Lactoferrin and lysozyme supplementation for long-term diarrhea sequelae

The Lactolyze Trial

(NCT05519254/ PACTR202108480098476)

A collaboration between the:
Kenya Medical Research Institute (KEMRI) &
University of Washington (UW)

Statistical Analysis Plan
Version 1.5 (11 Aug 2025)

Table of Contents

SECTION 1. ADMINISTRATIVE INFORMATION

Title: Statistical Analysis Plan Lactoferrin and lysozyme supplementation for long-term diarrhea sequelae (the Lactolyze Trial).

ClinicalTrials.gov Registration: NCT05519254

Pan African Trials Registration: PACTR202108480098476

SAP Version: 1.5 (11 Aug 2025)

Protocol Version: 2.0 (01 June 2022)

SAP Revision History:

SAP version (date)	Justification for Revision	Timing of SAP in relation to interim analysis
31Oct 2022	Review of stopping guidance with DSMC	Prior to trial initiation
20 Jan 2023	Decision to update primary endpoint related to diarrhea to specifically capture moderate and severe disease rather than care-seeking	Prior to trial initiation
26 July 2023	Decision to revise the cut-off for the primary endpoint definition of moderate-to-severe diarrhea to make it more specific, based on feedback from DSMC and subsequent literature review. Also clarified the name of the proposed severity score to be consistent with original publication. Adding further rationale for the stopping rules as per DSMC's suggestion.	Prior to interim analysis
17 Feb 2025	Changed modified intention-to treat to intention to treat analysis as per feedback from co-investigators. Added a new category for high adherence to conduct stratified analysis.	Post interim analysis
11 Aug 2025	Added analysis method for diarrhea-related secondary endpoints Added a new secondary endpoint-proportion of children who recovered from wasting during the follow-up. Added adherence cut-off for per-protocol analyses.	Post interim analysis, before unblinding

Roles and Responsibilities in SAP and Signatures

Name	Trial Role	SAP Role
Dr. Patricia Pavlinac, PhD, MS	Principal Investigator	Chief Investigator
Dr. Benson Singa, MBBS, MPH	Principal Investigator	Co-Chief Investigator
Dr. Barbra Richardson, PhD	Trial Statistician	Senior Statistician
Dr. Kirkby Tickell, PhD, MBBS	Project Director	Epidemiologist
Dr. Ruchi Tiwari, PhD	Post Doctoral Scholar	Epidemiologist

SECTION 2. INTRODUCTION

Background and Rationale

More than half a million children die each year from diarrhea, mostly in low- and middle-income countries (LMICs). Over half of these deaths occur more than 7 days after diarrhea presentation. Children who survive are at increased risk of enteric dysfunction, recurrent diarrhea, and malnutrition, including wasting and stunting. Malnutrition, in turn, further increases vulnerability to infection and is associated with delayed cognitive development.

Current diarrhea management strategies in low- and middle-income countries (oral rehydration solution, ReSoMal and zinc) focus primarily on the management of dehydration and micronutrient replacement and appear to have negligible impact in preventing future diarrheal episodes or improving nutritional outcomes. Lactoferrin and lysozyme are milk-derived nutritional supplements that may reduce the risk of diarrheal episodes and accelerate nutritional recovery by treating or preventing underlying enteric infections and/or improving enteric function. Children with moderate or severe wasting are at particularly high-risk of death, diarrhea recurrence, and nutritional deterioration following a diarrheal episode.[1–5]

Aims

1. **Aim 1.** To determine whether a 16-week course of lactoferrin, lysozyme or a combination of both shortens time to WHO-defined recovery from wasting (MUAC ≥12.5cm) and reduces the incidence of moderate-to-severe diarrhea during the subsequent 6-months following presentation to a health facility with diarrhea among children with moderate/severe childhood wasting
2. **Aim 2.** To explore whether a 16-week course of lactoferrin, lysozyme or combination therapy improves secondary clinical, nutritional, enteric pathogen, and enteric function outcomes.
3. **Aim 3.** To evaluate acceptability, adherence and cost-effectiveness of lactoferrin and/or lysozyme administration in Kenya.

SECTION 3. STUDY METHODS

Trial Design

This will be a factorial, double-blind, placebo-controlled, randomized trial to determine the efficacy and mechanisms of lactoferrin and lysozyme supplementation in promoting enteric and nutritional recovery among children seen at outpatient clinics or being discharged from hospital following management for comorbid diarrhea and wasting. Kenyan children aged 6-24 months with a mid-upper arm circumference (MUAC) <12.5cm returning home from an outpatient visit or inpatient hospital stay for diarrhea will be randomized to 16-weeks of lactoferrin, lysozyme, a combination of the two, or placebo.

Randomization

Block randomization (1:1:1:1) in random sized blocks of no more than 12 will be used to assign treatment groups at study enrollment. Treatment allocation (once assigned) will remain blinded to the participant, the study staff, the hospital clinicians, and the investigators during all data collection and analysis phases of the study. To account for the differences in severity of wasting and dehydration between hospitalized children and those seen as outpatients, we will stratify

randomization by hospital admission status (i.e. inpatient vs outpatient). At the proposed study hospitals we expect approximately 60% of our screened population to be outpatients.

Sample Size

The total sample size required (600 children, 150 per arm) was calculated based on the comparison of moderate-to-severe diarrhea incidence between any single intervention arm (lactoferrin, lysozyme or combined therapy) and the control arm (placebo-treated children). We assumed the incidence of moderate-to-severe in the placebo-arm to be approximately 100 episodes/ 100 child-years based on the Global Burden Disease 2019 estimates of <5 diarrhea incidence of 167/100 child-years [6] and assuming 60% of these episodes would meet the moderate-to-severe definition based on previous data.[7] We assumed a 35% reduction in this incidence in any of the interventional arms based on previous data.[8,9] Using an alpha of 0.05 and assuming 80% power, we will require, at minimum, 134 children per intervention arm. Assuming 2% of children die during follow-up based on estimates of mortality during convalescence from medically-attended diarrhea in Kenya [10], and a 10% loss-to-follow-up, we will enroll an additional 16 children per treatment arm, recruiting 600 children in total (150 per arm).

With 134 surviving children in each treatment arm with complete follow-up, we determined the minimum detectable difference in time to nutritional recovery between each intervention arm and the placebo arm assuming an alpha of 0.05 and 80% power. This conservative estimate is based on not being able to include data prior to censoring so represents the minimal detectable times. Data from CMAM programs suggests that approximately 70-85% of children in CMAM programs recover to a MUAC ≥12.5, with recovery taking between 4 and 8 weeks with standard deviations ranging from of 0.2 to 3.2 weeks, based on frequency of anthropometric assessments (studies with less frequent assessments result in larger SD of time to recovery).[11–15] Based on these assumptions, we will be powered to detect a difference of between 1 day (assuming a 0.2 SD) and 1 week (assuming a 3.2 SD) difference in time to nutritional recovery, respectively.

Framework
All hypotheses are tested for superiority.

Interim Statistical Analysis and Stopping Guidance
A single interim analysis for moderate-to-severe diarrhea incidence will be performed using an O'Brien-Fleming boundary for harm when 50% of expected person time (150 child-years) has been accrued. Interim analyses will be based on any two-way comparison between an intervention arm and placebo. For 37.5 child-year accrued in each arm at the interim analysis, we expect to see 37 events of moderate-to-severe diarrhea in the placebo arm (expected moderate-to-severe diarrhea incidence of 100 episodes/100 child-years, 100/100*37.5=38) and 24 events in each of the three intervention arms (35% reduction in this incidence in any of the interventional arms [8,9], 0.65*37.5=24). Assuming 62 events will be available at half of the person-time accrual per pairwise comparison, a z-score critical value of 2.797, or p-value < 0.005, from Wald chi-square tests of the pairwise intervention arm comparisons with placebo calculated using Poisson regression models will determine the cut-off of statistical significance. An interim analysis for harm based on time to nutritional recovery will not be conducted because it is not clear that days difference in time to nutritional recovery is harmful. Time to nutritional recovery and adverse events will be reported descriptively.

The DSMC will consider the totality of evidence from the interim analysis and descriptive data to make a determination about continuing the study. Futility will not be a basis for stopping rules because of the trials' value in understanding mechanisms of post-diarrhea nutritional recovery and recurring diarrheal episodes. Benefit will also not be a basis for interim stopping because the investigational product is not an immediate life-saving treatment and the placebo arm, or a non-beneficial arm, can still seek care and treatment.

Assuming the DSMC decides to continue the trial after the interim analysis, an alpha of 0.04 will be used as the statistical significance boundary at the final analysis for the primary hypothesis tests.

Timing of Final Analysis
The first main report/ publication of the trial will be prepared for the primary aim when every enrolled child has completed their 24-week follow-up visit or is deemed lost to follow-up and all primary endpoint data has been cleaned (anticipated publication in December 2027).

Timing of Final Outcome Assessment
The schedule of study procedures is outlined in Table 3. Regularly scheduled visits include those at Enrollment, and at home (W2, W6, W8, W12, W14) and in the clinic (W4, W10, W16, W24). Participants are actively followed for 7 days following their missed visit for all visits other than the final visit, which will be given a 14-day window. If a visit occurs after the 14-day window, relevant data will be ascertained when possible, but may not be included in the analysis (as described in Table 3). The start date/time for each participant is the date/time of randomization.

Table 3. Allowable windows for primary outcome assessment

Primary Outcome	**Visit Window**
Incidence of moderate-to-severe diarrhea	Follow-up time censored 183 days (6 months) after enrollment or at the time of the last follow-up visit (if <183 days). Data collected (including vital status) from visits that occur with the 14-day window following the 183 day (6 month) follow-up period will be included but events and person-time will be censored at 183 days.
Time to nutritional recovery	Follow-up time censored 183 days (6 months) after enrollment or at the time of the last follow-up visit (if <183 days). Data collected (including vital status) from visits that occur with the 14-days window following the 183 day (6 month) follow-up period will be included but events and person-time will be censored at 183 days.

SECTION 4. STATISTICAL PRINCIPLES

Confidence Intervals and P-values

Level of Statistical Significance
All statistical tests will be 2-sided using a 5% significance level (alpha of 0.05). For the primary aim (aim 1), an alpha of 0.04 will be used for hypothesis tests that were part of the interim analysis to account for alpha spending.

Type I Errors
We will not adjust the alpha for multiple testing in the primary aim. Instead we will clearly state primary and secondary analyses and interpret secondary analyses as hypothesis-generating rather than confirmatory.

Confidence Intervals to be Reported
For the primary analysis of medically attended diarrhea incidence in Aim 1, two-sided 96% confidence intervals will be used to account for the 0.01 alpha spent at the interim analysis. All other analyses will utilize two-sided 95% confidence intervals.

Adherence and Protocol Deviations

Definition of adherence intervention and assessment including extent of exposure
At enrollment, trained study counselors will provide counseling on the importance of adhering to the intervention. [16] Adherence will be assessed on all study participants with each course (14 days) of IP by measuring the number of

returned containers at the home (W2, W6, W8, W12, W14) and clinic visits (W4, W10) as well as evaluating the caregiver-reported daily IP administration in the pictorial adherence logs provided to caregivers. The logs will have spaces for caregivers to mark when they administer the IP each day, with the goal of supporting high adherence amongst all participants. Use of pictorial logs ensures that literacy is not a barrier to use.

Description of how adherence to the intervention will be presented
Two definitions of adherence include: (1) the proportion of caregivers self-reporting that their child consumed some or all of the IP ≥95% of the time over six months follow-up using daily pictorial adherence logs, (2) adherence to the recommended dosing based on objectively measured container consumption (+/- 10% consumption of prescribed IP in all weeks). We will additionally report the mean/median # of days of adherence and evaluate adherence as a continuous measure to determine whether there is a dose-response relationship.

For those missing self-reported adherence data, such as due to a death occurring prior to a scheduled follow-up visit, we will assume all adhered and none adhered and present data for both situations to establish the range of adherence to the investigational product.

Definition of protocol deviations for the trial
The following are pre-defined major protocol deviations with a direct bearing on the primary outcome:

- Unblinding of the study participant or study team to randomization allocation
- Errors in applying inclusion/exclusion criteria that are discovered after randomization

The following are pre-defined minor protocol deviations:

- Loss to follow-up
- Withdrawal of consent
- Missed sample collection (stool/rectal swab, blood spot) due to participant refusal or other barrier to sample collection (such as visit occurring over phone).
- Missed anthropometry assessment due to follow-up visit occurring over the phone

Description of which protocol deviations will be summarized
Protocol deviations will be classified as major and minor prior to unblinding of randomization allocation. The number (and percentage) of participants with major and minor protocol deviations will be summarized by study arm in relevant analyses with details of the deviation provided. The patients that are randomized will be used as the denominator to calculate percentages. No statistical tests will be performed.

Analysis Populations
Analysis of primary outcomes will be by intention-to-treat (ITT). The ITT population will include all randomized children according to the treatment they were randomized to receive. Children who were completely loss to follow-up, that is have fewer than one follow-up visit, will be included in sensitivity analysis assuming they have had the primary outcome (for the diarrhea analysis) and assuming they did not have the event (for nutritional recovery). In per-protocol analyses (secondary to the ITT), we will compare treatment effects in groups defined by self-reported adherence to the intervention. Per protocol analyses will also exclude children who were ineligible, those who were lost to follow-up (defined as missing all follow-up visits), those who had ≤99% adherence, and those who withdrew consent.

SECTION 5. TRIAL POPULATIONS

Screening Data
The total number screened will be reported along with summary of reasons for exclusion into the trial.

Eligibility
Children age 6 to 24 months old, managed as an outpatient or inpatient for diarrhea (3 or more abnormally loose or watery stools per 24 hours) at one of the seven recruiting sites who are ready to return home will be screened for eligibility in the trial (Table 4). Eligible children will include children managed as outpatients and children who were admitted and now ready for discharge. Children discharged against medical advice will not be screened.

Table 4. Description of study population and criteria for the inclusion and exclusion of study participants.	
Study Population:	Children age 6 to 24 months old, managed as an outpatient or inpatient for diarrhea at one of the recruiting sites who are ready to return home will be screened for eligibility in the trial
Inclusion Criteria:	An eligible child will be aged 6-24 months, seen as an inpatient or outpatient for diarrhea who is ready to return home, with a MUAC <12.5 cm at the time of screening whose caregiver consents, plan to remain in study area > 6 months, is not enrolled in another study, and is no longer exclusively breastfeeding.
Exclusion Criteria	Children younger than 6 months or older than 24 months, accompanying caregiver does not provide consent to study participation, history of 2 or more blood transfusions in the past 12 months, history of allergy to dairy products, caregiver reports the child will not stay within the study area for the next 6 months or greater, enrollment in another study, child is exclusively breastfeeding at the time of enrollment, child is not ready to return home (is not yet discharged), unwilling to participate in the dual sugar permeability sub-study if selected, child was discharged against medical advice, or child has a history of congenital defect or syndrome that prevents age-appropriate feeding (e.g. cleft palate)

Recruitment
Children will be recruited from outpatient and inpatient departments. In both settings, prior to the screening process, potential participants will be pre-screened for eligibility by the study staff. For inpatient recruitment, after a child has been scheduled for discharge from the hospital and ready to return home, a study staff will approach the primary caregiver. For outpatient recruitment, when a child is deemed ready to return home after being seen in the outpatient department, their caregiver will be approached by a study staff.

Per CONSORT guidelines, we will report the number of individuals who:
1. Underwent screening
2. Met inclusion criteria
3. Did not meet inclusion criteria (and reasons)
4. Enrolled in the study and were randomized
5. Were included in the ITT
6. Were included in the per-protocol population

Withdrawal/Follow-Up

Level of withdrawal (from intervention and/or from follow-up)
Withdrawal of consent will be tabulated using the following categories: withdrawal from follow-up but allow prior collected data/ samples to be used; withdrawal from follow-up and disallow already collected data/samples to be utilized; withdrawal from study intervention but continue with follow-up/data collection; withdrawal from study intervention and discontinue with follow-up/data collection.

Timing of withdrawal/lost to follow-up data
Tabulation of withdrawals will include withdrawals by each follow-up timepoint (W2, W4, W6, W8, W10, W12, W14, W16, W20, W22, W24)

Reasons and details of how withdrawal/lost to follow-up data will be presented

The numbers and reasons (if available) of losses to follow-up and withdrawals will be summarized by treatment arm.

Baseline Participant Characteristics

Characteristic	Lactoferrin+Lysozyme Arm (N=)	Lactoferrin Arm (N=)	Lysozyme Arm (N=)	Placebo Arm (N=)
	n (%) Median (IQR)	n (%) Median (IQR)	n (%) Median (IQR)	n (%) Median (IQR)
Recruitment site				
Homa Bay County Referral				
Mbita Sub-County				
Kendu Adventist Mission Hospital				
Kisii Teaching and Referral Hospital				
Rongo Sub-County				
Awendo Sub-County				
Isebania Sub-County				
Child age				
6m to 11m				
12m to 24m				
Female				
Wealth quintile				
Lowest				
Second				
Middle				
Fourth				
Highest				
Caregiver education (primary school or less)				
Household crowding				
Unimproved water source				
Reports treated drinking water				
Toilet type				
Flush				
Pit Latrine				
Open Defecation				
Dehydration status at presentation				
None				
Some				
Severe				
Blood in stool at presentation				
Breastfeeding status in the first 6 months of life				
Exclusively breastfed				
Partially breastfed				
Never breastfed				
Unknown				
Currently breastfeeding				
Stunting (LAZ/HAZ <-2)				
Acute malnutrition				
Severe (WHZ < -3 or MUAC <11.5cm or edema)				
Moderate (-3 ≥ WHZ < -2 or 11.5 ≥ MUAC <12.5cm)				
None				

HIV status				
HIV-infected				
HIV-exposed, uninfected				
HIV-exposed, unknown infection status				
Received all age-appropriate vaccines				

SECTION 6. ANALYSIS

Outcome Definitions

Primary Study End-Points:

1. **The incidence of moderate-to-severe diarrhea** will be defined as total number of new diarrhea episodes (>48 hours after a diarrhea-free period) deemed moderate or severe, divided by the child-time at risk during the 6-month follow-up period. Time at risk will be censored at the date of last follow-up for children who have died or are lost to follow-up. Also, child-time during a diarrheal episode, including during the index diarrhea, and the 48 hours after, will not be included in the denominator. Moderate to severe diarrhea will be defined using the CODA (Community DiarrhoeA) diarrhea severity score or dysentery (evidence or reported visible blood in stool)[17]. The CODA diarrhea severity score was developed to assess diarrhea severity in the context of community-based studies and is validated against outcomes of weight and length. A conservative moderate-to-severe diarrhea cut-off of ≥ 3 (instead of CODAs original ≥ 1) will be used, as caregiver report of anorexia (defined as the child not willing to eat as usual) that is included in the CODA score has been found to lead to heterogeneity in severity scoring [18], and similar conservative cut-off has been used in a previous study that used the CODA score.[19] Moderate-to-severe diarrhea (instead of all diarrhea) will be used because it is associated with poorer outcomes and thereby most important to prevent, and incurs costs to health care systems and families. Diarrhea information during the follow-up period will be ascertained through follow-up visit questionnaires administered every two weeks and enhanced by caregiver-completed daily diarrhea diary entries and if care is sought at a study facility, through physical examination and caregiver interview at the time of care seeking.
2. **Time to nutritional recovery** will be defined as the number of days since enrollment to the date of the 2nd of two consecutive MUAC measurements ≥12.5. For participants who do not reach nutritional recovery, due to death, loss to follow-up, or completion of the study prior to reaching recovery, they will be censored at the date of their last visit. Recovery by MUAC and WHZ (depending on which is used to identify acute malnutrition by the nutrition program) is the definition used by WHO to inform discharge from malnutrition programs making it a policy-relevant outcome. We focused on MUAC instead of WHZ because MUAC is less sensitive to hydration status than weight. A child will be considered lost to follow-up if they did not attend at least one of the follow-up clinic visits (at weeks 4, 10, 16, 24) and were not available at any of the home visits at week 2 or beyond (at 2, 6, 8, 12, 14, 18, 20, and 22 weeks).

Secondary Study End-Points:

1. **Severe diarrhea** will be defined by the CODA diarrhea severity score of 7 or more. Episodes will be defined as per the primary outcome.
2. **Dysentery** will be defined as evidence or reported visible blood in stool. Episodes will be defined as per the primary outcome.
3. **Diarrhea (any severity)** will be defined as diarrhea during follow-up, irrespective of severity, ascertained through follow-up visit questionnaires and a diarrhea diary. Episodes will be defined as per the primary outcome.
4. **Medically-attended diarrhea** will be defined as diarrhea that led to and outpatient or inpatient visit at a health facility or hospital that is typically attended by a nurse, clinical officer, and/or physician. Episodes will be defined as per the primary outcome.
5. **Cumulative duration of diarrhea** will be defined as cumulative days of diarrhea ascertained from follow-up

visit questionnaires and a diarrhea diary.

6. **Proportion of children who recovered from wasting** will be defined as the proportion of children who achieved two consecutive MUAC measurements ≥12.5 at any point during the follow-up period**.**
7. **Incidence of hospitalization** will be defined as any inpatient admission that results in an overnight stay (irrespective of diagnosis) in a health-facility and time to hospitalization or death analyzed as a combined outcome.
8. **Hemoglobin concentration** will be determined at each of four time points.
9. **Growth**: Length and weight measurements at each timepoint will be used to create age-standardized z-scores. Z-scores will be calculated using WHO-established reference standards and the WHO ANTHRO software. Linear growth will be defined as change (Δ) in length for age z-score (LAZ). Ponderal growth will be defined as change (Δ) in weight for length z-score (WLZ) and Δ MUAC.
10. **Concentrations of specific markers of enteric function** will include fecal alpha antitrypsin (mg/g), myeloperoxidase (ng/mL), neopterin (nmol/l) at baseline, 4, 16 and 24 weeks; and the lactulose:rhamnose ratio at 16 and 24 weeks. Baseline samples that were collected after administration of the investigational product (if for example whole stool could not be collected within 1 hour of enrollment) will be excluded in sensitivity analyses.
11. **Prevalence of enteric infections** will be determined by qPCR at or below the minimum limit of detection (cycle thresholds [CT] <35) at week 4 for all participants and at week 16 and 24 for a subset of 200 participants (50/arm). We will additionally group bacteria shown to be associated with growth faltering (*Campylobacter* species, LT-ETEC, EAEC, typical EPEC and/or *Shigella* [20] as a single variable.
12. **Acceptability** is measured as the proportion of caregivers reporting that administration of the IP was desirable or satisfactory along with perceived trust, safety, and comfort in the IP via 5-point Likert scale responses in surveys administered at follow-up visits and via emerging qualitative FGD thematic content from both caregiver and health worker FGDs.
13. **Adherence:** Two definitions of **adherence** include: (1) the proportion of caregivers self-reporting that their child consumed the IP ≥95% of the time over six months follow-up using daily pictorial logs, (2) adherence to the recommended dosing based on objectively measured container consumption (+/- 10% consumption of prescribed IP in all weeks).
14. **Incremental cost-effectiveness**: We will estimate the **incremental cost-effectiveness** of lactoferrin, lysozyme, and a combination of the two as compared to the standard-of-care. Outcomes for the cost-effectiveness analysis include the incremental costs and cost-per-episode of diarrhea averted in each IP arm, compared to the placebo arm. Because all children in the study also receive standard-of-care diarrheal and malnutrition management, the placebo arm serves as an appropriate standard-of-care proxy.

Analysis Methods

Statistical Analysis of Study End-Points

1. **Aim 1.** To determine whether a 16-week course of lactoferrin, lysozyme or a combination of both shortens time to WHO-defined recovery from wasting (MUAC ≥12.5cm) and reduces the incidence of moderate-to-severe diarrhea during the subsequent 6-months following presentation to a health facility with diarrhea among children with moderate/severe childhood wasting.
    a. *Statistical Analysis:* The rate ratio (RR) of each intervention arm compared to placebo will be determined using a Poisson model with number of episodes as the outcome and time at risk (defined above) as the model offset and Wald chi-square tests of the two-way intervention arm comparisons (example tale below). The median time to nutritional recovery will be compared between intervention arms using Kaplan Meier (K-M) survival analysis and associated log-rank tests for each two-way comparison of intervention group to placebo. Cox-proportional hazards regression will be used for analysis of time to nutritional recovery with adjustment for inpatient/outpatient status (because randomization was baseline covariates (e.g. baseline nutritional status, co-morbidities, HIV-status) if needed. We will conduct sub-group analyses to evaluate the effect of interventions in sub-groups a) severe vs. moderate wasting, b) high/low adherence to the intervention (<85%, 85-99%, >99%), and c) age <11 months vs. 12-24 months. These will be presented as Forest plots and evaluated visually.

Additionally, in analyses secondary to the primary analysis, we will test for effect modification by lactoferrin and/or lysozyme using a Wald test. If there is no evidence of effect modification using a p-value of a conservative alpha of 0.1, we will test the two lactoferrin and two lysozyme arms independently in comparison to the non-lactoferrin and non-lysozyme arms, respectively, to determine the efficacy of each supplement capitalizing on the factorial design.

Primary Outcome Table

	Lactoferrin+Lysozyme Arm (N =)				Lactoferrin Arm (N =)				Lysozyme Arm (N =)				Placebo Arm (N =)		
Outcome	n	Person-time	Incidence rate†	RR/HR‡ (95.5% CI)	n	Person-time	Incidence rate†	RR/HR ‡ (95.5% CI)	n	Person-time	Incidence rate†	RR/HR ‡ (95.5% CI)	n	Person-time	Incidence rate†
Moderate-to-severe diarrhea															
Nutritional recovery															

† Per 100 child-years. ‡ Relative risk (RR) for diarrhea outcome or hazard ratio (HR) for nutritional recovery, compared to placebo arm

Statistical Analysis of Secondary Aims

1. **Aim 2.** To explore whether a 16-week course of lactoferrin, lysozyme or combination therapy improves secondary clinical, nutritional, enteric pathogen, and enteric function outcomes.
    a. *Statistical Analysis:* All the secondary outcomes related to diarrheal episodes will be analyzed using Poisson regression modelling similar to the analysis of primary outcome moderate-to-severe diarrhea. The cumulative duration of diarrhea will be compared between each intervention arm and placebo arm using linear regression model. The confidence interval for the regression coefficients will be determined with the bootstrap method to obtain robust estimates without assuming a specific distribution for the data or the coefficients. The proportion of children who recovered from wasting will be compared between each arm and placebo descriptively to inform the interpretation of the results of the primary outcome (time to nutritional recovery). Risk of death or first re-hospitalization among children will be compared between each intervention arm and placebo using Cox proportional hazards regression. Participants will be censored at the date completion of the study or loss to follow-up. We will compare the mean change in hemoglobin concentration, linear growth, ponderal growth, antitrypsin, myeloperoxidase, calprotectin, and neopterin using multiple generalized linear mixed effects (LME) models with random intercepts. Because L:R ratio is only measured after diarrhea resolution, mean L:R ratio will be compared using a similar approach but omitting the baseline timepoint. Finally, GEE with Poisson link & independent correlation structure will be used to evaluate the prevalence of enteric bacteria, grouped by those associated with linear growth faltering, and individually, over time in each intervention arm compared to placebo. All analyses will be adjusted for multiple comparisons using the Benjamini and Hochberg method using a false discovery proportion (FDP) of 0.05 within each distinct potential mechanism (clinical, nutritional, enteric pathogen, and enteric function.[21]

2. **Aim 3.** To evaluate acceptability, adherence and cost-effectiveness of lactoferrin and/or lysozyme administration in Kenya.
    a. *Statistical Analysis:* (*1) Acceptability:* Quantitative acceptability measures will be presented as an average per visit by arm. Time series analysis will be used to understand increases, decreases, and correlation between acceptability measures over time, by treatment arm, thus serving as a potential explanatory variable for adherence. FGDs will be transcribed verbatim into the local language, if not conducted in English. Five one-minute quality assurance spot checks will be conducted on each transcript by an independent researcher fluent in the local language to determine quality of the transcription. Transcribed data will be translated into English and reviewed by an independent reader to confirm the quality of the translation. We will code the qualitative data in ATLAS.ti using thematic coding and a mix of inductive and deductive coding to identify the constructs influencing acceptability

and adherence. [22] A codebook will be prepared prior to data collection and will be added to and updated iteratively. The two intendent coders will meet weekly to review coded transcripts, ensure inter-coder agreement, and to add to or refine the codebook accordingly. *(2) Adherence:* The proportion of caregiver/child dyads who were judged to be adherent will be reported according to each definition above. Multivariate logistic regression will be used to determine if baseline IP acceptability as reported on caregiver questionnaires influences adherence to (1) self-reported adherence of ≥95%, (2) objective container monitoring adherence of ≥95%. (3) *Cost-effectiveness*: We will estimate the incremental cost-effectiveness ratio (ICER) for each IP compared to the placebo. Analyses will be conducted in Treeage Pro 2018 Healthcare Module [23] to estimate moderate-to-severe diarrheal events averted amongst a static population of each trial intervention group compared to the placebo group. We will use a 6-month time horizon to estimate the cost-effectiveness of the intervention on short-term moderate-to-severe diarrheal episodes over the duration of the trial. Deterministic and probabilistic sensitivity analyses will be conducted, including to determine if broadening treatment criteria from children with malnutrition to non-wasted pediatric populations affects observed ICERs.

Missing Data

We will assess the pattern of missingness in the data (Missing at Random, Missing Completely at Random, etc.). If there is substantial missing covariate data (>10%), multiple imputation using the Markov chain Monte Carlo (MCMC) method will be used to impute covariate information where appropriate. Missing outcome data will not be imputed, but participants will be censored at the last follow-up visit therefore contributing some person-time to the analysis.

Harms

Adverse and severe adverse events (SAEs) will be descriptively presented by randomization arm as described in Table 6:

Adverse events by randomization arm among ITT population

	Lactoferrin+Lysozyme Arm (N=)		**Lactoferrin Arm (N=)**		**Lysozyme Arm (N=)**		**Placebo Arm (N=)**	
AE Grade[i]	N[ii](%)	Weeks since enrollment 0-16 17-24	N[ii](%)	Weeks since enrollment 0-16 17-24	N[ii](%)	Weeks since enrollment 0-16 17-24	N[ii](%)	Weeks since enrollment 0-16 17-24
Serious AE Total								
Death								
Life Threatening								
Non-serious AE totals								
Severe								
Moderate								
Mild[iii]								

i. Defined according to 2014 Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events
ii. Events are placed in age categories based on age at enrolled, reports generated prior to July 2018 were based on age at event.

Statistical Software

All analyses will be conducted using STATA or R and the software used reported in all analysis write-ups

References to be Provided for Non-standard Statistical Methods

All methods being proposed are standard.

Data Management Plan

Procedures relating to data entry, management, QA/AC are outline in the Data Management Trial Standard Operating Procedure.

Trial Master File and Statistical Master File

The Statistical Master File is maintained by the Study Statistician, and Trial Master File by the Study Coordinator.

Reference List

1. Tickell KD, Pavlinac PB, John-Stewart GC, et al. Impact of Childhood Nutritional Status on Pathogen Prevalence and Severity of Acute Diarrhea. Am J Trop Med Hyg. **2017**; 97(5):1337–1344.
2. Tickell KD, Sharmin R, Deichsel EL, et al. The effect of acute malnutrition on enteric pathogens, moderate-to-severe diarrhoea, and associated mortality in the Global Enteric Multicenter Study cohort: a post-hoc analysis. Lancet Glob Health. **2020**; 8(2):e215–e224.
3. Talbert A, Ngari M, Bauni E, et al. Mortality after inpatient treatment for diarrhea in children: a cohort study. BMC Med [Internet]. **2019** [cited 2022 Oct 14]; 17(1):20. Available from: https://bmcmedicine.biomedcentral.com/articles/10.1186/s12916-019-1258-0
4. Brander RL, Pavlinac PB, Walson JL, et al. Determinants of linear growth faltering among children with moderate-to-severe diarrhea in the Global Enteric Multicenter Study. BMC Med [Internet]. **2019** [cited 2022 Oct 14]; 17(1):214. Available from: https://bmcmedicine.biomedcentral.com/articles/10.1186/s12916-019-1441-3
5. Black RE, Victora CG, Walker SP, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. The Lancet [Internet]. **2013** [cited 2022 Oct 14]; 382(9890):427–451. Available from: https://linkinghub.elsevier.com/retrieve/pii/S014067361360937X
6. Global Burden of Disease Collaborative Network. Global Burden of Disease Study 2019 (GBD 2019) Results [Internet]. Seattle, United States: Institute for Health Metrics and Evaluation (IHME); 2019. Available from: https://vizhub.healthdata.org/gbd-results/
7. Platts-Mills JA, Babji S, Bodhidatta L, et al. Pathogen-specific burdens of community diarrhoea in developing countries: a multisite birth cohort study (MAL-ED). Lancet Glob Health. **2015**; 3(9):e564-575.
8. Ochoa TJ, Chea-Woo E, Baiocchi N, et al. Randomized double-blind controlled trial of bovine lactoferrin for prevention of diarrhea in children. J Pediatr. **2013**; 162(2):349–356.
9. Zavaleta N, Figueroa D, Rivera J, Sánchez J, Alfaro S, Lönnerdal B. Efficacy of rice-based oral rehydration solution containing recombinant human lactoferrin and lysozyme in Peruvian children with acute diarrhea. J Pediatr Gastroenterol Nutr. **2007**; 44(2):258–264.
10. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet. **2013**; 382(9888):209–222.
11. Annan RA, Web P, Brown R. Management of Moderate Acute Malnutrition (MAM): Current Knowledge and Practice. 2014.
12. Moramarco S, Amerio G, Ciarlantini C, et al. Community-Based Management of Child Malnutrition in Zambia: HIV/AIDS Infection and Other Risk Factors on Child Survival. Int J Environ Res Public Health. **2016**; 13(7):E666.
13. Trehan I, Manary M. Management of moderate acute malnutrition in children in resource-limited countries. UpToDate [Internet]. Available from: https://www.uptodate.com/contents/management-of-moderate-acute-malnutrition-in-children-in-resource-limited-countries
14. Medoua GN, Ntsama PM, Ndzana ACA, Essa'a VJ, Tsafack JJT, Dimodi HT. Recovery rate of children with moderate acute malnutrition treated with ready-to-use supplementary food (RUSF) or improved corn–soya blend (CSB+): a randomized controlled trial. Public Health Nutr [Internet]. **2016**; 19(2):363–370.
15. Kabalo MY, Seifu CN. Treatment outcomes of severe acute malnutrition in children treated within Outpatient Therapeutic Program (OTP) at Wolaita Zone, Southern Ethiopia: retrospective cross-sectional study. J Health Popul Nutr [Internet]. **2017** [cited 2022 Oct 15]; 36(1):7. Available from: http://jhpn.biomedcentral.com/articles/10.1186/s41043-017-0083-3
16. Rowe AK, Onikpo F, Lama M, Osterholt DM, Deming MS. Impact of a malaria-control project in Benin that included the integrated management of childhood illness strategy. Am J Public Health. **2011**; 101(12):2333–2341.
17. Lee G, Peñataro Yori P, Paredes Olortegui M, et al. An instrument for the assessment of diarrhoeal severity based on a longitudinal community-based study. BMJ Open. **2014**; 4(6):e004816.
18. Lee GO, Richard SA, Kang G, et al. A Comparison of Diarrheal Severity Scores in the MAL-ED Multisite Community-Based Cohort Study. Journal of Pediatric Gastroenterology & Nutrition. **2016**; 63(5):466–473.

19. St Jean DT, Chilyabanyama ON, Bosomprah S, et al. Development of a diarrhoea severity scoring scale in a passive health facility-based surveillance system. Gurgel RQ, editor. PLoS ONE. **2022**; 17(8):e0272981.
20. Rogawski ET, Liu J, Platts-Mills JA, et al. Use of quantitative molecular diagnostic methods to investigate the effect of enteropathogen infections on linear growth in children in low-resource settings: longitudinal analysis of results from the MAL-ED cohort study. Lancet Glob Health. **2018**; 6(12):e1319–e1328.
21. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society: Series B (Methodological). **1995**; 57(1):289–300.
22. Bernard HR, Ryan GW. Analyzing qualitative data: systematic approaches. Los Angeles [Calif.]: SAGE; 2010.
23. TreeAge Pro 2019, R2. TreeAge Software, Williamstown, MA. Available from: http://www.treeage.com