take parasite samples at endline
- 375 LNS-eligible aged siblings in LNS households who will also receive LNS (10% of target households in nutrition arms)
- 113 LNS-eligible children of promoters who will receive LNS (estimated 20% in 2 nutrition arms)
- 1,125 younger siblings born to mothers during the course of the study (measured at endline)
- 11,250 mothers of target children
- 22,500 other mothers who live in compounds where target children live who serve as secondary respondents for questions on health outcomes (one per compound at baseline and endline)
- 1,500 promoters (1.5 on average per cluster, up to 125 clusters in 8 arms, excluding the passive control)

We arrived at this sample size using Monte Carlo simulations that assume our outcomes arise from a mixed model data generating distribution that is the basis for conventional power calculation formulas [Feiveson 2002, Arnold 2011]. To inform the calculations, we used measures of variability and cluster-level correlation that we calculated from a large randomized trial of water quality interventions in Western Kenya. We repeated the power calculations for two of our primary outcomes for which we have detailed information: height-for-age Z-scores (HAZ) and diarrhea.

HAZ Sample Size Calculations:

In our pilot cohort in rural, Western Kenya, the mean (SD) HAZ among 405 children < 28 months with height data is -1.23 (1.35). The cluster level ICC is 0.02. There are two hypotheses of interest: (H1) comparison of individual treatment arms vs. control, and (H3) comparison of the combined nutrition+WSH arm to the nutrition arm. Both comparisons are statistically equivalent for power calculations. We identified the minimum detectable effect (MDE) for the two comparisons with > 80% power. We have assumed a one sided α of 0.05. With the current design, we will have power to detect differences of +0.15 HAZ; this is approximately half the mean effect size observed in supplemental feeding intervention studies (Dewey 2008). We have targeted a smaller effect size because we expect the impacts from water, sanitation, and handwashing interventions to be smaller than from nutritional interventions.

Diarrhea Sample Size Calculations:

In the same Kenyan cohort, the estimated 48 hour period prevalence is between 12.2% and 13.7%. For all calculations, we have assumed that the prevalence in the control group will

be 12.0%. For comparisons of the combined WSH arm to any single treatment arm we assume that the single treatment arm prevalence is 8.0% (a 33% relative reduction from the control). There are two hypotheses of interest: (H1) comparison of individual treatment arms vs. control, and (H2) comparison of impacts in the combined WSH arm with the individual treatment arms. We identified the minimum detectable effect (MDE) for the two comparisons with > 80% power (see Table 1 below). We have assumed a one sided α of 0.05.

| Minimum Detectable Effect (MDE) Sizes for Height-for-Age Z-scores (HAZ) and Diarrhea | | | |
|--|---------------------------------|-------------------------------|--|
| MDEs with >80% power for treatment minus control for H1 (treatment v control), H2 (<i>WSH</i> v single treatments) and H3 (<i>N</i> v <i>NWSH</i>). HAZ calculations assume 1 post treatment measurement. Diarrhea calculations assume 2 post-treatment measurements. | | | |
| Clusters per arm | HAZ | Diarrhea | |
| | H1, H3 Single Arm Comparison | H1 Single Tr (12% base) | H2 <i>WSH</i> v (<i>W</i> <i>S</i> <i>H</i>) (8% base) |
| 90 | 0.16 | 2.3% | 2.0% |
| 100 | 0.15 | 2.2% | 1.9% |
| 125 | 0.14 | 2.0% | 1.7% |
| 150 | 0.13 | 1.8% | 1.5% |

Environmental Enteropathy Subgroup Sample Size Calculations:

We will collect blood, urine, saliva, and stool specimens from a subsample of 1,500 children in the study to measure biomarkers for environmental enteropathy including allostatic load and telomere length. Environmental enteropathy is one of the key hypothesized mechanisms for intervention impact on child growth and development. The sample of 1,500 children will be distributed equally over four arms in the study – 375 children in each of the active control arm, LNS alone arm, combined WSH arm, and the LNS+ combined WSH arm. Specimen collection will take place 6 months following baseline, at midline (15 months following baseline), and endline (27 months following baseline). We plan to collect specimens on the entire subsample at all three time points.

We arrived at a sample size of 1,500 children (375 children per arm) using two outcome measurements we collected in our Bangladesh environmental enteropathy pilot study. In the pilot, the 119 children ranged in age from 10 to 48 months and lived in 83 villages across rural Bangladesh. Using estimates of variability in the lactulose:mannitol (L/M) ratio and Endotoxin Core Antibody (EndoCAb), we estimate that this design will have greater than 80 percent power to detect differences between groups of –0.20 SDs in the L:M ratio and –0.25 SDs in IgG EndoCAb antibodies. These detectable differences are smaller than those observed between children in poor versus improved hygiene households in the pilot study: –0.42 SDs for L:M and –0.29 SDs for EndoCAb. In these calculations, we assumed a village-level intra-class correlation of 0.05 for L:M and 0.27 for EndoCAb, and child-level intra-class correlations (for repeated measures within children) that range between 0.5 and 0.9.

We plan to collect samples from 1500 infants at the first survey round, which will take place approximately 6 months after baseline. However, the entire sample of 1,500 will likely not be present for the 1 year (midline) and 2 year (endline) assessments due to the high number of

refusals and absences between rounds of measurement. Thus, at the midline and endline assessments, we will need to increase the sample size by potentially 500 children per round for a total subset sample size of 2000 at each time point.

Repeat diet recall will be conducted as a quality control measure in the EE sub-study. Specifically, of the EE –sub a further sub-sample of ~60 will be asked repeat diet recall questions as a quality control mechanism.

Breastmilk Sample Size Calculations (to be nested within the EE subgroup):

To test the hypothesis that greater proportion of long chain omega 3 polyunsaturated fatty acids in breastmilk will be inversely correlated with lactulose:mannitol ratio, we assume a type I error (α) of 0.05, power ($1 - \beta$), of 0.80, a design effect of 1.5 (based on an ICC=0.1 and 6 women/cluster) and a correlation > 0.2 to be meaningful.

To detect differences between groups in the breastmilk riboflavin and B12 concentrations > 0.55 SD, 296 breastmilk samples are required, though to account for missing data, a 15% oversampling buffer is included.

Thus, as a project target, 340 breastmilk samples will be collected during visit 1 of the EE subsample. The sample of 340 mothers will be distributed evenly over the four intervention arms (85/arm) included in the subsample.

Women in the nutrition or WASH + nutrition groups will be compared to women in the WASH alone or control groups. One hundred and fifty breastmilk samples in both groups are required to detect an effect size of at least 0.4 in breastmilk nutritive biomarkers (riboflavin and vitamin B12 concentrations, and the proportion of n-3 PUFAs in total fat) with 80% power, $\alpha=0.05$ and a design effect=1.5 (based on an ICC=0.1 and 6 women/cluster). Linear regression will be used to model the continuous outcomes of interest, including the nutrition intervention effect variable, hunger score variable, and their interaction along with potential confounders. Generalized estimated equations will be used to account for correlated observations at the cluster level when conducting hypotheses testing. Secondary analyses of observational data, categorizing women by ASF intake and overall diet diversity are also planned.

We hypothesize that docosahexaenoic acid (DHA, 22:6 n-3), riboflavin, B-12, and lactoferrin will vary based on treatment arm.

Intestinal Parasite Measurement in Target Children and Older Children:

At baseline, we plan to collect stool specimens and blood spot samples (finger prick) from all 18-27 month olds residing in the same compound as the study child (approximately 2 children per cluster; 2250 total). At the endline follow-up, we will collect stool specimens and blood spot samples from target children as well as one or more older siblings (14,000 total) assuming a 70% recovery rate for stool samples among all arms other than the passive control. We define older children as children between the ages of 3 and 15 years old. If the target child does not have an older, sibling in the age range, then we will randomly select an older child from among the children who live in the same compound. The purpose of the stool collection is to measure the presence and intensity of intestinal parasite infections.

Older children are more likely to be infected with intestinal parasites and will provide important information about within-household transmission of the parasites (or the interruption of transmission). We will also attempt to collect samples from the same children who contributed samples at baseline, who will be at least 3 years old at endline. A mother may consent to having one or more children that meet our criteria provide samples.

The eventual goal of the blood spot collection will be to analyze the samples at some point in the future for intestinal helminthes, protozoans, and other markers of disease using antigen-based assays.

We informed our sample size calculations using parasite infection data collected from 700 children < 24 months in India and Peru. Our power calculations assume 50% prevalence in the control arm, a village intraclass correlation of 0.14, and 71% successful stool collection and analysis (10 / 14 samples per village), which is highly conservative. We estimate that these samples will be sufficient to detect a relative reduction of 15% in infection prevalence of any parasite with a single, post-intervention measure.

Child mortality sample size calculations

We propose to conduct verbal autopsies with the caregivers of as many deceased study children as is possible. These data would be purely descriptive – sample size will be driven by budgetary and logistical constraints and we will use a convenience sample. The local IRB for the study has requested additional information on the causes and circumstances of deaths of study children, which is best accomplished by means of a formal verbal autopsy. The project has hired a registered nurse who will visit caregivers of deceased study children for the rest of the study. When a new death occurs, we will check our database of previous deaths to determine if there are any other caregivers of deceased children living nearby who the nurse could interview on the same day. We will prioritize children in the EE cohort since this additional information could help with interpretation of the rich dataset of biomarkers we are already collecting for these children.

- c) **If any proposed subjects are children/minors, prisoners, pregnant women, those with physical or cognitive impairments, or others who are considered vulnerable to coercion or undue influence, state rationale for their involvement.**

The proposed subjects include very young children, pregnant women and educationally and economically disadvantaged subjects. The goal of the WASH Benefits study is to generate rigorous evidence about the impacts of sanitation, water quality, handwashing, and nutrition interventions on child growth and development in the early years of life. There is abundant evidence aggregated over more than 325,000 children from around the world that the window for interventions to improve growth is in the first 1,000 days of life, including the 9 months before birth (Victora 2010). Meeting the study goals requires intervention in the middle of this development window. The nature of the research study requires recruitment of subjects in poor rural areas of Kenya. The populations in these areas are both economically and educationally disadvantaged.

6. Recruitment

- a) **Explain how, where, when, and by whom prospective subjects will be identified/selected and approached for study participation. If researcher is subject's**

instructor, physician, or job supervisor, or if vulnerable subject groups will be recruited, explain what precautions will be taken to minimize potential coercion or undue influence to participate.

The intervention trial will be implemented in Western Province, Kenya. Potentially eligible villages will be identified through screening phone calls conducted by IPA staff. The village elder (Liguru) will be called by IPA staff, who will introduce the study and ask if the village would like to participate. With the Liguru's assistance, IPA staff will gather the information necessary to determine if the village meets the eligibility criteria by conducting a door-to-door census. If the village is eligible, and the Liguru would like for the village to participate, then that village will be censused to identify all eligible pregnant women. Based on the census data, the village may be assigned to a cluster. When a cluster is enrolled, our staff will coordinate with the village elder(s) to have a meeting with pregnant women and primary caregivers of children under three years to nominate potential promoters who will be enrolled a few weeks later at training sessions sponsored by IPA. On the day of the meeting, the IPA staff will also visit eligible study compounds that have children aged 18-27 months old for the parasite sample consent and specimen collection process. For this activity the caregiver of the target child will be approached by a trained IPA staff member who will describe the study and the parasites component. The following day the survey staff will visit all eligible compounds. Those compounds with pregnant women will be approached by trained IPA staff, who will describe the study to the eligible pregnant women and other heads of the household. If the mother agrees to participate, she will be taken through the consent process. IPA staff will enroll and randomize compounds into the intervention trial until we reach sufficient sample size. We will enroll compounds from clusters that meet our eligibility criteria of having at least 6 eligible surveyed women.

Children born into existing study compounds, other than those born to pregnant mothers enrolled at baseline, will also receive the same intervention as the target child in the compound, with some minor exceptions (we will not provide additional potties after the initial distribution, we will not install additional handwashing stations although everyone in the compound is encouraged to use the two that will already be provided, and LNS will only be provided to siblings of the target child). This will be essential to maintain a coherent and consistent intervention within each compound. Since LNS is a more targeted (and expensive) intervention, we plan to limit LNS provision to additional children born to the same mother as target children. For children who are born into existing study compounds, we will attempt to enroll them at midline and baseline and if enrolled we will measure diarrhea, anthropometry (length, weight, head circumference) and child development outcomes (when possible: children would need to be > 4 months old to administer most tests). Due to cost and logistics, we do not plan to measure environmental enteropathy biomarkers or parasitic infections in the additional enrollees. We do not plan to include the additional newborn children in our primary analysis because the exposure to intervention for this subset will be shorter than for our index children, and their inclusion would increase the variability of intervention exposure length in our population (and would make defining what constitutes the "intervention" more difficult). However, children born into existing compounds may provide additional information about the impact of our interventions on very young children who are born into cleaner environments.

- b) **Describe any recruitment materials (e.g., letters, flyers, advertisements [note type of media/where posted], scripts for verbal recruitment, etc.) and letter of permission/cooperation from institutions, agencies or organizations where off-site subject recruitment will take place (e.g., another UC campus, clinic, school district). Attach these documents in 17. Attachments.**

Recruitment is integrated in to the consent process. There are no separate materials just for recruitment. Trained field staff will approach the eligible household and introduce themselves and describe the study and the participant involvement, should they choose to participate. The consent forms are attached.

7. Screening

- a) **Provide criteria for subject inclusion and exclusion. If any inclusion/exclusion criteria are based on gender, race, or ethnicity, explain rationale for restrictions.**

The study will be conducted in communities in Western Province, Kenya. These communities must meet all of the following criteria:

- The water source located in a rural area
- The majority of the community members relies on unimproved sanitation facilities
- The majority of village members collect their water from communal sources
- There are at least 2 eligible target children in the village whose parents own their home and have no plans to move away in the next 12 months
- The village cannot already have a chlorine dispenser installed
- The respondent can speak either English, Kiswahili or Luhya

Because of efficiencies of scale in intervention delivery, villages with larger numbers of pregnant women will be prioritized for enrollment. Two or more neighboring villages may be combined to create one cluster as long as the pair of villages has at least 8 eligible pregnant women and shares a border. Compounds (within eligible communities) will be eligible to participate if they include at least one pregnant woman in her second or third trimester living in the compound. Within each enrolled compound, we will collect information from three types of children:

(1) Infants (target child) will be eligible to participate in the study if:

1. They were in utero or less than 3 months old at the time of the village census
2. Their parents/guardians own their home and are planning to stay in the study village for the next 12 months

(2) Children < 36 months at baseline that are living in a compound of a target child will be eligible to participate in diarrhea measurement if:

1. They are 3 – 36 months old at the baseline survey

(3) An older sibling or older child from each enrolled compound that includes a target child will be eligible to participate in the intestinal parasite specimen measurement if:

1. They are between the ages of 18-27 months at baseline or 3 -15 years at endline

There will be no exclusion criteria based on gender, race, or ethnicity.

- b) **If prospective subjects will be screened via tests, interviews, etc., prior to entry into the "main" study, explain how, where, when, and by whom screening will be done. NOTE: Consent must be obtained for screening procedures as well as "main" study procedures. As appropriate, either: 1) create a separate "Screening Consent Form;" or 2) include screening information within the consent form for the main study.**

Based on a list of potentially eligible mothers from the village census, the same field officer who will administer the survey will first visit the compound, and immediately upon entering, introduce him or herself and request permission to screen the pregnant woman for participation in the study. If permission is granted, the same field officer will attempt to verify eligibility, and if eligible, the same field officer will request permission for the main survey and begin it immediately.

8. Compensation and Costs

- a) **Describe plan for compensation of subjects. If no compensation will be provided, this should be stated. If subjects will be compensated for their participation, explain in detail about the**

Subjects who participate in structured interviews, focus groups, or household surveys will be given a small token of appreciation for their time, such as a kanga or a small tub of cooking fat, after each data collection session (3 visits total). Subjects in the environmental enteropathy subgroup will be given a small cooking pot (a sufuria) in recognition of the longer duration of sample collection. The small gifts are not so extravagant as to unduly influence subjects' decisions about whether or not to participate in any aspect of the study. No other compensation will be provided.

In addition, participants in treatment arms will receive free chlorine dispensers, sanitation and handwashing hardware as part of the study, which they will retain after the completion of the study. Participants with target children in the household will additionally receive free nutritional supplements (LNS), detergent for making soapy water, and water treatment supplies for the duration of their participation in the study.

If qualitative (focus groups and in-depth interviews) follow up is necessary as described below, depending on the nature of the interaction we may reimburse transportation costs for participants for attending a discussion in a central location in or near their village.

Those who meet the criteria for referrals will be reimbursed for transport costs to travel to the nearest health facility with the capacity to provide care.

- b) **Discuss reasoning behind amount/method/terms of compensation, including appropriateness of compensation for the study population and avoiding undue influence to participate.**

Budgetary constraints limit what we are able to offer participants in terms of compensation for their time. In addition, larger gifts might unduly influence the study population to participate.

- c) **Costs to Subjects. If applicable, describe any costs/charges which subjects or their insurance carriers will be expected to pay. (If there are no costs to subjects or their insurers, this should be stated.)**

Participation in the study will not result in any costs to subjects, other than cases in which subjects elect to devote their own time or resources to improving their water quality, sanitation or hygiene practices, as encouraged and facilitated by the interventions.

9. Study Procedures

- a) **Describe in chronological order of events how the research will be conducted, providing information about all study procedures (e.g., all interventions/interactions with subjects, data collection procedures etc.), including follow-up procedures.**

Please see Figure 1 for the overall chronology of the study. The interventions will require about 3 months from the baseline to implementation. The follow-up rounds are planned for 12 and 24 months after intervention delivery. The major activities involved in the study include:

- 1) **Census and Enrollment**
- 2) Promoter Selection and Training
- 3) Baseline Assessment
- 4) Intervention Implementation
- 5) Midline Assessment
- 6) Endline Assessment

1) Census and Enrollment

After obtaining permission from the Liguru to work in a given village, IPA field staff will conduct a door to door census of every compound in the village, following the boundaries delineated by the Liguru. After obtaining verbal consent to ask about the population of the household, a respondent in each compound will be asked for the total number of people residing there, the number of households, the number of pregnant women, and the number of children under 5 years of age. If there is an under-5 in the compound the age in years of each child will be asked, as well as the name of primary and secondary drinking water sources that the household accesses. Compounds with pregnant women will be visited at a later date for enrollment into the study and administration of the baseline survey. IPA staff will also ascertain the presence or non-presence of an 18-27 month old child in the compound for participation in parasites analysis (see item 3, below). GPS coordinates of all compounds will be recorded for use in computing population density and spillovers (see section 7.2.3, below). Identifying information such as names of respondent, children under five, mothers of children under five, and compound head name will also be obtained in compounds with a child under the age of 5 in order to better understand the benefits to non-enrolled villagers either via spillovers, or in the case of the water intervention, direct benefit through water chlorination. In addition, we will visit all water sources in the village and record information such as names of the sources, GPS coordinates, turbidity

and seasonality. This information is used to assess the eligibility of the sources for dispenser installation in the water, WASH, and WASH+ villages. GPS location of schools and markets as well as estimates of attending populations will also be recorded during the census as an additional method of measuring spillovers.

2) Promoter Selection, Training, and Community Activities

For all study arms including the passive control, local promoters will be nominated by pregnant women and primary caregivers of children under 3 from clusters that are participating in the study. We must hold these meetings in the passive control arm where there is not a promoter because randomization will yet have occurred at the time we need to identify the promoter due to other logistical constraints. However, we are explicit that there is a possibility that no one who is nominated will be selected. One or more candidates from each cluster will be invited to an initial 2 day training workshop conducted by the research organization's staff on interpersonal communication, introduction to behavior change communication strategies, basic adult learning theory, time management/planning, mid-upper arm circumference (MUAC) measurement and reporting procedures. Each promoter will have approximately 10 households assigned to them but the exact number will be based on the total number of enrolled respondents per cluster. Upon successful completion of the initial training, promoters from all intervention arms will subsequently attend a 1-to-5-day training specific to the intervention assigned in their cluster. For example, in villages assigned the nutrition intervention arm, the training will address the importance of good nutritional practices during pregnancy and lactation, and appropriate infant feeding practices, such as early initiation of breastfeeding, exclusive breastfeeding through 6 months, continued breastfeeding through at least 24 months, and timely introduction of healthy complementary foods. Additionally, modules have been developed describing how LNS can be incorporated into healthy complementary feeding practices. In addition there will be refresher trainings at six and 12 months that will last for one to two days and present a review of general themes as well as any new behavior change communication strategies that have been developed. The refresher training will serve as a venue to share ideas, lessons learned and best practices among the promoters and their supervisors.

Shortly after the training workshops, the promoter will be presented to the community and mothers participating in the study by a representative from the research organization. The study representative will then accompany the promoter on his/her first 1-3 participant interactions (lasting ~1 hr each) and provide the promoter with feedback on his/her techniques. The promoter supervisors will each oversee ~100 promoters for the duration of the study. They will stay in touch with promoters through monthly phone calls and periodic site visits each year. In order to ensure that study households are adequately supported, promoters will be asked to make frequent contact with study households in the first days and weeks after the intervention is launched, with interactions then tapering off after the first 6 months of the study to a long-term pattern of monthly visits by promoters to deliver LNS, soap and/or bottled chlorine depending on intervention arm, provide ongoing behavior change support, and check on uptake. Promoters will be compensated for their efforts at a rate that is commensurate with the requested assistance they provide to their community (~1000 Kshs in the initial months, scaling back as study participants develop new habits and the promoter's behavior change work load diminishes), with a strong emphasis on the prestige of being selected as a promoter (actualized in the form of a diploma from the training, an official promoter t-shirt and household visit kit, and potentially a program cell

phone to facilitate communication between research staff and promoters). There will also be in person supervision visits at approximately four preset intervals.

We will conduct a brief survey with promoter nominees to collect information on their pre-training knowledge, behaviors and observations of living conditions (including an inspection of the latrine and presence of soap in the house and a test for chlorine in stored drinking water), with an additional module covering demographic and socio-economic characteristics administered to trained promoters during a later interaction due to logistical constraints. These data will allow us to describe our pool of promoters and test for interaction effects between promoter characteristics and outcomes among study participants. We will also conduct surveys or qualitative follow up with promoters at mid and endline survey rounds and the questions will be drawn from approved instruments. We will obtain written consent from all promoters (and nominees) who participate in the survey.

3) Baseline Assessment (age -6 to 0 mo)

After a pregnant woman has been enrolled in the study, our trained staff will conduct a baseline assessment. Mothers will be asked standard questions about their (and spousal) education, activities, occupation, household assets, and current sanitation, nutrition, water, sanitation, and hygiene practices. All households will be asked to provide phone numbers to allow for tracking of study respondents throughout the study. The baseline assessment in all participating households will include bar soap and detergent powder consumption measurements, latrine use (visual inspection), and numerous spot-check hygiene indicators (e.g. presence of animal or human feces in the household environment). The field team will collect these measurements at the three measurement rounds of the study (baseline, 1-year follow-up, 2-year follow-up).

The baseline assessment also will include measurements of free chlorine residuals (water treatment), water quality (enumeration of fecal indicator bacteria in source and in stored water of households, molecular analysis of fecal contamination origin), availability of soap in the home, visual inspections of hands, handwashing demonstrations, measurements of environmental contamination using sentinel objects, child hand rinses, soil, food, and measurement of fly density in households (enumeration and speciation of flies at the latrine and at the eating area over a specified time period), latrine use (visual inspection, including latrine depth measurement, infrared latrine-use and/or tippy tap sensors), and numerous spot-check sanitation indicators (e.g., presence of animal or human feces in the household environment). The field team will collect these measurements at the three measurement rounds of the study (baseline, 1-year follow-up, 2-year follow-up). Additionally, intervention promoters will collect a subset of the indicators (LNS sachet consumption, hardware use indicators) on a monthly basis which will be provided to IPA staff on regular check-in phone calls. We will also ask mothers to provide a sample of blood for testing of nutrition and other biochemical parameters, and a stool sample to permit future testing of parasites or pathogens. Although we are not currently funded to analyze these maternal samples, we envision future interest in assessing the impact and controlling in the analysis for maternal nutritional

deficiencies or other biochemical factors on child development, and so we may collect and archive this prenatal sample.

We will select 54 clusters (375 children) from each of four arms – the active control group, the combined WASH interventions, the nutrition intervention and the combined WASH and nutrition interventions) for detailed measures of environmental enteropathy (detailed below).

Parasite Assessment (an older sibling aged 18-27 months old)

Stool and blood spot samples will be collected from all 18-27 month old children in the study compound at baseline. If the target child has no siblings, another age-eligible child from the compound will be randomly selected to provide stool and blood spot (consent will be obtained first). Stool collection will require two visits to each household. On day 1, the field team will deliver to each caregiver a stool collection kit and instruct them how to collect stool from their children. Caregivers will be instructed to collect stool from their children on the following morning in the event that the child defecates before the field team arrives at their household. Caregivers will be instructed to have their child defecate on a sheet of provided plastic or aluminum foil, to use a provided plastic scoop (integrated into a storage container) to collect ~10 mL of fresh stool from the top of the pile, and to bring the collected specimens to the centralized test location. On day 2, field staff will return to the household to collect the stool sample. Field staff will aliquot stool specimens to allow for the analysis of intestinal protozoan parasites (*Giardia*, *Entamoeba histolytica*, *Cryptosporidium* sp.) using antigen-based tests (ELISA). We will maintain a cold chain of 4°C until the stool are transported to a sample processing center, where they will be stored at –20°C until they are shipped on dry ice to the ESAICPAC/KEMRI lab in Nairobi to be analyzed.

Paired with each stool sample we collect we will also collect a finger prick blood sample. One of the child's fingers will be cleaned using the disinfectant liquid and after drying completely prick to adapt to 0.25 mm using a spring-loaded disposable handset (BD Microtainer®). Six drops of blood (about 60 µl) will be collected using a filter disk. We will store the filter disk at 4°C. The filter disk will then be frozen and transported to the study's labs in each country, where they will be stored at –80°C. The eventual goal of the serum specimen collection will be to analyze them for parasite infectious and other markers of infection using antigen-based assays (Luminex), but this protocol does not include the serum analysis activities.

Subsample Environmental Enteropathy (EE) Assessment

Environmental enteropathy biomarkers will be collected from a subset of 1,500 infants in approximately 216 village clusters. During mid and endline an additional 500 participants may be enrolled to account for loss to follow up for a total subset sample size of 2000 at each time point. This data collection will be implemented over two days per cluster. On the first day, consent, anthropometric measurements, blood pressure, heart rate, provision of stool collection materials, saliva and blood collection take place. On the second day we will collect urine from the study children, pick up the stool from the study children, and interview

the mothers about their diets as well as their infant's diet during the past week and the previous 24 hours, and interview the mothers about their health, and exposure to depression and stress and that of their child over the period of pregnancy to the present time. On a non-consecutive day, a subsample of mothers (n=60) will be revisited to be interviewed about their diet as a quality control measure.

We will also measure the child's length, weight, MUAC and head circumference using standardized measurement techniques. Our anthropometric teams will have been trained and standardized in measurement techniques according to the FANTA and WHO guidelines (Cogill 2003, deOnis 2004). The child will be weighed using a calibrated scale and will have his/her length or height measured using a height board and head circumference using a tape measure. We will also measure maternal height, MUAC and weight. We will begin environmental enteropathy data collection approximately 6 months after baseline data collection.

Urine specimen collection and analysis: Our field teams will collect urine samples from all eligible children in a study cluster (up to 20 children) in one day per cluster. The field team will request that mothers not feed their children for at least one hour before they receive the lactulose-mannitol. The children will be weighed and measured using the same anthropometric procedures as described above. The lactulose-mannitol solution will be prepared at the field office using lactulose syrup and mannitol powder secured from international pharmaceutical suppliers. The lactulose-mannitol will be mixed with sterile water to produce a solution with a concentration of 250 mg of lactulose, and 50 mg of mannitol. Field workers will administer 2mL of the solution per kilogram of body weight of the child. The lactulose-mannitol assay requires the collection of an additional pre-LM urine sample to serve as a "control or baseline" urine for comparison with the post-LM urine. A pre-LM urine sample is a sample of urine (12 ml) that is collected during the 1-hour fasting period preceding the administration of lactulose-mannitol solution to the child. This additional pre-LM urine sample does not change the amount of time we are present in the household. For assay standardization and QA/QC purposes, we also plan to spike these pre-LM urine samples with fixed concentrations of lactulose, mannitol, or a known interfering compound during analysis. A urine collection bag equipped with a drainage tube will be attached to the infant at the beginning of the fasting period. Thirty minutes after the infant consumes the lactulose-mannitol solution, mothers will be encouraged to breastfeed infants <6 months to help their urination. Children over 6 months will be given purified drinking water 30 minutes after taking the sugar to help urination. Whenever the child urinates, the urine will be removed from the bag and placed in a container marked with 0.1% thimerosal (1 drop per 5 ml), a preservative. The total volume of urine collected after 5 hours will be noted, a 24 mL well mixed sample will be stored at -80 degrees C, and the urine bag will be removed from the child.

Since the mannitol/lactulose concentration measurements necessitate the use of high performance liquid chromatography and mass spectrometry (LC-MS/MS), which is presently unavailable in Kenya, the child urine samples will be shipped to the US and Bangladesh, where the LC-MS/MS labs are located. We plan to collaborate with Dr. Mohammad Alauddin and his team at Wagner College to analyze these urine samples. Oxidative stress and hypothalamic-pituitary-adrenal axis markers will also be measured in the urine samples from the mother and child: F2-isoprostanes will be analyzed using gas

chromatography (GC)/negative ion chemical ionization (NICI) mass spectrometry (MS) (Morrow and Roberts 2002), 8-hydroxy-2'-deoxyguanosine (8-OHdG) will be measured in using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (Weimann, Belling et al. 2002), and catecholamines (epinephrine, norepinephrine, and dopamine) will be measured using a commercial ELISA kit (Parks, Miller et al. 2009).

Blood specimen collection and analysis: In addition to the urine collection, trained phlebotomists will collect up to 5 mL of venous blood from each child (< 2.5% of total blood volume for infants > 2 kg). Blood spots will be collected for luminex testing of antibodies to intestinal parasites. Blood samples will be centrifuged within two hours of collection to separate the plasma and serum from the red blood cells. The plasma, serum and packed red blood cells will be separately aliquotted and will then be frozen and transported to the study's labs in-country, where they will be stored at –80°C. Commercially available ELISA kits will be used to measure total IgG, IgG endotoxin core antibodies (Coaset EndoCAb), C-reactive protein (CRP), alpha-1 acid glycoprotein (AGP), interleukin 6 (IL-6), interleukin 1 (IL-1), tumor necrosis factor (TNF), and insulin-like growth factor 1 (IGF-1). At least 50 microliters of each serum sample will be reserved for nutritional markers (iron, vitamin A, folate, and B12) and the rest of the sample will be frozen to allow for the analysis of future parasite, environmental enteropathy, and stress biomarkers. Aliquots of the blood samples may be shipped to the WASH Benefits lab at icddr,b (Bangladesh) and Stanford University (USA) and other countries for the nutrient panel, telomere analyses, and EE biomarker testing to ensure comparability between the WASH Benefits Kenya and Bangladesh sites.

Stool specimen collection: Stool collection will require two visits to each household. On day 1, the field team will deliver to each caregiver a stool collection kit and instruct them how to collect stool from their children. Caregivers will be instructed to collect stool from their children on the following morning in the event that the child defecates before they report to a central location in the cluster for urine and serum sample collection. Caregivers will be instructed to have their child defecate in a plastic diaper or on a sheet of provided aluminum foil, to use a provided plastic scoop (integrated into a storage container) to collect ~10 mL of fresh stool from the top of the pile, and to either remain in their household for sample pickup or bring the collected specimens to the centralized test location. On day 1, staff will either collect at the household level or set up a central testing station for the blood sample collections in each cluster (details above). On day 2, staff will collect the stool samples. Field staff will aliquot stool specimens (either collected by the caregiver in the early morning, or collected by field staff at the central location) for the analysis of intestinal protozoan parasites (*Giardia*, *Entamoeba histolytica*, *Cryptosporidium* sp.) and environmental enteropathy stool biomarkers using ELISA. We will maintain a cold chain of 4°C until the samples are transported to central lab (<48 hrs) where they will be stored at –80°C until they are analyzed. Aliquots of the stool samples may be shipped to the WASH Benefits labs at icddr,b (Bangladesh) and Stanford University (USA) and other countries for the EE biomarker testing to ensure comparability between the WASH Benefits Kenya and Bangladesh sites.

Saliva specimen collection and allostatic load measurements: On day 1, the field team will collect a total of 4 saliva specimens from the study children (before the blood draw, immediately following the blood draw, and at a later time point after the blood draw) using a soft sponge and tube. To measure allostatic load, blood pressure measurements will be

taken using a standard sphygmomanometer and heart rate will be measured using a finger pulse oximeter. The mother will receive the blood pressure and heart rate results for herself and her child. The saliva samples collected from day 1 will be used to measure cortisol reactivity, cortisol recovery, and cumulative cortisol levels using a commercial ELISA kit. Telomere length will be measured in saliva samples collected from the children and mothers. Aliquots of saliva will be shipped to Johns Hopkins University and UC Irvine for EE biomarker analysis (e.g., secretory IgA, pathogen specific IgA and IgG multiplex assays, cortisol, etc.) Aliquots of the saliva samples may be shipped to another country for telomere analysis.

Stress surveys: During the 6-hour period when the field workers are already present in the household collecting urine samples in the EE subsample, interviewers will administer the Cohen's Perceived Stress Scale (PSS) (Cohen, Kamarck et al. 1983) during the 6-hour period. The PSS test asks questions regarding stressful life experiences in the past month and has been widely used in many countries (Hamad, Fernald et al. 2008). The Patient Health Questionnaire (PHQ) will be used to measure maternal depression. Guardians, mothers, and fathers will also be interviewed to obtain general information on perceptions of social norms that may affect child health.

Breastmilk sample collection: Breastmilk collection will occur during the household visit on the second day. Mothers with infants between 1 and 4 months, who have anthropometry data from the previous day, agreeing to answer questions about recent food intake and able to give written informed consent are eligible for breastmilk sample collection. Children will be requested to fast for one hour prior to receiving the lactulose/mannitol/ solution and liquids are recommended to be given 30 minutes after the dosing, so to standardize time since last feed, we recommend mothers to breastfeed just prior to the fasting period. When the mother next chooses to breastfeed her baby after the fasting period, we will suggest that she feed her child for 1 minute from the same breast as the last feed, and then switch the baby to the other side. From the first breast, the mother will be asked to hand express 10 mL of milk into a plastic cup. The sample must be collected before 11am.

Milk will later be aliquoted into 5 cryovials and be maintained on a cold chain of 4°C until the samples are transported to a field lab, where they will be stored at -20°C. All samples will be frozen at -80°C after they move from the field lab to the storage freezer in Nairobi. These samples will be shipped on dry ice, and the amount of time that breastmilk samples can remain at temperature between 4 and -20°C is < 48 hours. Breastmilk will be shipped to the University of California Davis for analysis of B-vitamins, fatty acids, and lactoferrin, and for archiving.

The milk collection procedure will be standardized to match technique used in multiple ongoing studies that collect “casual milk” for nutrient content comparison. The steps for collection follow:

Observe the mother feeding her infant prior to the lactulose: mannitol dosing and fast period. Record which breast the feed was from, and the time that the feed ended. If no observation is possible, ask the mother when she last fed the infant from the right breast and record this information.

*The field worker may need to ask probing questions to determine the time of last feed from *each breast* and not just the time of last feed.*

Examine the right breast to ensure that the areola or nipple area is not broken or cracked. If the skin is broken, red, or inflamed, wipe the area with a soft cloth and apply petroleum jelly or medicated salve. Check to assess whether a sample can be obtained from the left breast.

Clean the areola and nipple area using water and a soft cloth. Make sure the area is clean and dry before proceeding.

Allow the infant to feed from the right breast (the breast being sampled) to stimulate 'let-down'. *[Note: It is also acceptable if the mother prefers to express milk without the infant suckling at the same time.]*

Approximately 60 seconds (use a watch with a second hand to estimate the time) after the infant begins to suckle, ask the mother to remove the infant (or switch the infant to the left breast). Begin milk collection from the right breast as described below.

If the mother prefers to express milk without the infant suckling at the same time, collect the first ~2ml of milk into a paper cup and discard or feed to the infant; then collect the next 10ml of milk into the plastic cup.

Milk collection by manual expression: Allow the mother to express her milk by hand. Hold the cup for her and allow her to express her own milk. If the mother has difficulty manually expressing milk, ask the mother to hold the bottle under the nipple. With clean hands, stimulate the lactiferous ducts by gently sweeping the breast beginning from the top (above the mammary glands) towards the areola. Provide counter-pressure with the other hand on the opposite side of the breast.

If the mother has difficulty letting down:

Remove (as much as possible) any sources of stress in the environment: crying babies, heat, crowding, thirst/hunger (have water/plain biscuits on hand), etc.

Some mothers with no experience in manually expressing milk may feel more comfortable if the field worker expresses milk for them. Ask the mother which she prefers.

Apply a warm compress (tissue soaked in warm water) over her breast near the under-arm for 10 minutes.

Following collection, use sterile bulb pipets to aliquot 2 mL into 5 different pre-labeled cryovials, and transfer them into the cooler contained in a Ziploc bag that holds all of the moms aliquots together. Record the exact time that the 'casual' milk sample was collected on the Milk Collection form (that is, the time that the mother began expressing milk into the cup).

Record the exact time that the aliquots were placed into the cooler and the cooler box temperature data logger ID.

Sample transport and handling: All samples will be frozen at -80°C after they move from the field lab to the storage freezer in Nairobi. These samples will be shipped on dry ice, and the amount of time that breastmilk samples can remain at temperature between 4 and -20°C is < 48 hours. Breastmilk will be shipped to the University of California Davis for analysis of B-vitamins, fatty acids, and lactoferrin. A sample will also be maintained for archival purposes.

Water quality, sentinel toy, child hand rinse, soil, and food microbial assessments:

At baseline, a sample of stored drinking water will be collected from all households. In addition, all community public water sources used by study respondents for drinking will also be sampled. The field team will collect 100ml of water from each container of stored drinking water container and water source sampled. A subset of households will be selected for measurements of child exposure to environmental contamination using a sentinel toy, mother and child hand rinses, stored drinking water collection, and food microbial assessments. Each household will be given a sentinel toy (plastic toy ball) on day 1 (the same day the household received the stool sample collection container) by the field team. Every toy ball will be sterilized and stored in a sterile bag until it is distributed. The field team will

return on day 2 to collect the toy ball (~24 hours later). He/She will ask the mother to locate the toy ball without touching it to avoid hand contamination. The field research assistant will use sterile gloves to retrieve the toy and place it in a sterile Whirlpak bag containing 200ml of recovery media (sterile water with salts). The toy will be immersed and rinsed for 30 seconds. The field research assistant will remove the toy, place the sealed bag on ice packs, and then return it to the household. The field team will also collect a hand rinse sample from the mother and/or target child. They will be asked to insert his/her hands, one at a time, into a 69 oz Whirlpak bag containing 350 ml of recovery media. The field enumerator will massage each hand through the bag for 15 seconds. In each study compound, ~100 grams of soil will be excavated using a disposable sterile scoop from approximately a 25cm by 36cm area (Pickering et al., 2012). Any previously prepared food being stored in the household for consumption by the index child will be sampled by collecting ~50-100g directly from the storage pot using a sterile spoon, then placed into a sterile plastic bag. Produce items in the household will be sampled by rinsing in a sterile bag filled with clean water and soap. All samples will be placed on ice, then transported to IPA's field microbiology laboratory. All samples will be analyzed for levels of *E. coli*, fecal coliform, and/or enterococci per 100 ml of sample by a process of dilution with sterile water and membrane filtration. An aliquot of the food, hand, water, and soil samples will also be processed by microscopy for detection of *Ascaris*, hookworm, and *Trichuris* ova (Albonico et al., 2012) In addition, aliquots from a subset of hand rinse, soil rinse, diluted food, and water samples will be vacuum filtered and archived for subsequent DNA/RNA extraction, molecular fecal source tracking analysis. The archived filters will be transported to Stanford University for molecular analysis.

Validation of microbial source tracking methods

Fecal contamination has been predominately measured by culture-based methods targeting fecal indicator bacteria, such as *E. coli* and enterococci. However, fecal indicator bacteria can originate from various sources, including human, livestock, and wildlife. Culture-independent methods to rapidly detect fecal sources are being developed using molecular assays that target the 16S rRNA gene of enteric bacteria. The effectiveness of MST fecal markers in identifying contamination by particular hosts' feces varies by region, as the enteric organisms found in the gut of organisms may vary by geography. To evaluate the efficacy of microbial source tracking markers in detecting and distinguishing human, ruminant, and avian fecal contamination in rural Kenya fecal samples will be collected from participants in the WASH Benefits study. Sources will include 20-30 humans (adults and older siblings in study compounds, see consent in Annex 17). The fecal specimens will be made into fecal slurries to allow for filtration of the sample for *E. coli* and enterococci enumeration. Additionally, each slurry will be filtered for DNA collection, and the filter will be archived and stored at -20°C until transported to Stanford University for further molecular processing. At Stanford, each sample will be processed using human, ruminant, and avian MST molecular assays. The assays to be conducted on the samples will be qPCR MST assays, giving quantitative measurements of source-specific genetic markers.

Piloting of improved fly density measurements

In order to identify the best method for measuring fly densities at latrines and food preparation areas at households, we will conduct pilot measurements in approximately 200 households in villages that are censused but not enrolled into official clusters for the WASH Benefits study. The piloting will entail comparing the use of a fly grill rapid observation

technique versus the hanging of sticky tape for enumerating fly densities. The fly grill technique, introduced by Scudder in 1947, is a widely utilized and versatile technique for determining fly concentrations in a given area. The grill usually consists of 16-24 slats arranged to form a square of between 20cm and 80 cm, which is painted yellow. The design of the grill attracts flies in the area, which are then observed and counted over a period lasting 30 – 90 seconds. The fly sticky tape method involves hanging 3 horizontal strips of Revenge sticky fly tape in the kitchen and latrine areas. The following day field officers take down the fly tape and count the number of flies captured. In addition to an exploration of the Scudder Grill as a more time efficient and cost effective fly enumeration method, this pilot seeks to establish the variety and types of fly species seen in our study area. Speciation will be done using both the sticky tape and fly grill methods. For the sticky tape method the number of each species of fly will be enumerated by counting the flies on the tape when it is removed and disposed of after 24 hours. Staff will receive a detailed speciation training, and will have species fact sheet guides with photos of local flies to refer to in the field. Staff enumerating flies with the fly grill will also have the same materials and training, but will note just the presence/absence of the target species. Genera/species to be targeted are: *Musca domestica*, *Fannia canicularis*, *Calliphora*, *Lucilia*, *Stomoxys*, and *Sarcophaga*.

Quality Assurance / Quality Control (Back check) : We will include biological and technical replicates to ensure data validity. Aliquots from the same biological sample will be analyzed separately and compared. For example, the ELISA test can be performed twice on two separate days for the same sample. We will set aside an aliquot of each batch of L/M solution for further testing in case there are any batch inconsistencies. Multiple field research assistants can record anthropometric, blood pressure, and heart rate measurements for the same child to measure human error. A subset of respondents will be resurveyed (back checked) for quality control purposes.. After establishing that we are speaking to the person who participated in the survey we will ask select questions from the survey whose answers should not change over time. This “backcheck” activity will occur within ~1 month of the survey and be conducted by trained enumerators. I would add this on page 28. For our diet recall portion of the study, on a non-consecutive day, a subsample of mothers (n=60) will be revisited to be interviewed about their diet as a quality control measure.

A list of the modules/instruments that will be utilized at baseline include:

- Module 0. Tracking information
- Module 1. Birthdate & age measurement
- Module 2. Diarrhea and illness symptoms
- Module 3. Deworming
- Module 7. Handwashing assessment
- Module 8. Sanitation assessment
- Module 9. Child defecation and feces disposal assessment
- Module 10. Water access, storage, and treatment practices
- Module 11. Food insecurity
- Module 13. Measures for spillover
- Module 15. Environmental sampling (subset of households)
- Module 17. Maternal intelligence
- Module 18. Maternal food frequency questionnaire

Module 20. Environmental enteropathy assessment (subset of study population)
Module 21. Intestinal parasites assessment (for older siblings of target children)

4) Intervention Implementation

There will be period of up to 3 months between the baseline assessment and the intervention implementation. Field staff will return to the intervention compounds after the baseline assessment and randomization into intervention arms. IPA staff or a sub-contractor will implement latrine improvements, build handwashing stations, and/or install chlorine dispensers, depending on the study arm. Field staff will discuss the process of intervention implementation and messaging with study participants in each community as the interventions are being rolled out and participants will provide informed consent before receiving interventions. We will distribute hardware components of the interventions according to the following criteria:

| | |
|---|--|
| Chlorine dispensers | Available to anyone who uses the water source |
| Bottled chlorine | To all households in enrolled compounds; additionally (in water, WASH, and WASH+ arms only) to all households with children under 5 for at least the first year of intervention delivery |
| Tippy-tap handwashing station and soap | One near the primary latrine used by enrolled pregnant women; one near the enrolled pregnant woman's cooking area (both can be used by anyone in the compound) |
| Sani-scoop hoes | To all households in enrolled compounds |
| Potties | To all households with children under 3 at baseline in enrolled compounds |
| Plastic latrine slab with drop hole cover | One for the enrolled pregnant woman's primary latrine (if it is not cement) |
| Drop hole cover | One for each latrine in the compound that does not have a cover at baseline and is not receiving a plastic slab |
| Latrine construction | One for each compound with pregnant women that does not already have a latrine |
| LNS | To all target children from ages 6-24 months; additionally to siblings aged 6-24 months of target children during the period when the target child is receiving LNS |

A trained, local health promoter will deliver the behavior change communication intervention. The health promoter will work with authority figures in the community to communicate central messages depending on the assigned intervention, for example that child feces should be disposed in a latrine, that it is the occasional unseen contaminant in both water and on hands that needs to be protected against, and that key times to wash hands with soap include after defecation, after cleaning a child who has defecated, before preparing food and before eating or feeding their infants.

The nutrition intervention will be implemented in two phases. Before birth, and during the first 6 months of the child's life, study promoters will promote healthy nutrition during pregnancy and lactation, and encourage mothers to exclusively breastfeed their children. When children turn 6 months of age and are starting to eat solid foods, mothers/guardians

will be instructed to continue breastfeeding along with offering complementary food. They will be instructed to mix 1 sachet (10 mg) of the supplied nutrition supplement (LNS) into the baby's meal (i.e. porridge) 2 times per day. The mother/guardian will be given a 1-month supply of the supplement at a time. The local health promoter will deliver the monthly supplies, will be responsible for delivering behavior change communication (BCC) messages encouraging continued breastfeeding, feeding of nutrient rich complementary foods, feeding frequency, and proper use of the supplement. In addition to messages about the preparation and mixing of LNS into children's complementary foods, we plan to deliver a series of messages about breast feeding and the consumption of micronutrient-rich complementary foods to households receiving LNS, modeling these messages on those recommended in the Guiding Principles for Complementary Feeding of the Breastfed Child and the recent UNICEF Program Guide for Infant and Young Child Feeding Practices. General messages will include (1) practice exclusive breastfeeding from birth to 6 months of age and introduce complementary foods at 6 months of age while continuing to breastfeed; (2) continue breast feeding as you did before receiving LNS (inserting a local term for LNS); (3) provide your child micronutrient-rich foods such as meat, fish, eggs, and vitamin A rich fruits and vegetables (adapted to locally available food examples); and (4) feed your child at least 2-3 times per day when 6-8 months old and 3-4 times per day when 9-24 months old.

For all intervention arms, the health promoter will visit the participating household frequently (i.e. 2 visits per month) early in the study; later in the study, the promoter visits will taper off to one visit per month. Once per month, promoters in intervention arms will check on the status of the hardware and talk by phone with their supervisor at IPA with the results of these hardware checks. If we find that take-up drops below our acceptable threshold based on records provided to IPA by the promoters, or random spot-checks to confirm such records, then we may intensify the intervention, for example by holding additional community meetings, organizing some sort of contest that would motivate villages with prizes for higher take-up. In the hygiene arms, we might also opt to start providing free soap.

We will also use the phone calls from promoters to assess their performance and trigger closer oversight or additional training, and to collect information on births, deaths, and participants who have moved. In all but the passive control arm, promoters will measure the study child's MUAC (or the pregnant mother's belly circumference) monthly, so that there is also a performance measure for promoters in the active control arm. Promoters will share the result of these measures with the enrolled mother on each visit. For study children who are 6 months or older, promoters will refer malnourished subjects to their local health facility based on the Ministry of Medical Services (MOMS) and Ministry of Public Health and Sanitation (MOPHS) guidelines for use of MUAC measurements as specified in the Handbook on Integrated Management of Acute Malnutrition (GOK, 2012b). All children > 6 month old with MUAC <11.5cm will be referred to health facilities treating Severe Acute Malnutrition. For those study participants who migrate we will attempt to visit them and provide at least a part of the intervention package and have our staff provide similar information and checks that a promoter would but much less frequently.

Promoters will be instructed not to attempt to provide any other health information, but rather to refer study participants to their local Community Health Worker or health facility for assessment. IPA has made arrangements with health facilities in the study area to accommodate these referrals.

Monitoring and Process Documentation. Valid evaluation of the study hypotheses requires a consistent intervention. While an intervention of this scope and complexity involving this many individuals will inevitably have some deviation from optimal implementation as planned, meaningful interpretation of the results requires a rigorous assessment of how consistently the intervention activities were delivered. This assessment will include a monitoring system that tracks procurement and distribution of commodities and technologies, recruitment, as well as training and supervision of promoters. Uptake indicators provided by promoters (i.e. presence of soap in tippy tap) will be monitored monthly and staff will conduct random spot checks at a subset of households (also called a rapid uptake assessment). In addition, trained field workers will conduct a five hour structured observation in a subset of participating households (up to 1000) to observe how the interventions are being used. The structured observation will include observation of defecation events, child feeding events, handwashing events, and water extraction events. Qualitative (focus group discussions and in-depth interviews) follow up may be necessary with the study communities (compound, promoters, etc.) depending on the outcome of the process monitoring. For example, if there is low uptake of a specific intervention or a challenge with a distribution system the project leadership may determine that more details are needed that can't be obtained quantitatively. Questions developed for the individual arms will be aggregated for implementation in the combined arms. If the phone call, spot check, or structured observation data suggest important difficulties in uptake and use of the interventions, then the project leadership will consider changes to the intervention.

Use of sensors to monitor uptake. To objectively and accurately monitor usage of tippy-taps and latrines, we will employ remote sensors to assess frequency of handwashing and latrine use among a subset of households post-intervention delivery. The sensors are remote battery-powered monitoring devices that can transmit data in real-time via a mobile phone network. The sensors will be provided by Dr Evan Thomas of SweetLab at Portland State University, who has a great deal of experience designing and deploying sensors to monitor usage of health-related products. The latrine motion sensor will capture when individuals enter and exit latrines, providing an objective indicator of per-capita latrine use in compounds. The tippy-tap sensor will incorporate an accelerometer and pressure transducer to allow for detection of the tippy-tap motion, as well as determination of the volume of water used per handwashing event. The two types of sensors will be deployed together among a subset of households in the hygiene, sanitation, WASH, and WASH+N arms (however only the latrine sensor will be deployed in the sanitation arm). Up to 800 households will be invited to have sensors installed on their tippy taps and latrines (N~200 per arm) for a period of 1- 2 weeks. We will compare the objective sensor usage data with other measured outcomes of uptake to better understand the potential social desirability bias associated with observational and self-reported indicators of handwashing and latrine use frequency.

Promotion of Chlorine Dispensers to Other Households in the Community with Children Under 5. Although we do not currently have plans to collect any data on older children in the study communities, as a community-level intervention the chlorine dispenser could possibly have positive health benefits for other members of the community, particularly children under 5. In order to maximize this potential, as part of the WASH Benefits study IPA will support a Community Dispenser Promoter (CDP) in each cluster enrolled in the water, WASH, and

WASH+ arms of the study. The CDP program will be modeled on the WASH Benefits promoter program, but will be slightly less intensive. In order to facilitate long-term sustainability of the chlorine dispensers installed through the WASH Benefits study, for each dispenser, a Chlorine Dispenser Volunteer will be selected and trained to monitor the functionality of the dispenser and alert the promoter when a refill is necessary.

5) Midline Assessment

Field teams will measure outcomes at 1 year following the initiation of intervention. Children will be between 9 and 15 months at the 1-year survey. This will be the first round in which the field team measures anthropometry (aside from in the EE subsample). The field teams will measure length, weight, MUAC and head circumference using standardized measurement techniques. Our anthropometric teams will have been trained and standardized in measurement techniques according to the FANTA and WHO guidelines (Cogill 2003, deOnis 2004). The child will be weighed using a calibrated scale and measure his/her length or height using a height board, MUAC and head circumference using a tape measure. We will also measure maternal height and weight. The common modules that will be utilized at the midline assessment include:

- Module 0. Tracking information
- Module 1. Birthdate & age measurement
- Module 2. Diarrhea and illness symptoms
- Module 3. Deworming
- Module 4. Anthropometry
- Module 5. Vaccination history
- Module 6. Child food frequency questionnaire
- Module 7. Handwashing assessment
- Module 8. Sanitation assessment
- Module 9. Child defecation and feces disposal assessment
- Module 10. Water access, storage, and treatment practices
- Module 13. Measures for spillover
- Module 14. LNS Assessment (in WASH +Nutrition and Nutrition arms only)
- Module 15. Environmental sampling (subset of households)
- Module 16: Child development
- Module 19: Patient Health Questionnaire (PHQ) – depression screening
- Module 20. Environmental enteropathy assessment (subset of study population)
- Module BF: Breastfeeding Practices

The uptake assessment of the sanitation interventions will include spot checks of latrine structures to assess type, cleanliness, stated use, and state of repair, latrine depth, as well reported disposal methods for children's feces and the presence of child feces or other feces that appears to be human in or near the compound. Measures of fly density and sentinel object (Child Toy) contamination, will also be assessed.

The uptake assessment for the handwashing intervention will be the presence of soap and water at the handwashing station, the per capita consumption of soap in the compound, and the assessment of visible dirt on mothers and children's hands.

The primary uptake assessment of drinking water quality will be through the use of household drinking samples to test for free chlorine residuals. In unannounced visits, field microbiologists will collect drinking water samples (250 ml) from a subset of households that receive water treatment interventions, by asking participants to provide a glass of water that they would give to their child to drink. Collected water samples will be analyzed using membrane filtration for the fecal indicator bacteria *E. coli* and total coliform. We will also assess the presence of free chlorine residual in reportedly treated water.

The primary assessment of compliance with the LNS intervention will be through monitoring the remaining LNS sachets unused at the end of every month. In addition, caregiver knowledge, attitudes and practices related to LNS usage and complementary feeding practices will be assessed.

The midline survey will also include questions that address the major behavior change constructs for social cognitive theory, for new habit formation and for community mobilization. Key behavioral determinant questions will gauge the respondents' perceptions of health risk, disgust, nurturing, habits, and attitude towards water, sanitation, and hygiene practices.

The midline survey will also include water sample collection in all water (water, WASH and WASH+) clusters. Water samples will be analyzed using membrane filtration for the fecal indicator bacteria *E. coli* and total coliform as described earlier in the protocol.

At midline, child development will be assessed using the World Health Organization motor milestones to determine if the child has achieved normal physical motor skills for his/her age (such as sitting up). In addition, the Ages and Stages Questionnaire (ASQ) may be implemented to understand if the child's cognitive development is on a normal schedule.

Environmental Enteropathy, telomere length, allostatic load, and micronutrient biomarkers will also be measured, as described above (3 Baseline Assessment). Within the EE sub-study, a subset of respondents (n=60) will be re-visited to collect a second day of detailed dietary data on the participant. This activity will involve a separate consent.

A subset of households in the active control and intervention arms will be selected for environmental sampling at midline. This sampling will include collecting/testing the target child drinking water, sentinel toys, target child hand rinses (measure of contamination likely to be ingested by child from hands), food to be consumed by the child, and soil collected from the child's compound/play area (methods described above). We will also measure fly densities and conduct fly speciation in the latrine and food preparation areas in the same households.

6) Endline Assessment

The final assessment will take place 2 years after the initiation of intervention. Children will be between 18 and 27 months in age. The common modules to be included are:

- Module 0. Tracking information
- Module 1. Birthdate & age measurement
- Module 2. Diarrhea and illness symptoms
- Module 3. Deworming
- Module 4. Anthropometry
- Module 5. Vaccination history
- Module 6. Child food frequency questionnaire
- Module 7. Handwashing assessment
- Module 8. Sanitation assessment
- Module 9. Child defecation and feces disposal assessment
- Module 10. Water access, storage, and treatment practices
- Module 13. Measures for spillover
- Module 14. LNS Assessment (in WASH +Nutrition and Nutrition arms only)
- Module 15. Environmental sampling (subset of households)
- Module 16: Child development
- Module 19: Patient Health Questionnaire _ (PHQ) – depression screening
- Module 20. Environmental enteropathy assessment (subset of study population)
- Module 21. Intestinal parasites assessment (target children and older children)
- Module BF: Breastfeeding Practices
- Module PS: Perceived Stress Scale

Trained field staff will repeat the anthropometric, child development, and diarrhea morbidity assessments. Spot checks and behavior change assessments will be performed as described above (see Midline Assessment). A short depression screening (PHQ) will be administered which has been used in Western Kenya and many other parts of the world.

Chronic exposure to stressors, such as overcrowding, poor quality housing, pollution, and family turmoil, is associated with poorer health and developmental outcomes across the lifespan (Shonkoff et al 2009). Early adversity may impact health and development by disrupting the child's ability to regulate stress response and recovery. Sustained physiological response to stress in animals has been linked with damage to the hippocampus, a brain structure important for learning and memory (National Scientific Council on the Developing Child, 2005/2014). For this measure, videotapes of child reactivity to a stressful event (the blood-draw, as described in the Environmental Enteropathy substudy above) will be made for the children enrolled in that substudy. The videotapes will be used to code child emotional reactivity made before and after the blood draw. There is growing evidence that very high or very low reactivity to a stressful event (e.g., blood draw) is related to difficulties in regulating emotions and developing executive function abilities in young children, which in turn has been related to poorer school performance and cognition during primary school years (Blair, 2010; Blair et al 2011). It is believed an underlying mechanism for poorer development of executive function abilities is that high or low reactors have either too much or too little cortisol available for optimal brain growth in regions governing development of these skills. Gathering information on children's emotional reactivity will contribute to our understanding of how biological factors (i.e., cortisol regulation) help shape child development and behavior. This will also enable us to explore the relative inputs of the

WASH and nutrition interventions on other child outcomes being gathered in the study (language, motor, personal-social and executive function skills).

The endline survey will also include water sample collection in all water (water, WASH and WAHS+) clusters. Water samples will be analyzed using membrane filtration for the fecal indicator bacteria *E. coli* and total coliform.

Environmental Enteropathy, telomere length, allostatic load, and micronutrient biomarkers will also be measured in a subset of participants, as described above (Baseline Assessment). At endline, hemoglobin will also be measured in the field using a portable spectrophotometer (Hemocue) for the assessment of anemia status. In addition, rapid diagnostic tests will be used to test for malaria. Results will be immediately available and will be provided to mothers during the visit. Children with severe anemia Hb<70 g/L or malaria (positive malaria test plus fever) will be referred to the local health facilities. An aliquot of the packed cells will be sent to KEMRI/Wellcome Trust Laboratory in Kilifi that is headed by Dr. Thomas Williams for the assessment of genetic markers of inherited hemoglobin disorders (sickle cell and thalassemia). Children with a positive sickle cell disease test result will be re-visited to have a second blood draw in order to conduct a confirmatory test. This re-visit will involve a permission statement. Those who test positive will be provided with transportation to a local health facility with the capacity to provide care.

Intestinal parasitic infections will also be measured at endline. We will collect stool and blood spot samples from all target children per cluster and one or more older children (3 – 15 years old) living in the same household compound. The procedures will be the same as described in the Baseline Assessment section, but will also include a stool aliquot used to measure the presence and intensity of soil transmitted helminths (*Ascaris*, *Trichuris*, hookworm) using the Kato-Katz microscopy method. Extra stool kits will be delivered to compounds in order to maximize stool collection from each enrolled individual. Stool may also be collected at a central location instead of at individual household. One stool aliquot will be used to measure the presence of protozoan parasites using ELISA assays (*Giardia*, *Entamoeba histolytica*, *Cryptosporidium sp.*). A second aliquot will be used to measure the presence and intensity of soil transmitted helminths using Kato-Katz microscopy. Stool samples will also be analyzed by multi-parallel qPCR for soil transmitted helminths, including targets for *Necator americanus*, *Ancylostoma duodenale*, *Ascaris lumbricoides*, *Trichuris trichiura*, and *Strongyloides stercoralis*. qPCR assays will be performed because they have been shown to have better sensitivity than microscopic methods (Mejia, 2013). Both Kato Katz and qPCR will be used to detect soil transmitted helminths because these qPCR assays have not been validated in our study area in Kenya yet. Microscopy assays on stool will be performed within 4 - 8 hours at our sample processing centers in Kakamega and Bungoma in collaboration with ESACIPAC and will yield quantitative counts of eggs per gram of stool tested (Knopp 2011; Albonico 2012). ELISA and qPCR assays will be conducted at ESACIPAC at KEMRI in Nairobi. Training of ESACIPAC staff on ELISA and qPCR assays will be provided by study collaborators. A small subset of stool aliquots will be shipped to the United States for quality control analysis using qPCR. At the time of our final stool specimen collection round, in which we test for soil transmitted helminths, we will provide all compound members a dose of deworming medication (albendazole). [We will not provide deworming

medication at the time of stool collection in earlier rounds because we will not test for helminth infection in earlier rounds.] A subset of stool and blood spot samples may be shipped to Stanford University, Smith College, or other institutions in the United States (e.g. for quality control purposes to ensure comparability with assays run at KEMRI).

Endline Quality Assurance/Back Check: We will follow the same quality assurance / quality control measures as described for the baseline assessment above.

A subset of households in the active control and intervention arms will be selected for environmental sampling at endline. This sampling will include the target child's drinking water, sentinel toys, target child hand rinses (measure of contamination likely to be ingested by child from hands), primary caretaker hand rinses, food or fresh produce from the household, and soil collected from the child's household (samples will be analyzed for fecal bacteria and soil transmitted helminthes; methods described above). We will also measure fly densities and conduct fly speciation in the latrine and food preparation areas in the same households.

Assessing child mortality. A verbal autopsy would be conducted with the primary caregiver of each deceased study child. The World Health Organization has developed a standard verbal autopsy questionnaire to assess cause of death among children 1 month to 14 years of age. The tool takes 45 minutes for a trained interviewer to administer. Following the verbal autopsy, a computer program (InterVA) can be used to determine the most likely cause of death. If funded, as a cross-check, approximately 5% of verbal autopsies would be reviewed and coded by a physician familiar with causes of child mortality in rural Kenya. Verbal autopsies would be conducted 1-36 months after a death. Recommended optimal recall periods have ranged from 3 to 24 months (Fottrell 2010), however, there is evidence that length of recall period up to five years does not affect data quality, since death is a salient event (Baqui, 1996).

Data from health facilities. The current study design focuses on individual-level outcomes, measured in the household, as functions of the various interventions. While child-level outcomes are clearly of utmost interest where health and human wellbeing are concerned, from a public health perspective there is good reason to also measure the cost effectiveness of the study interventions. Evidence of cost savings from prevention, relative to the cost of treating diarrhea and other diseases after presentation at a health facility, is especially useful for governments facing tight budget constraints for health care. If funding is obtained, we will plan to compile data from health facilities in the study area in order to: i) to measure the effects of interventions on demand for formal health care among the target population; ii) to measure spillovers from improvements in the sanitation environment, or from changes in village norms, on otherwise unsurveyed and un-treated members of the village population; and iii) to inform a robust cost-benefit analysis, oriented toward the Government of Kenya, showing the relative costs to the public health care system of prevention and treatment.

Data for the clinic survey will be gathered directly at all of the health clinics, hospitals, and larger dispensaries, including both private and public sector facilities, in the study area. We will collect information from all patients presenting with cases of diarrhea, other waterborne illnesses, respiratory illness, malnutrition, and an unrelated condition for conducting falsification tests. In order to minimize disruption at the facilities, our aim is to gather only the data necessary

to measure the rates of health care demand associated with the evaluation arms. The survey will gather the following data points: date and time, age, gender, location, sub-location, village, confirmed diagnosis, registration book from which record was drawn, whether other facilities have been visited for this case of this illness, which other facilities have been visited, and whether the patient lives in a treated or treatment-eligible household. Village names will be pre-coded to ensure accurate matching of cases to treatment arms.

To assemble the data of interest from health facilities, we will maintain a separate record book next to the main triage registration book at each facility. When patients with a condition of interest return to the registration desk to have their diagnosis recorded in the registry, if oral consent is granted by the patient, the necessary information will be recorded in the study record book. At multiple-service facilities, a small number of additional records on cases of diarrhea and other conditions of interest will be maintained in the inpatient registration book, Child Welfare Clinic (CWC) records (which record malnutrition), and Comprehensive Care Center (CCC) files. Staff in the inpatient and CWC wings will be trained to notify the field officer responsible for the study record book when a relevant case appears, and the field officer will obtain oral consent from patients before collecting data. A daily check of the registration book for these wings will be conducted to ensure that all cases were recorded, and additional training will be provided if reporting rates are low. To doubly protect CCC patient anonymity, a short form will be provided to the CCC staff each day, and they will be trained to obtain consent and provide the relevant information without granting IPA field officers access to the patient files. These additional procedures related to the inpatient records, CWC, and CCC will account for only a small fraction of total cases, and final protocols will be developed with the input of clinic staff to ensure that the data collection system is not disruptive.

IPA field officers will rotate between facilities, rather than remain stationed at one facility for the duration of the project. Each field officer will be assigned a set of facilities, and she/he will visit them in sequence, one each day. The ratio of staff to facilities will vary with the size of the facilities; dispensaries will be visited less frequently than the larger clinics. One or more current clinic staff members will receive training and ongoing support related to the study records, and will be compensated with a small stipend to maintain the study record book on the days when our staff is not present. All clinicians and nurses will be made aware of the study record book and the required data so that they can assist in ensuring that the collected data is accurate and complete. In-kind compensation in the form of medical supplies (yet to be determined, but likely rubber gloves, masks, cleaning supplies) will be provided to each participating facility each month. The study record book will be anonymized (will not contain any identifying patient information). The study record book will take the form of a binder with ringed, rather than stapled, pages. Field staff will remove all records each time they leave the clinic, and deliver the data to their respective IPA offices. Data entry will be ongoing throughout the study.

b) Explain who will conduct the procedures, where and when they will take place. Indicate frequency and duration of visits/sessions, as well as total time commitment for the study.

Trained field staff will conduct all diarrhea morbidity, and child development assessments in the participant's home or central location at the beginning of the study, one year following intervention implementation, and 2 years following intervention implementation.

Anthropometry measurements will take place in centralized location in the village a few minutes' walk from the household. These assessments will take about 1 hour to complete, and we expect the baseline/enrollment visit to require 2 hours total.

Local health promoters will visit households biweekly early in the study to encourage families to adopt the new behaviors, gradually tapering off to at most monthly by the end of the two year period. In the arms receiving the nutrition intervention, the promoters deliver the supplement to target households, teach the mother/guardian about proper use of the supplement, and will deliver behavior change communications to encourage breastfeeding and proper feeding of complementary foods after 6 months of age. In arms receiving water, sanitation, and/or handwashing interventions, the messaging will vary depending on the group assignment.

Environmental Enteropathy assessment will be conducted by trained staff, including phlebotomists. This assessment will take place at the beginning, middle and end of the study. The assessment will take about 10 and 15 minutes per participant and will occur over two days (approximately 3 hours and 15 minutes the first day and 7 hours the second day)

Stool specimen collection to test for intestinal parasites will be conducted by trained field staff during the final survey. Stool collection will require two visits to each household. The first visit will last about 20 minutes (not including consent time) and will involve delivery of the stool collection kit and provision of instructions to the caregiver regarding stool collection. The following day, field staff will return to collect the stool specimen. This visit is expected to take 10 minutes, and if logistically feasible will be combined with the household survey.

Our study staff will interact with a sub-set of households as part of our Monitoring and Process Documentation Monitoring activities. The uptake assessment will occur with a randomly selected sub-set of households who are consented and take about 20 minutes. The qualitative components will only be done if there is a need to further understand a specific component of the study. These interactions will take between 60 and 90 minutes.

- c) **Identify any procedures that are experimental/ investigational and explain how they differ from standard procedures (medical, psychological, educational). If applicable, distinguish between procedures that the subject would undergo regardless of enrollment in the study and procedures done specifically for study.**

The element of this study that is not a standard public health interventions is the intensive data collection required to learn from the experience. Most of this data collection involves observation and collection of information that is not particularly culturally sensitive.

The LNS used in the study will be a slightly modified variant of Nutributter, which is a commercially-available supplemental feeding product sold by Nutriset. The specific LNS formulation that we will use has been developed by our nutrition colleagues at UC Davis and has been tested extensively in Bangladesh, Malawi, Ghana and Burkina Faso.

- d) **If a placebo will be used, provide rationale and explain why active control is not appropriate.**

The control group will not receive a placebo. However, the active control group will receive promoter visits. These promoters will measure upper-arm circumference.

- e) **If any type of deception or incomplete disclosure will be used, explain what it will entail, why it is justified, and what the plans are to debrief subjects. See [CPHS Guidelines on Deception and Incomplete Disclosure](#) for more information. Any debriefing materials should be included in the Attachments section.**

N/A

- f) **State if audio or video taping will occur. Describe what will become of the tapes after the project (e.g., shown at scientific meetings, erased) and final disposition of the tapes.**

Audiotaping, photography and videotaping may be done during assessment visits to record interviews and observations. All media will be digitized and securely stored for up to 20 years. At that point, the media will be destroyed.

In addition, some videotapes and photographs may be used in public presentations and on project websites. Specific consent for these uses will be obtained from study participants.

10. Alternatives to Participation

Describe appropriate alternative resources, procedures, courses of treatment, if any, that are available to prospective subjects. If there are no appropriate alternatives to study participation, this should be stated. If the study does not involve treatment/intervention, enter "N/A" here.

Prospective subjects are free to carry on with their current sanitation and hygiene practices, regardless of whether or not they chose to participate in the study. Those who choose not to participate can purchase most materials that will be subsidized during the course of the study (i.e. latrine components, potties, and soap are available in local stores). Information about advisable sanitation and hygiene practices can be obtained from the Government of Kenya's public health system, by visiting local hospitals or district clinics.

11 – 14 to be filled in online

15. Risks and Discomforts

- a) **Describe all known risks, discomforts associated with study procedures, whether physical, psychological, economic or social (e.g., pain, stress, invasion of privacy, breach of confidentiality), noting the likelihood and degree of potential harm.**

There is minimal risk of physical, psychological, social, or legal injury from participation in this study.

No severe allergic or other reactions to Nutributter (a product with similar ingredients to the LNS used in this pilot study) were observed in similar studies in Ghana or Bangladesh

(conducted by our UC Davis team members), and none are expected in this study. In the LNS arms, when the children are age 6 months we will ask about peanut allergies and when children first receives LNS, a small test sample will be provided by the promoter and children observed for at least 10 minutes. If the child experiences any immediate reaction, the child will be referred to a health facility and transportation costs will be provided if necessary. If a child is allergic, we will not give them LNS but we will retain them in the intention to treat analysis. Community health workers will be trained to tell mothers the symptoms of allergic reactions children may have after using the LNS supplement, and in the event of an allergic reaction, not to give the supplement and to call the IPA office. For any adverse events related to the evaluation study procedures, the project manager and local staff in Kenya will report them to the principal investigator at UC-Berkeley and Innovations for Poverty Action.

The intervention hardware, improved latrines, child potties, handwashing stations, and sodium hypochlorite dispensers are interventions that are widely promoted and used in a variety of contexts. Chlorine treatment of drinking water is the most common water disinfectant used globally, and does not present an increased risk to pregnant women/fetuses. At the time of distributing bottled chlorine, field workers will mention that it is important not to directly ingest bottled chlorine and to keep bottled chlorine away from small children. The interventions involve a behavior change component that involves developing communication messages and training a local health promoter to deliver these messages. This is a standard approach to public health promotion used throughout Kenya and other contexts.

Some aspects of the interventions and data collection activities might be uncomfortable for subjects to discuss, given cultural sensitivities surrounding the topic of defecation. Currently many young children defecate in the open and parents sometimes do not clean this up and sometimes clean it up with a hoe. When using a potty or sani-scoop, parents may be exposed to their children's feces in different ways than they previously were.

There is the slight risk of breach of confidentiality. Community members may see study staff entering study compounds or other homes in their own compounds, and may overhear interviews. We will make every effort to ensure that household surveys and structured interviews are conducted in privacy. There is also the slight risk of breach of confidentiality in the small subset of people from whom we will collect video data. We will make every effort to ensure that household surveys and structured interviews are conducted in privacy, and all video data stored securely.

The measurements for the environmental enteropathy activity involve administration of naturally occurring sugars, and small children seem to enjoy the flavor. There is the risk that some children will not like the flavor and will be upset when it is administered. There is the minimal risk of short-term loose stools that typically resolve within a few hours of LM dosage. Collection of urine and stool may be uncomfortable to the parent or child. There is also the risk of short-term discomfort and pain during the collection of venous blood and blood spot samples.

b) Discuss measures that will be taken to minimize risks or discomforts to subjects.

We will discuss the objectives of the research with participants as part of delivering the intervention. All participants will be informed of potential discomforts during the consent process, and may decide not to participate at any time during the study. The study team will

request verbal informed from all persons (or guardians for children) whom the study will collect data from. Individually identified information will be kept confidential.

Potential harms of the study include that people will give time to the study that would be better given to address other issues. We will address this risk by securing informed consent, and clarifying that study participants can drop out at any time, even in the middle of an interview or group discussion.

Discomfort during venous blood draw will be minimized by using trained phlebotomists to collect the specimens. Needles used to collect blood will be disposed of in a safe manner and will not be re-used. Field staff will be carefully trained to collect blood spot samples. We will explain the procedures to the parents and will be available to answer any questions they may have.

We will streamline the data collection procedures as much as possible in order to take as little of the subjects' time as possible. We will make every effort to put subjects at ease during discussions of sensitive topics such as defecation, by using culturally appropriate terminology or euphemisms as possible, and by reminding subjects at the outset that they are free to withdraw from the study activities at any point. It will be made clear during promotion activities that individuals are free to continue their current sanitation practices, and that no one should be coerced to adopt latrine usage. When parents are trained in use of the child's potty, they will be taught the importance of handwashing with soap afterwards.

To minimize the risk of breach of confidentiality, every effort will be made to conduct interviews in the privacy of the participant's home. Data collected during interviews and observations will be kept secure by study staff.

- c) **If applicable, indicate if a particular study treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject is or may become pregnant) that are currently unforeseeable.**

N/A

- d) **If applicable, describe the Data safety Monitoring Plan (DSMP). [NIH](#) may require a DSMP for some projects.**

An independent Data Safety Monitoring Board (DSMB) will be assembled to monitor adverse events and to advise investigators. The Committee will include a multi-disciplinary team that will track adverse events in the study. This board will meet twice each year, either by phone or in person. Since this is an efficacy study designed to identify proof of principal, even if a marked early benefit is identified with one or more of the intervention, neither the study implementers nor the Government of Kenya will be in a position to immediately scale up effective interventions. Thus, the social benefit of early stoppage is limited. However, if at the midline, child length for age Z- score in any of the intervention arms is more than 2.0 standard deviations above the control arm we will look to the DSMB to decide on the appropriateness of continuing the trial.

- e) **Explain how unanticipated negative outcomes/experiences or serious adverse events will be managed. (NOTE: This may apply in social-behavioral as well as biomedical research, e.g., undue stress or anxiety of subject, breach of confidentiality via loss of laptop computer with study data. Provisions should be made and described here if applicable.)**

In the event of an unanticipated negative outcome/experience or serious adverse event, caregivers will be told to contact IPA staff, who will be trained on how to respond. The event will be documented on an adverse event form and will be submitted to the study investigators at IPA and UC Berkeley. The IPA investigator will report the event to the Data Monitoring Committee and to the local Ethical Review Committee.

- f) **Discuss plans for reporting unanticipated problems involving risks to subjects or others, or serious adverse events to CPHS. (This applies to all types of research.) See [Adverse Event and Unanticipated Problem Reporting](#).**

Adverse events will be reviewed by the UC Berkeley PI. The event will be reported to CPHS if the event 1) is unexpected; 2) is related or possibly related to study participation; AND 3) suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized. If the event is determined by the PI to be reportable, an initial report will be submitted via email to the Director, Research Subject Protection at CPHS. The initial report will be submitted as soon as possible, but no later than 7 days after the PI learns of the event. The initial report will be followed by a formal written report within 14 days of learning of the incident. The formal report will be submitted to CPHS via eProtocol.

- g) **Describe plans for provision of treatment for study-related injuries, and how costs of injury treatment will be covered. If the study involves more than minimal risk, indicate that the researchers are familiar with and will follow University of California policy in this regard, and will use recommended wording on any consent forms (see [CPHS Informed Consent Guidelines](#)).**

This study does not involve more than minimal risk. In the LNS arms, health promoters will recommend that caregivers stop using the LNS and notify one of the IPA staff immediately should their child have any adverse reactions shortly after ingesting the supplement (such as vomiting, stomach pain, rash, breathing problems with wheezing). In the event of an adverse reaction, IPA staff will assess the child's condition and, if necessary, provide transport to the closest medical facility for treatment.

In the anthropometry and enteropathy assessment survey, children who are found to be severely wasted (MUAC < 115mm) will be referred to the appropriate existing treatment programs.

16. Benefits

Describe any potential benefits to the individual subject, group of subjects, and/or Society. If subjects will not benefit directly from study procedures, this should be stated.

NOTE: Do not include compensation/payment of subjects in this section, as remuneration is not considered a "benefit" of participation in research.

Participants will receive the results of all anthropometry assessments and referrals to

appropriate treatment will be made as necessary. The promoters will refer children with MUAC < 11.5cm for management of severe acute malnutrition. Staff will refer children with WLZ<-3 and/or bipedal edema based on EE subsample anthro data, and main study midline, and endline anthro data will also be referred for management for severe acute malnutrition. Children found to have Hb<0.70 g/L (the cutoff used to define severe anemia) will also be referred for treatment and/or medical advice. Children found to have Sick Cell Disorder will be referred to the nearest health facility which can provide care. This will benefit the individual and his or her family in that the early diagnosis of SCD will reduce confusion around the symptoms the child may exhibit, and provide knowledge regarding what actions to take if the child is in crisis. In addition, this testing will help us to gain knowledge about the prevalence of sickle cell disorder in this population. All compound members in study compounds will receive a dose of deworming medication (albendazole), which is used in mass-deworming campaigns across rural Kenya. Households will additionally benefit from free sanitation, handwashing and water quality improvements, and nutrient supplements provided by the study. In the long term, the results of this study could benefit other children in Kenya and elsewhere by helping us understand the effects of providing nutrition supplements in combination with WASH interventions.

17. Confidentiality

NOTE: See [CPHS Data Security Policy](#) before completing this section.

- a) **If reviewing or accessing [Protected Health Information](#) from the Tang Center, Optometry Clinic or Psychology Clinic for activities preparatory to research, describe the process and confirm that the health information will not be removed from the facility.**

N/A

- b) **What identifiable data will you obtain from participants? Note: Audio, photo, and video recordings are generally considered identifiable unless distinguishing features can be successfully masked.**

We record the following identifiable data: study participant and their relative's names, phone numbers, compound location, dates of birth, and GPS coordinates. Blood, stool, saliva, and urine samples will be labeled with the same numerical ID code used on the household surveys and the forms used to collect additional information will include names, phone numbers, compound location, and dates of birth. We will also collect, audio (qualitative research), video and photo images for specific activities.

- c) **If obtaining existing data/specimens, will you have access to identifiers?**

N/A

d) **Explain how the confidentiality of subject information will be maintained. Include:**

- 1. Who will have access to study records/specimens? If the study is subject to FDA regulations, include a statement that the FDA might inspect the records of the study.**

Study specimens will be stored in Kenya and later transported to Bangladesh, Europe (Germany) or the US. Only study staff at these labs (and Investigators who have traveled to these labs) will have access to the specimens. Study records will be digitized and all investigators will have access to the data from questionnaires administered in conjunction with the specimen collection.

- 2. How the records will be secured (e.g., password-protected computer, encrypted files, locked cabinet). Response should be consistent with [CPHS Data Security Policy](#).**

Any data from data collection instruments, photographs, videotapes and audiotapes will be stored at Innovations for Poverty Action after study completion. All video recordings will be identified by a number only; no recordings will identify the respondent by name. These video recordings will be viewed only by trained personnel for coding. Any physical data and video recordings will be stored securely under in a locked storage cabinet or room. The Study PI and project management will have access. Electronic data will be stored on password-protected computers and servers and encryption will be used.

How long study data will be retained.

After study completion, these materials will be digitized, and the hardcopy will be destroyed. Digital copies of the data and video recordings will be stored indefinitely after the conclusion of the study, for the purposes of additional analysis and informing future research project design.

- 3. When audio/video recordings will be transcribed and when they will be destroyed (if ever).**

Digital audio/video files will be transcribed as soon human resources allows, likely within 2 months of data collection. The audio/video files will be kept indefinitely.

- e) **Identifiers should be removed from data/specimens as soon as possible following collection, except in cases where the identifiers are embedded (e.g., voices in audio or faces in video recordings). If data are coded in order to retain a link between the data and identifiable information, explain where the key to the code will be stored, how it will be protected, who will have access to it, and when it will be destroyed.**

Identifiers will be removed from the data at the time that the data is sent out for cleaning and analysis. Identifiers will not be destroyed, but will be stored indefinitely at IPA, as we may return at some future date to evaluate long term intervention effectiveness.

The key to identifiers will be stored in field offices in a locked cabinet and/or password

protected server. Only study personnel that require the key to complete the study will have access to the locked cabinet. Once the study is completed, the key to identifiers will be stored at IPA in a locked cabinet and/or secure server. The identifier key will be both password protected and encrypted when stored electronically.

- f) **Describe how identifiable data will be transferred (e.g., courier, mail) or transmitted (e.g., file transfer software, file sharing, email). If transmitted via electronic networks, describe how you will secure the data while in transit (e.g., prior encryption). If not applicable, enter N/A.**

Data are encrypted before being uploaded to the pass-word protected shared storage server, for which each users account must be approved by the Research Manager, Theodora Meerkerk or PI.

- g) **Will subjects be asked to give permission for release of identifiable data (e.g., for future studies, publications, presentations, etc.), now or in the future? If so, explain here and include appropriate statements in the consent materials. See [Media Records Release Form](#) template for guidance.**

Participants will be asked for permission to use their images in photographs and/or videotapes for public presentations and/or on project websites. No other identifiable data will be included within or along with these photos/videos (i.e. no names or other identifiable data). Appropriate statements have been included in consent materials.

- h) **Explain how subject [privacy](#) will be protected (e.g., conducting interviews in a discreet location).**

Interviews will be conducted in discreet locations by staff who have received standardized training on the sensitive instruments.

Appendix XX: Comparison of nutrient content of Nutributter vs. the LNS produced for WASH-Benefits

| Nutrient | Unit | WHO/FAO RNIs ¹ for children 1-3 y | <u>Nutributter</u> | | <u>WASH-B LNS</u> | | Chemical form of n |
|---------------|------|--|--------------------|-------|-------------------|-------|--------------------|
| | | | Content | % RNI | Content | % RNI | |
| Dose | g | | 20 | | 20 | | |
| Energy | kcal | | 108 | | 118 | | |
| Fat | g | | 7 | | 9.6 | | |
| Linoleic acid | g | | 1.29 | | 4.46 | | |

| | | | | | | | |
|------------------------|----|------|------|------|------|------|--|
| Alpha-linolenic acid | g | | 0.29 | | 0.58 | | |
| Ratio of LA to ALA | | | 4.4 | | 7.7 | | |
| Protein | g | | 2.6 | | 2.6 | | |
| <u>Vitamins</u> | | | | | | | |
| Vitamin A | µg | 400 | 400 | 100% | 400 | 100% | Retyinyl acetate |
| Vitamin D | µg | 5 | NA | 0 | 5 | 100% | Cholecalciferol (D3) |
| Vitamin E | mg | 5 | NA | | 6 | 120% | DL-alpha-tocopherol |
| Vitamin K | µg | 15 | NA | | 30 | 200% | Phylloquinone (K1) |
| Vitamin C | mg | 30 | 30 | 100% | 30 | 100% | L-ascorbic acid |
| Biotin | µg | 8 | NA | | NA | | |
| Folic acid | µg | 150 | 80 | 53% | 150 | 100% | Pteroyl monoglutamate |
| Thiamine (B1) | mg | 0.5 | 0.3 | 60% | 0.5 | 100% | Thiamin hydrochloride |
| Riboflavin (B2) | mg | 0.5 | 0.4 | 80% | 0.5 | 100% | Riboflavin |
| Niacin | mg | 6 | 4 | 67% | 6 | 100% | Niacinamide |
| Pantothenic acid (B5) | mg | 2 | 1.8 | 90% | 2 | 100% | Calcium pantothenate |
| Vitamin B6 | mg | 0.5 | 0.3 | 60% | 0.5 | 100% | Pyridoxine hydrochloride |
| Vitamin B12 | µg | 0.9 | 0.5 | 56% | 0.9 | 100% | Cyanocobalamin (B12) |
| <u>Minerals</u> | | | | | | | |
| Calcium | mg | 500 | 100 | 20% | 280 | 56% | Tri-calcium phosphate |
| Copper | mg | 0.56 | 0.2 | 36% | 0.34 | 61% | Encapsulated copper |
| Iodine | µg | 90 | 90 | 100% | 90 | 100% | Potassium iodide |
| Iron | mg | 11.6 | 9 | 78% | 6 | 52% | Encapsulated ferrous sulfate |
| Magnesium | mg | 60 | 16 | 27% | 40 | 67% | Magnesium citrate |
| Manganese | mg | 1.2 | 0.08 | 7% | 1.2 | 100% | Manganese sulfate |
| Phosphorous | Mg | 460 | 82 | 18% | 190 | 41% | Tri-calcium phosphate & Di-potassium phosphate |
| Potassium | Mg | | 152 | | 200 | | Di-potassium phosphate & potassium chloride |
| Selenium | Mg | 17 | 10 | 59% | 20 | 118% | Sodium selenite |
| Zinc | Mg | 8.3 | 4 | 48% | 8 | 96% | Zinc sulfate |

1. RNI, Recommended Nutrient Intake

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