Lactobacillus Rhamnosus GG vs. Placebo for Pediatric Acute Gastroenteritis

Supplement

This supplement contains the following items:

1. Original protocol, final protocol, summary of changes.
2. Original statistical analysis plan, final statistical analysis plan, summary of changes.

Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis (Probiotics Study) PECARN Protocol Number 032

Pediatric Emergency Care Applied Research Network
National Institute for Child Health and Human Development (NICHD)

Protocol Version 1.00
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PROTOCOL TITLE:

Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis

Short Title: Probiotics Study
PECARN Protocol Number: 032

Lead Investigator and Author:
David Schnadower, M.D.
Washington University

Protocol Version: 1.00
Version Date: January 24, 2014

I confirm that I have read this protocol, I understand it, and I will conduct the study according to the protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and will adhere to the Ethical and Regulatory Considerations as stated. I confirm that if I or any of my staff are members of the Institutional Review Board, we will abstain from voting on this protocol, its future renewals, and its future amendments.

Principal Investigator Name: _____

Principal Investigator Signature: _____

Date: _____

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Abstract

Practice guidelines do not endorse routine use of probiotics in pediatric acute gastroenteritis. However, probiotic manufacturers aggressively purvey probiotics using health claims that have not been supported by rigorous research, and the world-wide market for probiotic products is equivalent to $32.6 billion per year. The Food and Drug Administration and the European Food Safety Authority remain concerned about probiotic value and safety.[1–3] Some institutions now recommend the routine use of probiotics based on potentially flawed or limited evidence,[4] and parents of patients with acute gastroenteritis often administer probiotics to their children without guidance from medical professionals.[5] Since the use of probiotics is increasing without adequate evidence to support its use in the United States, the study described in this protocol was designed and successfully submitted to the National Institute for Child Health and Human Development (NICHD) for funding.

The overall objective of this randomized, placebo-controlled, double-blind study is to determine if probiotic administration reduces the severity of acute gastroenteritis episodes in children aged 3 to 48 months. The probiotic agent that will be used in this trial is *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG).

Participants will be randomized to receive a five-day course of probiotic or placebo. The trial is anticipated to enroll 900 children, age 3-48 months, treated in participating emergency departments for acute gastroenteritis. We define acute gastroenteritis as the presence of three or more watery stools in a 24-hour period. Children with underlying illness that pose potential increased risk from probiotic therapy (e.g. indwelling vascular access line, structural heart disease, immunosuppressive therapy or known immunodeficiency, chronic gastrointestinal disease such as inflammatory bowel disease, or household contact with immunodeficiency or immunosuppressive therapy) will be excluded. The age range of 3-48 months was selected because this age group has the highest incidence of acute gastroenteritis and the greatest morbidity. Subjects will be recruited at the time of an emergency department visit. All eligible subjects who present for treatment when study personnel are available will be approached for participation. Those agreeing to participate will be randomized, using block randomization stratified by study site, to treatment groups. The study will be conducted at multiple sites within the Pediatric Emergency Care Applied Research Network (PECARN). We will also collect and freeze pre- and post-treatment bulk stool specimens at the lead study site to assess the mechanism of action of the specific probiotic agent, *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG).

1 Study Summary

This randomized, double-blind, placebo-controlled trial will quantify the benefits and potential side effects associated with probiotic administration in ambulatory children presenting to the emergency department with acute gastroenteritis. This will provide the first definitive evidence in the United States for, or against, using probiotic therapy for this condition. This study will use the most commonly employed probiotic agent (*Lactobacillus rhamnosus* GG, ATCC 53103 (LGG)) approved for use in the United States. The results of this multicenter study will guide the standard of care: if probiotic administration is associated with benefit, it offers an inexpensive, safe and easy to administer treatment to reduce morbidity from acute gastroenteritis. If the trial does not demonstrate probiotic efficacy, healthcare, caregiver and societal resources may be refocused on alternative therapeutic interventions.

1.1 Hypotheses

The hypotheses of this study are:

1. In children with acute gastroenteritis, probiotic administration in the emergency department will be associated with a clinically-important decrease in the proportion of children suffering from moderate-severe disease, defined by a validated Modified Vesikari Score ≥ 9, compared to placebo.

2. In children with acute gastroenteritis, probiotic administration will not be associated with serious adverse events, and will have a similar rate of side effects (e.g. bloating, fever, abdominal distention) as compared to placebo-treated children.

1.2 Specific Aims

This project has the following Specific Aims:

Specific Aim 1. Determine the clinical effectiveness of probiotic administration to reduce morbidity in children presenting to the emergency department with acute gastroenteritis.

Specific Aim 2. Determine the safety and side effect profiles of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) in children presenting to the emergency department with acute gastroenteritis.

1.3 Trial Endpoints

Primary Endpoints

- The **primary efficacy endpoint** of this study is the presence of moderate-severe acute gastroenteritis, as defined by a total post-enrollment Modified Vesikari Score ≥ 9 during the 2-week follow-up period.

- The **primary safety endpoint** of this study is the occurrence of invasive disease (including meningitis and bacteremia) from *Lactobacillus rhamnosus*.

Secondary Endpoints

1. **Diarrhea duration:** Time from randomization until the appearance of the last watery stool as reported during daily surveys.

2. **Vomiting duration:** Time from randomization until the last vomiting episode as reported during daily surveys. Vomiting duration is only evaluated in children who vomited ≥ 3 times during the 24 hours prior to the emergency department visit.

3. **Return visits:** Return visits for unscheduled care to a healthcare provider related to vomiting, diarrhea, dehydration, fever, or fluid refusal, within 2 weeks of the index visit. Scheduled visits (e.g. reassessment, vaccinations, unrelated issues) will not be included.

4. **Missed daycare:** Days of daycare missed by subjects who attend daycare.

5. **Missed work:** Days of work missed by caregivers who work outside of the home.

6. **Household transmission rate:** A household census will be obtained at the time of enrollment, and information about household contacts symptoms will be obtained during daily surveys to determine household transmission rate.

7. **Adverse Events:** Occurrence of side effects such as bloating, gas, intestinal rumbling, diarrhea, blood in stool, abdominal pain, abdominal cramps nausea, vomiting, loss of appetite, abnormal taste, heartburn, constipation, skin rash, fever, nasal congestion, sore throat, cough, headache, muscle aches, chills, etc..

1.4 Subject Eligibility, Accrual and Study Duration

Eligible participants will be identified by on-site study staff. Inclusion criteria are:

1. Age 3-48 months (have not yet reached their fourth birthday); AND
2. Presence of 3 or more watery stools within 24 hours of screening; AND
3. Duration of vomiting or diarrhea less than 7 days; AND
4. Symptoms consistent with acute intestinal infectious process.

We will exclude subjects with any of the following:

1. Presence of an indwelling vascular access line; OR
2. Presence of structural heart disease excluding non-pathological heart murmurs; OR
3. Receiving immunosuppressive therapy or history of immunodeficiency; OR
4. Hematochezia in the preceding 48 hours; OR
5. Chronic gastrointestinal problems (e.g. short gut syndrome, inflammatory bowel disease); OR
6. Patients with known pancreatitis; OR
7. Critically ill patients; OR
8. Family member with an indwelling vascular access line, or on immunosuppressive therapy, or with a known immunodeficiency; OR
9. Bilious emesis; OR
10. Probiotic use (supplement) in the preceding 2 weeks; OR
11. Previously enrolled in this trial; OR
12. Allergy to lactobacillus or Microcrystalline Cellulose (MCC); OR
13. Allergy to erythromycin, clindamycin, AND β-lactam antibiotics (all); OR
14. Patients who have already been enrolled in the study once; OR
15. Not available for daily follow-up while symptomatic; OR
16. Parent/guardian not speaking English or Spanish.

The trial is anticipated to enroll 900 subjects over four years, which will include four acute gastroenteritis seasons. An interim safety analysis will be prepared for Data Safety Monitoring Board (DSMB) review after the first 80 subjects (half of whom will be under 1 year of age) have been followed for 1 month. At the time of this review, the DSMB *may* alter the subsequent sample size based on overall event rates.

2 Rationale and Background

Acute gastroenteritis is a leading cause of malnutrition and death worldwide and affects millions of children in the United States each year.[6, 7] Acute gastroenteritis exerts high impact burdens on children and their families: each episode causes on average 6-8 days of diarrhea, 2-4 days of vomiting, and 2-4 days of fever.[8–11] In two prospective studies

conducted in 11 Canadian and 5 United States pediatric emergency departments, 433/683 (63%) children 3-48 months of age with acute gastroenteritis had moderate to-severe symptoms at presentation, resulting in substantial daycare and parental work absenteeism.[12, 13] The economic burden associated with such cases is considerable.[14] Unfortunately, current treatment options are limited to rehydration, symptomatic management and supportive care, prevention of severe dehydration and secondary infection control.[15–17]

Probiotic agents represent a novel approach to management of pediatric acute gastroenteritis. Probiotics are viable microbes that are generally considered to be safe and are relatively inexpensive, easily administered, and hypothesized to modulate disease processes.[18] They may also reduce symptoms (i.e. diarrhea, vomiting, fever)[19–22] and morbidity, thereby diminishing acute gastroenteritis-associated resource consumption (e.g. physician visits, hospitalization) and societal costs. Numerous studies report promising results in children with acute gastroenteritis treated with probiotics, but these studies have been inadequate to change clinical practice, because of flaws in methodology including small sample sizes, lack of probiotic quality control, and outcomes that are of minimal relevance to patients and their families.[19] Remarkably, few studies of probiotics have evaluated outpatients, a group representing >90% of acute gastroenteritis episodes in the United States.[23, 24] Moreover, only a single emergency department study of probiotic efficacy in acute gastroenteritis has been reported.[20]

To date, practice guidelines do not endorse routine use of probiotics in pediatric acute gastroenteritis.[5, 19, 25–27] However, current trends provide the motivations for the study described in this protocol. First, probiotic manufacturers aggressively purvey probiotics using health claims that have not been supported by rigorous research,[28–31] and the world-wide market for probiotic products is growing rapidly (projected growth 2009-2014 12.6%, now equivalent to $32.6 billion per year).[32] Second, the United States Food and Drug Administration and the European Food Safety Authority remain concerned about probiotic value and safety.[1–3] Third, some institutions now recommend the routine use of probiotics based on potentially flawed or limited evidence.[4] Fourth, parents of patients with acute gastroenteritis often administer probiotics to their children without guidance from medical professionals.[5]

2.1 Rationale for Probiotic Efficacy

Probiotics, defined as viable microbial preparations with beneficial effects on the health of the host,[33] are regarded as safe, and are generally affordable and convenient (i.e., as over-the-counter food supplements). Probiotics, and *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) in particular, promote colonization resistance and modulate immune responses in the host, and can be directly antimicrobial. Over 100 studies have studied how

probiotics can improve immune function in humans.[34] At the mucosal surface, probiotics:

- compete with pathogenic bacteria for nutrients and adhesion binding sites;[35–38]
- produce antimicrobial substances;[37, 39, 40]
- provide nutrients to colonocytes;[41]
- alter epithelial gene expression;[42] and
- reduce intestinal permeability.[43]

Additional mechanisms of action include enhancing phagocyte[44] and natural killer cell activity,[45] and increasing fecal,[46] salivary,[47]and systemic[48] IgA levels.

Preliminary probiotic studies in humans, including over 56 trials in children with gastroenteritis (17 of which used some form of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) , the agent employed in this trial) suggest that probiotics may improve the course of acute gastroenteritis. Meta-analyses[19, 21, 49–51] report reduced mean duration of diarrhea (by 25 hours), reduced proportion of diarrhea lasting four days (risk ratio 0.41), and reduced stool frequency on Day 2 (mean difference 0.8).[19] This has prompted some physicians and institutions to recommend probiotics in children with acute gastroenteritis. Careful examination of the data, however, shows significant methodological flaws in these studies. For these reasons, experts and agencies have continued to recommend large, unbiased, well designed definitive trials to inform practice.[19, 26]

2.2 Limitations of Research to Date

Most trials of probiotics for acute gastroenteritis have been methodologically flawed.[19] Published studies have used small sample sizes and non-standardized treatment regimens, probiotic preparations with variable production quality and non-verified colony counts. Outcomes have been focused almost exclusively on diarrhea duration, and use inconsistent (or unreported) definitions,[19, 52] without considering the more global (and relevant) assessment of the illness (e.g. fever, vomiting, repeat emergency department visits, hospitalization). Because the outcomes sought are heterogeneous, and/or not validated, and/or of minimal importance to participants,[53] and have not addressed clinical and socioeconomic efficacy,[49, 50] the value of the existing data has been questioned.[54] Moreover, few studies address side effects and adverse events systematically.[1–3]

Although >90% of children with acute gastroenteritis are treated as outpatients,[23] hospitalized children have been the main focus of most research in this area. Extrapolation to outpatients is problematic because hospitalized children are more likely to have

rotavirus infections,[55] severe disease, and to benefit from probiotics.[21, 52, 56] Emergency department patients are ideal for outpatient probiotic trials, as their acute gastroenteritis disease severity represents the entire spectrum in a normal distribution,[13] offering distinct advantages over primary care populations. Emergency department patients are at risk for more severe disease, there is a greater potential for benefit because of the disease acuity and the large sample size, and disease spectrum will enable identification of subgroups most likely to benefit. Emergency departments are also a high venue of presentation for acute gastroenteritis, enabling uniform trial design and compliance compared to non-emergency department ambulatory settings.[24, 57–59] Only one emergency department study has been conducted previously, but the study was underpowered and did not explore side effects.[20]

2.3 Validation of the Modified Vesikari Score

The Vesikari Score, a composite measure of seven items first described in 1990,[60] has been used in many clinical trials for treating acute gastroenteritis, particularly those evaluating rotavirus vaccines in infants, and in large international epidemiological studies.[8, 61–65] The score incorporates features of acute gastroenteritis that are important to parents, children and physicians, including

- diarrhea duration
- diarrhea frequency,
- vomiting duration,
- vomiting frequency,
- maximum daily temperature,
- medical interventions required, and
- degree of dehydration.

In outpatient studies, the degree of dehydration, measured as weight loss, is problematic because it requires in-person re-evaluations, can vary based on recent voiding, defecation, and liquid intake, and also might reflect inadequate caloric intake or catabolism during acute illness. In fact, the assessment of dehydration using signs or scales designed based on the use of post-illness weight as a gold standard has been questioned.[66] Because of these limitations, modifications to the original Vesikari score have been tested.[67, 68] The modification replaces percent dehydration with the need for an unscheduled future healthcare visit within the two week follow-up period (Table 1 on the facing page). This modification is supported by evidence that the demand for professional medical care correlates with disease severity.[67] The Modified Vesikari Score has been validated in two

Points	0	1	2	3
Diarrhea duration	0	1-96 hrs	97-120 hrs	$\geq$ 121 hours
Max # of diarrheal stools/24 hrs	0	1-3	4-5	$\geq$ 6
Vomiting duration	0	1-24 hrs	25-48 hrs	$\geq$ 49 hours
Max # of vomiting episodes/24 hrs	0	1	2-4	$\geq$ 5
Max recorded fever	$\leq$ 37° C	37.1-38.4° C	38.5-38.9° C	$\geq$ 39° C
Unscheduled healthcare visit	0	—	Primary Care	Emergency Department
Treatment	None	Rehydration	Hospital Admission	—

Table 1: Modified Vesikari Score

multicenter studies[12, 13] conducted during the planning of the current trial described in this protocol.

3 Study Design and Data Collection

3.1 Study Design Overview

This is a double-blind randomized placebo controlled trial of probiotic therapy for acute gastroenteritis. Children 3 months of age to 48 months of age who present to a participating emergency department with acute gastroenteritis will be assessed for eligibility. Children for whom parents provide permission to participate in the trial will be randomized to receive probiotic therapy (*Lactobacillus rhamnosus* GG, ATCC 53103 (LGG)) or placebo. The trial will be analyzed as an intention-to-treat study.

3.2 Participant Screening and Consent

Potentially eligible patients will be identified and screened for eligibility. The treating physician or other study personnel will discuss the details of the study with the patient's caregivers to explain the study and obtain parental permission.

For patients who are actively screened in real time and meet all inclusion criteria, data will be recorded that include yes/no answers to each of the exclusion criteria. If a patient is ineligible at this point, no further data will be recorded, and the patient's parents will not be approached for participation in the study. If a patient is eligible, the parents will be approached to request permission for their child to participate in the study. If parents

are not approached for permission, the reason the parents were not approached will be recorded. If the parents decline permission to participate, the reason for refusal will be documented (if offered by the parent). The recorded exclusion criteria and reasons for declining permission will enable the investigators to assess barriers to trial enrollment and identify potential biases in the study.

3.3 Baseline Data Collection

Trained study staff at each site will collect baseline demographic information (e.g. birthdate, gender, race, ethnicity) and relevant clinical variables, record the data on worksheets, and enter the data into the electronic data capture system provided by the Data Coordinating Center. Baseline clinical dehydration scale[69] and disease severity scores (Modified Vesikari Score)[13] will be assigned to enable baseline comparisons between treatment arms.

3.4 Follow Up Data Collection

Successful follow up will be maximized by obtaining multiple phone numbers for families and emergency contacts, scheduling calls and sending phone text reminders, allowing for electronic completion and centralizing all follow-up procedures at the lead institution (Washington University). Trained, experienced study staff will contact the family daily (including weekends) until both the diarrhea and vomiting have resolved and the treatment has been completed on day 5. Additional contact will be made on day 14 for outcome assessment, and months 1, 3, 6, 9 and 12 for long term safety outcomes. Study staff will use a standardized data collection form, and will inquire about ongoing symptoms, medical evaluations, treatments, child care, work absenteeism, and side effects. Detailed questioning will follow positive responses. A study diary will be provided to caregivers to use as a note-taking tool. Caregivers may also be provided an option of completing the follow-up collection electronically. Patient compliance with study drug administration will be assessed on day 5. To maximize compliance, caregivers will be reminded of the importance and method of administering the study drug. On day 14, the study site staff (not the central staff conducting the follow up) will perform a chart review for final data collection and identification of recorded adverse events during the 14 day study period. Adverse event reporting is described in detail in Section 8.3 on page 33.

Every effort will be made complete daily follow-up on the day necessary. However, if one or two days of follow-up contact is missed, data for those missed days may be collected on the next contact, if the caregiver used the home diaries or can recall the information. If all contact is missed but then the parent is contacted on day 14, follow-up

data for the daily calls will be collected only if the parent diaries were used. For day 5 and 14, contact may be made within + 3 days. For month 1, 3, 6, 9, and 12 follow-up, contact may be made within ± 2 weeks.

4 Study Procedures

4.1 Randomization (Enrollment)

Randomization will be accomplished through the use of an online randomization service. Subjects will be randomized to receive either probiotic (*Lactobacillus rhamnosus* GG, ATCC 53103 (LGG)) or placebo. Equal allocation randomization tables will be provided by the Data Coordinating Center to the central research pharmacy. The central research pharmacy will prepare consecutively numbered study kits according to the randomization schedule. Study kits will be sent to the clinical sites. Randomization tables will be created at the Data Coordinating Center using permuted-block randomization stratified by clinical site and duration of symptoms. This will ensure that variations (e.g. site specific practice patterns, gastrointestinal patterns) are comparably distributed across treatment arms. The randomization number will be recorded in the database.

4.2 Study Drug Administration

Study Drug Description

Lactobacillus rhamnosus GG, ATCC 53103 (LGG) is supplied in a gelatin capsule containing 10^{10} colony forming units of *Lactobacillus rhamnosus*(75 mg). The capsule also contains 250 mg of microcrystalline cellulose (MCC – purified partially depolymerized cellulose), an inert ingredient. Placebo capsules contain only MCC (to a total of 325 mg). Each capsule is wrapped in double foil to protect it against harmful light, air, and moisture. Blister packs are labeled with the lot number. The probiotic and placebo capsules have active Drug Master Files at the Food and Drug Administration (BB-MF 2 #13668 and MF2 # 13646).

Initial Study Drug Administration

Clinical staff in the emergency department will administer the first dose of study drug by sprinkling the capsule's contents into 30 mL of room temperature oral replacement solution (electrolyte solution, e.g. Pedialyte, used to prevent or treat dehydration). Oral fluid therapy will be encouraged according to sites' existing clinical guidelines.[26] At

discharge, parents will also be provided with clinical instructions concerning what and how much fluid to drink, criteria for returning to the emergency department or seeing their physician, and other standardized discharge instructions for acute gastroenteritis. Study research coordinators will provide the parents with instructions concerning study drug administration at home, completion of study forms, and a letter for their pediatrician that explains the study. The importance of administering all doses dispensed and the need to communicate with the study team on a daily basis until symptoms resolve will be stressed.

Home Study Drug Administration

All patients will consume one capsule every 12 hours for 5 days (total of 9 home doses). Patients will receive the medication at meal time, mixed with 30 mL of a room temperature non-carbonated liquid and ingested immediately to optimize probiotic viability. One extra dose/day will be provided (i.e. kits will contain 5 extra doses total of 15 capsules) to account for vomiting or wastage. The dose may be repeated once if vomiting occurs within 15 minutes of administration; this rarely happens more than once.[70] Oral fluid therapy will be encouraged according to established guidelines.[26]

Hospital Study Drug Administration

We estimate that only a small proportion (<5%) of enrolled subjects will be hospitalized. If that does occur, subjects will continue on the study protocol as described for home study drug administration, as done in previous studies.[71] Future unscheduled hospitalization at a non-study hospital site is even less common: none of the 274 patients enrolled in our pilot study were admitted to a different institution after evaluation.[12] To minimize the impact that such an event could have on this trial, caregivers will be provided with a letter that they will be instructed to share with their pediatrician and with the admitting physician. The letter will describe the study, the care-plan, and it will include site investigator contact information and the importance of adhering to the study protocol.

4.3 Discontinuation of Study Drug

Study drugs are sometimes discontinued because of intolerance or perception of a clinical adverse event from study drug administration. If any clinical abnormality, laboratory abnormality, intercurrent illness, new immunosuppressive therapy, or other condition occurs such that continued administration of study drug would not be in the best interest of the subject in the opinion of the investigator, study drug administration may be discontinued. When this occurs, regardless of the reason, the subject remains in the intention-to-treat

population, all data collection should proceed, and long term follow up for adverse events is carried out. Discontinuation of study drug will be documented and will be reported in the closed session of the next scheduled Data Safety Monitoring Board meeting. Clinical staff and parents should *not* report the early discontinuation of study drug to research staff who are conducting follow up data collection. The Principal Investigator should *not* be contacted by the site investigator, as intentional discontinuation of study drug implies a safety outcome, and the Principal Investigator should be blind to all study outcomes. *Discontinuation of study drug is not a withdrawal from the study.*

If study subjects are hospitalized after discharge to home from the emergency department, this is not a reason for discontinuation of study drug. We estimate that only a small proportion (<5%) of enrolled children will be hospitalized after discharge to home from the emergency department. If that does occur, subjects should continue on the study protocol unless they have developed an exclusion criterion for study drug administration (e.g. critical illness, immunosuppression, etc.). To facilitate continuation of study drug administration during hospitalization, parents are provided with a letter for their pediatrician. The letter will describe the study, the care-plan, and it will include site investigator contact information and the importance of adhering to the study protocol. The parents will be instructed to share this letter with their pediatrician and with the admitting physician.

Breaking the blinding of the study drug should not be necessary, but an unblinding procedure will be available. This will require contacting the Medical Monitor (PI of the Data Coordinating Center), who will have access to the study drug kit numbers. However, unblinding is almost never necessary. The primary anticipated reason for a clinician requesting unblinding is the suspected occurrence of invasive infection (bacteremia/septicemia or meningitis), and the natural desire to know if the subject was receiving active *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG). The initial recommendation from the Medical Monitor will always be that the clinician should *assume* that the subject was receiving active drug, and include *Lactobacillus rhamnosus* in the differential diagnosis (and treatment coverage) of the suspected invasive infection. *Lactobacillus rhamnosus* is readily susceptible to common antibiotics, and the differential diagnosis of subsequent invasive infection would include organisms other than *Lactobacillus rhamnosus*. Thus, future clinical treatment should not be dependent on unblinding information.

If the situation requires unblinding, the Medical Monitor and Data Coordinating Center staff will provide the information, and the unblinding will be documented. Study drug will be discontinued, *but the subject should not be withdrawn from the study!* All study

procedures (except study drug administration) should be continued, as the subsequent clinical course of the subject is critical to the science of the trial. All unblinding events will be reported in the closed session of the next scheduled Data Safety Monitoring Board meeting. Clinical staff and parents should *not* report the unblinding to research staff who are conducting follow up data collection. The Principal Investigator should *not* be contacted by the site investigator, as intentional unblinding of a subject implies a safety outcome, and the Principal Investigator should be blind to all study outcomes.

4.4 Withdrawal from Study

Parents may completely withdraw their child from participation in this study at any time, including discontinuation of data collection. However, withdrawal from study is a completely different issue from discontinuation of study drug, and true withdrawal from study is an exceedingly rare event. If parents demand withdrawal of their child from the study, research staff will attempt to obtain adverse event information as required by the Food and Drug Administration (all adverse events for 30 days, monthly follow up for long term adverse events).

4.5 Stool Sample Testing

Stool sample swabs will be collected for polymerase chain reaction (PCR) analysis. Once obtained, swabs will be frozen and sent to a central laboratory for analysis. A storage and shipping protocol will be provided in the study manual of operations. Samples will be tested with multiplex PCR.

In addition, at the lead site, Washington University, study research personnel will collect and freeze subjects' bulk stool specimens in the acute phase (within 24 hours of presentation) and 14 days after presentation using a previously tested bulk stool specimen collection protocol. Specimens may be collected in the emergency department or the families will be given a specimen collection kit, gel packs and a metallic envelope. When the specimen is ready for collection, a courier will pick up the specimen and cool pack at the patient's home and deliver it to a logistics collection center at Washington University School of Medicine. This service is available 24 hours a day, year round. The stools will be stored as a part of a future project to assess the potential mechanisms of action of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) in acute gastroenteritis.

5 Data Analysis

The hypotheses of this study are:

1. In children with acute gastroenteritis, probiotic administration in the emergency department will be associated with a clinically-important decrease in the proportion of children suffering from moderate-severe disease, defined by a validated Modified Vesikari Score ≥ 9, compared to placebo.

2. In children with acute gastroenteritis, probiotic administration will not be associated with serious adverse events, and will have a similar rate of side effects (e.g. bloating, fever, abdominal distention) as compared to placebo-treated children.

All analyses will be undertaken by the intention-to-treat (ITT) principle, except for adverse events, which will use the as-treated principle (compare the subjects based on the treatment regimen that they received). Patients who drop out or inadvertently crossover will be followed and included in the ITT analysis. All statistical tests of hypotheses will be two-sided. Baseline characteristics will be analyzed to determine if there is a need to adjust for differences between groups in exploratory analyses. Sensitivity analyses will be performed to assess the possibility and consequences of non-random loss to follow-up. The proportions of children experiencing an unscheduled healthcare visit or any potential adverse effect, as reported by the caregivers, will be compared between groups using the Mantel-Haenszel test, stratified by site. The analysis will evaluate the presence/absence of pre-specified side effects, as an aggregate outcome variable. A per-protocol analysis will be conducted to provide additional insight as non-compliance may result in an underestimation of the benefits of probiotics in the ITT analysis.[72]

5.1 Specific Aim Analyses

Specific Aim 1. Determine the clinical effectiveness of probiotic administration to reduce morbidity in children presenting to the emergency department with acute gastroenteritis.

The **primary efficacy endpoint** of this study is the presence of moderate-severe acute gastroenteritis, as defined by a total post-enrollment Modified Vesikari Score ≥ 9 during the 2-week follow-up period. Each of the 7 items in the score is tabulated individually (maximum of 20 points); the sum of these individual variables represents the total Modified Vesikari Score. At the time of randomization a baseline Modified Vesikari Score will be assigned based on symptoms prior to randomization. This baseline score will serve

as a covariate in a secondary analysis of the primary outcome. The post-enrollment Modified Vesikari Score which will be employed to determine the presence/absence of the primary efficacy outcome, is based only on symptoms that occur between randomization and day 14, the conclusion of the study period for this outcome. Only symptoms and outcomes that occur following randomization will be included in the post-enrollment score.

The post-enrollment score is calculated only once, on day 14. At that time, each of the seven variables will be assigned a score for the entire study period (from randomization to day 14). Each variable will be scored by 1 of 3 methods:

1. Worst 24 hour period - maximal number of episodes of vomiting in a 24 hour period, maximal number of episodes of diarrhea in a 24 hour period, and maximal temperature;
2. Total duration of symptoms, including the number of days on which any gastroenteritis-related symptom occurred;
3. Occurrence of a treatment outcome or unscheduled subsequent healthcare utilization. If the patient was admitted directly following their enrollment ED visit and that admission stay is longer than 48 hours, it will be included as treatment in the post enrollment MVS. If the patient's admission stay was less than 48 hours, it will not be included in any MVS calculation.

The primary efficacy endpoint (the presence of moderate or severe disease, as defined by a total Modified Vesikari Score ≥ 9 during the 2 week follow-up period) will be limited to symptoms and outcomes that occur after randomization. If a patient's symptoms of vomiting and diarrhea stop during the same 24 hours, and then recur, that is not included in the post-enrollment score since that will be considered a *new* illness. In the original score, severe disease was defined as ≥ 11[61, 62, 73–75] and moderate as ≥ 9.[76] In our derivation and validation pilot studies,[12, 13] construct validity was demonstrated and validated by using scores of ≥ 9 to define moderate and ≥ 11 to define severe disease. These cut-points were associated with significant increases in other measures of disease severity such as degree of dehydration, likelihood of admission and daycare ($p = 0.01$) and parental work absenteeism ($p < 0.001$).[12, 13]

The proportion of children with moderate-to-severe disease (i.e. Modified Vesikari Score ≥ 9), the primary outcome, will be analyzed by comparing proportions utilizing a Mantel-Haenszel test, stratified by participating center and by duration of symptoms at enrollment. Significance for this primary outcome measure will be set at 0.05. Secondary analyses of the primary outcome will use logistic regression methods to adjust for covariates

that may be imbalanced between groups, (e.g. age, pre-enrollment Modified Vesikari Score, hydration assessment, need for hospitalization at index visit). We will also analyze the outcome using Modified Vesikari Score as a continuous variable through a stratified Wilcoxon rank-sum test and compare the results with the primary analysis.

Specific Aim 2. Determine the safety and side effect profiles of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) in children presenting to the emergency department with acute gastroenteritis.

The **primary safety endpoint** of this study is the occurrence of invasive disease (including meningitis and bacteremia) from *Lactobacillus rhamnosus*. The secondary safety outcome will be the presence of any adverse events. For more information on adverse events, see Section 8.3 on page 33. Adverse events (including serious) will be tabulated by study arm for DSMB reporting and for final analysis of the safety of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) in this setting. Adverse events will also be coded using the MedDRA vocabulary.

5.2 Secondary Study Outcomes

Secondary outcomes will include three of the individual components of the Modified Vesikari Score considered independently and one additional measure of burden of illness. Specifically, they are:

1. **Diarrhea duration:** Time from randomization until the appearance of the last watery stool as reported during daily surveys.
2. **Vomiting duration:** Time from randomization until the last vomiting episode as reported during daily surveys. Vomiting duration is only evaluated in children who vomited ≥ 3 times during the 24 hours prior to the emergency department visit.
3. **Return visits:** Return visits for unscheduled care to a healthcare provider related to vomiting, diarrhea, dehydration, fever, or fluid refusal, within 2 weeks of the index visit. Scheduled visits (e.g. reassessment, vaccinations, unrelated issues) will not be included.
4. **Missed daycare:** Days of daycare missed by subjects who attend daycare.
5. **Missed work:** Days of work missed by caregivers who work outside of the home.
6. **Household transmission rate:** A household census will be obtained at the time of enrollment, and information about household contacts symptoms will be obtained during daily surveys to determine household transmission rate.

7. **Adverse Events:** Occurrence of side effects such as bloating, gas, intestinal rumbling, diarrhea, blood in stool, abdominal pain, abdominal cramps nausea, vomiting, loss of appetite, abnormal taste, heartburn, constipation, skin rash, fever, nasal congestion, sore throat, cough, headache, muscle aches, chills, etc..

The overall significance level for statistical tests on the secondary outcomes will be set at 0.05. Holm's method will be used to adjust for multiple comparisons.[77] The continuous variables of duration of diarrhea and duration of vomiting (measured in hours and analyzed with a Van Elteren test),[78] will be stratified by clinical center. Similarly, the number of days the child is absent from daycare and the caregiver is absent from work will be analyzed with a Van Elteren test, stratified by clinical center and etiology.

Dichotomous outcomes to be evaluated include emergency department acute gastroenteritis-related revisits, intravenous rehydration, and hospitalization. Additional analyses involving these outcomes will include linear and logistic regression models that adjust for possible effects of baseline characteristics.

5.3 Sample Size Calculations and Statistical Power

The primary analysis will be performed on a binary outcome: development of moderate-to-severe disease. The power of this analysis is based on the proportion of patients with moderate-to-severe disease. Our pilot data[12, 13] indicate that 25% of patients will have moderate to severe disease during the course of their illness. Furthermore expert surveys indicated that an absolute risk reduction of 10% would constitute a minimal clinically-important difference (MCID). Therefore our sample size calculation assumed a 25% event rate in the control group for which we desire to detect an absolute beneficial treatment effect of 10% with 90% power. Using a two-sided type I error (α) of 0.05 and the hypothesized proportions yields a required total sample size of 670 patients.[79] Our expected power, should we find different event rates in our 2 groups, is displayed in Table 2 on the next page.

Based on previous work by our group,[70, 71, 80] we assumed a 10% loss to follow up ($670/0.90 = 744$), 5% drop out, and 3% drop in (caregivers who buy a probiotic agent to administer to their child) rate ($744/(0.92)^2 = 879$). Adjustment for O'Brien-Fleming monitoring boundaries requires a further 2% increase. Thus, the total number randomized (final sample size) will be 897.

Outcome Control	Outcome Intervention	% Difference	Power
0.30	0.21	9%	0.76
0.30	0.20	10%	0.85
0.25	0.15	10%	0.90
0.25	0.16	9%	0.82
0.25	0.17	8%	0.72
0.20	0.10	10%	0.95
0.20	0.12	8%	0.81
0.20	0.13	7%	0.69

Table 2: Power Analysis

5.3.1 Enrichment Design

The above study design and power analysis are based on the assumption of homogeneous treatment effect. We may also incorporate an enrichment design[81, 82] to restore the statistical power in case the presence of a subpopulation with a substantially low treatment effect is identified. We are particularly interested in two potential subpopulations: participants with <2 days of symptoms and those with $\geq$ 2 days of symptoms. Based on our pilot data, each subpopulation accounts for approximately 50% of the total population. The decision for enrollment modification will be made at the first interim analysis for efficacy. Specifically, three statistics (based on a normal approximation of binomial distribution, or z-statistics) will be calculated to compare the primary efficacy endpoint between treatment and control groups for subjects in the total population and the two subpopulations, respectively. If the z-statistic from a subpopulation is <0.3 and also smaller than that in the total population, subjects from this subpopulation will no longer be considered in the subsequent enrollment. All subjects, regardless of symptom duration will be included in the final analyses. Our simulation studies have shown that such an enrichment design can increase the power considerably when the treatment effects are different across subpopulations, while it will have little impact on power when the treatment effects are similar.

5.3.2 Sub-group Analyses

The presence of a Modified Vesikari Score $\geq$ 9 will be analyzed by age <1 year, duration of symptoms, breast-feeding status, antibiotic usage, rotavirus and norovirus positivity. A subgroup effect will be considered significant if the interaction between subgroup and treatment in a logistic regression model is significant at a Bonferroni-corrected level.

5.4 Recruitment Estimates and Attrition

5.4.1 Recruitment

To estimate the number of subjects with acute gastroenteritis of eligible ages that would meet all inclusion criteria and be enrolled annually, we obtained discharge diagnoses from all study sites for 2005-2011. In 2011, the study sites evaluated 14,048 potentially eligible subjects (patients 3-48 months with ICD9 codes for diarrhea) out of 507,039 total emergency department visits (2.8%). In the past 7 years the lowest proportion of such patients per total emergency department volume was 2.3% (2008). Therefore, using 2011 emergency department volumes and lowest proportion of patients with diarrhea our conservative estimate for eligible patients would be 11,150 patients 3-48 months with diarrhea per year (or 30 patients per day network wide). Our best estimate is that 4.5% of children with acute gastroenteritis aged 3-48 months will be enrolled. We plan to enroll 225 patients/year and 900 subjects over 4 acute gastroenteritis seasons.

5.4.2 Potential for Bias

Reporting bias will be minimized by adhering to CONSORT recommendations including the use of third-party assignment.[83] Placebo capsules and active drug will be provided by I-Health Inc. The total weight of all capsules is 325 mg. The probiotic and placebo capsules and powder are identical in appearance, taste, texture, and odor. Participants, families, healthcare providers, data collectors, outcome adjudicators, and data analysts will be blinded as to intervention arm, thereby preventing bias in outcome assessment. Two DCC statisticians will be partially unblinded (with knowledge of group assignments, but not group identities) in order to present interim results to the DSMB. An intention-to-treat analysis will be performed to minimize bias associated with poor compliance and non-random loss of participants.[84] Co-interventions (e.g. antiemetic administration, intravenous rehydration) and other potential sources of confounding will be recorded. Our use of a published validated score as an outcome measure will protect against the introduction of bias in the assessment of treatment effects.[85]

5.4.3 Compliance

Noncompliance with probiotics is rarely reported in previous studies, and not expected to be problematic in this cohort.[86, 87] Participant withdrawal has been related mostly to the primary illness.[19] In the Canadian pilot study, compliance was 91%, We will track compliance by obtaining from parents unused capsule counts on day 5. If there are any unused capsules, we will request their return (a pre-stamped, pre-addressed envelope will be provided).

5.4.4 Loss to Follow-up

Our previous emergency department pediatric acute gastroenteritis research studies achieved telephone follow-up rates of 98% on day 3 and 96% on day 7.[70] Similar success has been documented in other PECARN multicenter studies (91%).[80] We estimate a 10% loss to follow-up. If daily contact does not occur, we will collect data from missed days on subsequent days when caregivers are contacted. The use of patient diaries (paper and/or electronic) and chart review will supplement parent contact. Based on our pilot trial we have devised the following strategies to maximize follow-up: all follow-up procedures will be centralized at the lead institution and conducted by experienced study staff; we will obtain multiple phone numbers from caregivers as well as emergency contacts, and we will schedule calls and send reminders prior to the call if preferred. Finally, electronic diary filing and transmission may be available for interested families.

5.4.5 Compensation

Financial compensation may be provided to compensate for parent's time completing follow-up. This compensation must be approved by each site's Institutional Review Board.

6 Data Management

6.1 Clinical Sites

Study data will be recorded on paper work forms, which will be retained at the clinical site. Data will then be entered into the electronic data capture (EDC) system provided by the Data Coordinating Center at the University of Utah School of Medicine.

The clinical investigator at each participating site will complete a Form FDA 1572, Statement of Investigator. That individual is responsible for all aspects of study implementation, including administration of study drug, collection of accurate study data, and correct entry of the data into the EDC. These tasks may be specifically delegated to other individuals at the clinical site, but the clinical investigator is responsible to supervise all aspects of the study, and is responsible to assure that all staff involved in this study are adequately trained to perform the delegated tasks. All local research records will remain in a locked file in a secure room unless being used by research staff, and all computerized information will be maintained on password protected computers.

6.2 PECARN Data Coordinating Center (Utah)

In addition to locally secured, identifiable information, partially identifiable information for all sites will be maintained at the PECARN Data Coordinating Center, located at the University of Utah in Salt Lake City, Utah. The Data Coordinating Center has a state-of-the-art computer infrastructure with a dedicated server room with a fire suppression system, air conditioning, cooling system and separate air filtering. The server facility is locked separately from the remainder of the Data Coordinating Center and access to the building is monitored by security personnel year round. The Data Coordinating Center coordinates its network infrastructure and security with the Health Sciences Campus (HSC) information systems at the University of Utah. This provides the Data Coordinating Center with effective firewall hardware, automatic network intrusion detection, and the expertise of dedicated security experts working at the University.

Network equipment includes four high-speed switches. User authentication is centralized with two Windows 2008 domain servers. Communication over public networks is encrypted with virtual point-to-point sessions using SSL or VPN technologies, both of which provide at least 128 bit encryption. OpenClinica (Web-based clinical studies data management system), eRoom^{TM} (Web-based collaborative workspace) and other web applications use the SSL protocol to transmit data securely over the Internet. Direct access to Data Coordinating Center machines is only available while physically located inside the Data Coordinating Center offices, or via a VPN client. All network traffic is monitored for intrusion attempts, security scans are regularly run against our servers, and IT staff are notified of intrusion alerts.

Production servers running mission critical applications are clustered and configured for failover events. Servers are backed up with encryption through a dedicated backup server that connects across an internal 10 gigabit network to a tape drive. Storage area networking (SAN) applications, clusters, and switch-to-switch links are also on a 10 gigabit network. Incremental backups occur hourly during the week. Incremental backups also are performed nightly with full system backups occurring every week. Tapes are stored in a fireproof safe inside the data center facility, and full backups are taken to an off-site commercial storage facility. Security is maintained with Windows 2008 user/group domain-level security.

Users are required to change their passwords every 90 days, and workstations time out after 5 minutes of inactivity. All files are protected at group and user levels; database security is handled in a similar manner with group level access to databases, tables, and views in Microsoft SQL Server. All portable computers are whole-disk-encrypted.

6.3 Data Confidentiality

The PI and other research personnel have all completed training and received certification in Human Subjects Research Protection and HIPAA. All project staff hired will also successfully complete this training prior to engaging in any research or treatment with study participants and renew this training as required by their institution.

The investigators and staff of the Data Coordinating Center are fully committed to the security and confidentiality of all data collected for PECARN studies. All Data Coordinating Center personnel at the University of Utah have signed confidentiality agreements concerning all data encountered in the center. Violation of these agreements may result in termination from employment at the University of Utah. In addition, all personnel involved with data coordinating center data systems have received Human Subjects Protection and HIPAA education.

The staff, reviewers and investigators involved with this study will be required to sign agreements from the Data Coordinating Center that relate to maintenance of passwords, information system security, and data confidentiality.

6.4 Data Quality Management and Monitoring

The Data Coordinating Center monitors PECARN studies on behalf of the investigators and the funding agency. The purposes of monitoring include demonstration of adherence to human subjects protection requirements and assurance of high quality study data. Monitoring is usually done remotely and may also involve physical site monitoring visits. Site monitoring is described in more detail in Section 9.2.

6.5 Record Access

The medical record and study files (including informed consent, permission, and assent documents) must be made available to authorized representatives of the Data Coordinating Center, upon request, for source verification of study documentation. In addition, medical information and data generated by this study must be available for inspection upon request by representatives of the the National Institutes of Health, Food and Drug Administration, and the Institutional Review Board (IRB) for each study site.

7 Protection of Human Subjects

7.1 Institutional Review Board (IRB) Approval

The Data Coordinating Center and each clinical center must obtain approval from their respective IRB prior to participating in the study. The Data Coordinating Center will track IRB approval status at all participating centers and will not permit subject enrollment without documentation of initial IRB approval and maintenance of that approval throughout subsequent years of the project.

7.2 Informed Consent

This protocol requires that parents or other legally empowered guardians sign a parental permission form. The parent or legal guardian will be informed about the objectives of the study and the potential risks. Parents or legal guardians of eligible children with symptoms of gastroenteritis will be approached to provide permission for their child's participation in the study. Parental or guardian permission will be obtained prior to initiation of study activities. Documentation of parental permission will be maintained at the study site. As the maximum eligible age is 48 months, child assent is not applicable.

7.3 Potential Risks

Lactobacilli are ubiquitous in the human diet and are a large part of the over 1 trillion live bacteria that reside in the gastrointestinal tract of healthy individuals. Overall, *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) is well tolerated in the pediatric patient population. Infections (bacteremia, endocarditis, pneumonia, deep abdominal abscesses) have been reported in sick neonates, severely debilitated and immune-compromised individuals given probiotics. The use of probiotics in these individuals continues to be controversial; however prospective studies have been conducted in adults and children with HIV as well as preterm neonates, with no reported systemic infections. Finally, there are 7 case reports of invasive disease after administration of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG). These occurred in patients in intensive care units, in patients with central venous catheters, or in patients who are on immunosuppressive therapy, have short gut syndrome, or are at risk for endocarditis. These risk factors are exclusion criteria from our study population.

The clinical risks of invasive infection from *Lactobacillus rhamnosus* are related to receiving *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG). There are risks associated

with acute gastroenteritis that are not related to study participation, including dehydration, electrolyte abnormalities, systemic infection and co-infection, and hospital admission. All subjects will be clinically treated for acute gastroenteritis in accordance with local site treatment protocols. There may be other unknown risks of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) in children with acute gastroenteritis.

Loss of confidentially is always a risk in a study, but safeguards are in place to protect against this.

7.4 Protections Against Potential Risks

Several steps will be taken to minimize the risks of participation in the study. All of the participating clinical centers are tertiary pediatric hospitals with highly trained pediatric staff. Subjects will be closely followed after discharge. Families will be contacted daily until both the diarrhea and vomiting have resolved and the treatment has been completed. Additional contact will be made on day 14, and months 1, 3, 6, 9 and 12 for long term safety outcomes. Parents or legally authorized representatives will also receive specific instructions as to when to see a health care provider. If an adverse event is reported at the time of follow-up, research personnel will refer subjects for appropriate medical care, when applicable.

Loss of confidentiality will be mitigated by the use of the PECARN Data Coordinating Center which has a highly secure IT infrastructure, and by the existence of trained research staff at participating sites. Data security is described in Section 6.

7.5 Potential Benefits

Subjects participating in the study may benefit directly by experiencing less severe disease, shorter duration of symptoms, and decreased need for further health care utilization. There may also be reduced symptoms (i.e. diarrhea, vomiting, fever) and morbidity, thereby diminishing acute gastroenteritis-associated resource consumption (e.g. physician visits, hospitalization) and societal costs. Additional benefits in the study include closer monitoring of symptoms and potentially earlier recognition of any worsening of acute gastroenteritis due to the frequency of telephone follow up and communication with the family. The knowledge gained in this study may lead to improved treatment options for gastroenteritis for other children and lead to new therapeutic options for future patients. Subjects and their families may therefore benefit indirectly by participating in research with the potential to provide subsequent benefit to others. Finally, future analysis of bulk

stool specimens will enable a better understanding of potential mechanisms of action of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG), which will likely provide benefit to future patients.

8 Data and Safety Monitoring Plan

8.1 Data Safety Monitoring Board (DSMB)

This study will have a Data Safety Monitoring Board (DSMB) appointed in accordance with instructions from the NICHD program officer. The DSMB will have a charter, will approve the protocol prior to implementation, and will review interim analyses for safety and efficacy.

The purpose of the DSMB is to advise the Federal funding agency (NICHD), the study Principal Investigator (Dr. Schnadower), and the PECARN Steering Committee regarding the continuing safety of study subjects and the continuing validity and scientific merit of the study. The DSMB is responsible for monitoring accrual of study subjects, adherence to the study protocol, assessments of data quality, performance of individual clinical sites, review of serious adverse events and other subject safety issues, and review of formal interim statistical analyses of treatment efficacy.

The Data Coordinating Center will send reports relating to these topics to DSMB members prior to each DSMB meeting. The Data Coordinating Center will staff DSMB meetings. The production and approval of DSMB minutes will be done in accordance with requirements of the NICHD. Each DSMB meeting will have a summary recommendation that will be provided to each participating clinical site for submission to the local Institutional Review Board (IRB). More detailed information from the DSMB meetings will not be routinely provided for local IRB submission.

8.2 Frequency of Interim Analysis:

The Data Safety Monitoring Board (DSMB) will meet after 80 patients (safety end points for 1 month), approximately 350 patients (end of 2nd year of enrollment) and 620 patients (end of 3rd year of enrollment) to review enrollment, study procedures, loss to follow-up, drop-in rate, and interim safety and efficacy results. The analyses will test the hypothesis that the probability of developing moderate-to-severe acute gastroenteritis in the probiotic arm is equal to that in the placebo arm. An analysis will also be conducted after 20 subjects under 6 months of age have been enrolled. The DSMB is to review

interim data and make recommendations regarding continuation or modification of the study based on safety in this age group. Conservative O'Brien-Fleming monitoring boundaries, implemented using the Lan-DeMets α-spending function approach, will be used as guidelines for early stopping for efficacy.

8.3 Adverse Event Reporting

8.3.1 Definitions, Relatedness, Severity and Expectedness

Definition: An adverse event (AE) is any untoward medical occurrence experienced by a subject. An event constitutes a disease, a set of related signs or symptoms, or a single sign or symptom. On each study day, the site investigators will evaluate adverse events. Study staff will obtain information on symptoms and adverse events on scheduled follow up calls. Adverse events not previously documented in the study will be recorded on the adverse event record form. The nature of each experience, date and time (where appropriate) of onset, outcome, course, and relationship to treatment should be established.

Relatedness: The suspected relationship between study interventions and any adverse event will be determined by the site investigator using the following criteria. *Relatedness may **not** be assessed by a research coordinator, and must be assessed by an investigator.*

Not Related: The event is clearly related to other factors, such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Possibly Related: The event follows compatible temporal sequence from the time of beginning the assigned study intervention, but could have been produced by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Probably Related: The event follows a reasonable temporal sequence from the time of beginning the assigned study intervention, and *cannot be reasonably explained* by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Some toxicities will be difficult to distinguish from abdominal symptoms related to acute gastroenteritis (such as bloating, abdominal pain, diarrhea, fever and diaper rash), and only at the time of analysis will we be able to determine whether these signs and symptoms are different between the groups.

Seriousness: The severity of clinical adverse events and laboratory abnormalities will be recorded by the site investigator and categorized. A serious adverse event (SAE) is an adverse event that:

- results in death; or
- is life-threatening (the patient was, in the view of the site investigator, in immediate danger of death from the event as it occurred); or
- requires inpatient hospitalization or prolongs an existing hospitalization; or
- results in persistent or significant disability or incapacity; or
- results in congenital anomaly/birth defect; or
- any other event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Expectedness of the Event: All adverse events, including serious adverse events, will be evaluated as to whether their occurrence was expected or unexpected. An adverse event is considered expected if it is known to be associated with acute gastroenteritis (acute gastroenteritis), other underlying medical conditions of the subject, is directly related to study outcome, or is otherwise mentioned in the protocol, informed consent, investigator brochure, or other study documents. Expected complications of acute gastroenteritis include loss of appetite, abnormal taste, heartburn, constipation, skin rash, fever, nasal congestion, sore throat, cough, headache, malaise, muscle aches, chills, blood in stool, bloating, gas, intestinal rumbling, abdominal distention, foul smelling stools, diarrhea, buttock rash, abdominal pain, abdominal cramps, nausea, vomiting, dehydration, electrolyte and other laboratory abnormalities, systemic infection and co-infection, seizures and hospital admission. Other expected adverse events are reactions known to occur with probiotic administration, specifically bloating, gas, intestinal rumbling, diarrhea, blood in stool, bacteremia, abdominal pain, abdominal cramps, nausea, vomiting, foul smelling stools, loss of appetite, abnormal taste, heartburn, constipation, skin rash, fever, nasal congestion, sore throat, cough, headache, malaise, muscle aches, other symptoms associated with viral syndromes.

Treatment or Action Taken: For each adverse event, the site investigator will record whether an intervention was required:

- Intervention: Surgery or procedure
- Other Treatment: Medication initiation, change, or discontinuation

- None: No action taken

Outcome of Event: Finally, the site investigator will record the clinical outcome of each adverse event as follows:

- Death
- Recovered and the patient returned to baseline status
- Recovered with permanent sequelae
- Symptoms continue

8.3.2 Time Period for Adverse Events

For purposes of this study, adverse events occur following randomization through 30 days after the last study drug dose will be recorded. Serious adverse events, unexpected medically attended events, and new onset chronic illnesses will be recorded from randomization through twelve months after the last study dose. Specifically, events that occur following parental permission to participate in the study, but prior to actual randomization, are *not* adverse events. These should be recorded as baseline conditions.

8.3.3 Data Collection Procedures for Adverse Events

After patient randomization, all adverse events (including serious adverse events), whether anticipated or unanticipated, will be recorded according to the date of first occurrence, severity, and their duration, as well as any treatment prescribed. Any medical condition present at the time of randomization, recorded in the patient's baseline history at study entry, which remains unchanged or improves, will not be recorded as an adverse event at subsequent evaluations. However, worsening of a medical condition that was present at the time of randomization will be considered a new adverse event and reported.

Abnormal laboratory values that are clinically significant will be recorded as adverse events and the site investigator will assess the severity and relationship to the study. Laboratory values that are abnormal at the time of randomization and that do not worsen will not be recorded as adverse events.

Adverse events will be coded using the MedDRA coding vocabulary. Coding will be done centrally at the Data Coordinating Center because this requires specific training.

8.3.4 Unanticipated Problems (UP)

Unanticipated problems (UP) are defined as incidents, experiences, or outcomes that are unexpected, related to participation in the study, and suggest that the research places subjects at a greater risk of harm than was previously known or recognized. The site investigator will report unanticipated problems to the Data Coordinating Center within 24 hours. A detailed completed report will be required to be sent to the Data Coordinating Center within 3 working days of the event. After receipt of the complete report, the Data Coordinating Center will report these unanticipated problems to the NICHD Program Official or Project Officer in an expedited manner (within 24 hours). In accordance with local IRB requirements, the site investigator may be required to report such unanticipated problems to the IRB in addition to notifying the Data Coordinating Center. In the event that the medical monitor believes that such an event warrants emergent suspension of enrollment in the trial, and NICHD staff cannot be reached expeditiously, the Data Coordinating Center will notify the study investigator (Dr.Schnadower) and all site investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the NICHD staff after discussion with the DSMB.

8.3.5 Monitoring Serious Adverse Events

The Principal Investigator of the Data Coordinating Center (Dr. Dean) will act as the medical monitor for this study. If Dr. Dean is unavailable, a qualified physician will be designated to fulfill this function. Site investigators and/or research coordinators will report serious adverse events to the Data Coordinating Center within 24 hours. A detailed completed report will be required to be sent to the Data Coordinating Center within 3 working days of the event, and the medical monitor will assess all serious adverse events reported from site investigators.

For each of these serious adverse events, the site investigator will provide sufficient medical history and clinical details for a safety assessment to be made with regard to continuation of the trial. The medical monitor will sign each SAE report after review. All SAE reports will be retained at the Data Coordinating Center, and all SAE reports will be available for review by DSMB members and NICHD staff.

In the unlikely event that the medical monitor believes an unexpected and study-related SAE warrants emergent cessation of enrollment in the trial, NICHD staff and the DSMB chairperson will be immediately consulted. If these individuals concur with the judgment of the medical monitor, or if the NICHD staff and the DSMB chairperson cannot be reached expeditiously, the Data Coordinating Center will notify the study investigator (Dr.Schnadower) and all site investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the NICHD staff after discussion with the

DSMB.

In accordance with local IRB requirements, the site investigator may be required to report such events to the IRB in addition to notifying the Data Coordinating Center.

After notification of the NICHD Program Official or Project Officer, and the DSMB chairperson, of *serious, unexpected, and study-related* adverse events or unanticipated problems (UP), decisions will be made whether to continue the study without change, and whether to convene the entire DSMB for an emergent meeting. If a decision is made to suspend enrollment in the trial, this will be reported to the study investigator (Dr. Schnadower) and all clinical investigators, who will be instructed to report this to their local IRB.

The DSMB will review all adverse events (not necessarily serious, unexpected, and study-related) during scheduled DSMB meetings. The Data Coordinating Center will prepare a Summary Report of Adverse Events for the DSMB meetings, classified with the MedDRA coding system.

8.3.6 Follow-up of Serious, Unexpected and Related Adverse Events

All serious, unexpected and related adverse events, that are unresolved at the time of the patient's termination from the study or discharge from the hospital, will be followed by the Clinical Center investigators until the events are resolved, subject is lost to follow-up, the adverse event is otherwise explained or has stabilized, or 12 months have passed from the time of last study dose.

9 Study Training and Monitoring

9.1 Study Training

A formal training program for investigators and research staff will be held prior to the start of enrollment. The training program will cover regulatory topics and Good Clinical Practice. The training will also provide in depth explanations regarding study procedures, clinical care, adverse event reporting, data entry procedures, quality assurance, site monitoring, and the informed consent process. A manual of operations will be provided to each Clinical Center investigator prior to the start of enrollment. The manual will detail specific information about the study procedures, regulatory information, safety reporting, and other necessary information. Updates and revisions to the manual will be made available electronically. The Data Coordinating Center, in collaboration with the study investigator (Dr. Schnadower), will be the main contact for study questions.

9.2 Study Monitoring

The investigators recognize the importance of ensuring data of excellent quality. Site monitoring is critical to this process. Site monitoring has been a very effective tool for maintaining data quality in previous PECARN studies, and we will utilize this process to ensure excellent quality data in the proposed study. Our site monitoring plan is designed to identify problems with sites and methods for handling problems that arise. Site monitors must be provided with full access to study materials and the medical records for study subjects. If the medical records are in electronic form, the clinical investigator or an authorized individual must provide any assistance necessary to facilitate the site monitor's review of data in the electronic medical record.

9.2.1 Site Monitoring Plan

A supplemental study-specific monitoring plan, separate from the protocol will be completed which outlines specific criteria for monitoring. This plan will include the number of planned site visits, criteria for focused visits, or additional visits, a plan for chart review and a follow up plan for non-compliant sites. The monitoring plan also describes the type of monitoring that will take place (e.g. sample of all subjects within a site; key data or all data), the schedule of visits, how they are reported and a time frame to resolve any issues found. Remote site monitoring schedules will be determined by the Data Coordinating Center in coordination with the study principal investigator.

9.2.2 Clinical Site Monitoring

Site monitoring visits will be performed by a trained site monitor during the study period to ensure regulatory compliance, patient safety, and to monitor the quality of data collected. Essential document binders, regulatory documents and data collection forms may be reviewed. Interim visits will take place depending on grant budget, site enrollment, and compliance issues identified. The site monitor will provide each site with a written report, and sites will be required to follow up on any deficiencies. It is anticipated that the study monitoring visits for this protocol will consist of a site initiation visit (prior to patient enrollment), interim visits, and a close out visit. The site initiation may take place as group training made up of site investigators and research assistants.

9.2.3 Remote Monitoring

The Data Coordinating Center may supplement on-site monitoring with remote monitoring activities. Remote monitoring involves detailed review of the data entered by the Clinical Center and consultations with the Clinical Center investigator and/or research coordinator

to review safety and data quality. This may require uploading de-identified copies of specific parts of the medical record, patient study file, regulatory documentation, or other source documents to the Data Coordinating Center staff, who review those materials against the data recorded in the electronic data capture system. This helps assure protocol compliance and accurate data collection. The Data Coordinating Center may conduct more remote monitoring activities early in the trial to assure protocol compliance and identify any training issues that may exist. Remote monitoring the documents will be retained in accordance with federal requirements. Safety of subjects will be monitored and ensured in accordance with the Data and Safety Monitoring Board (DSMB) plan.

9.2.4 Pharmacy Monitoring

The Clinical Center pharmacy must maintain adequate records of all dispensed study drug. Each pharmacy will be monitored and may be requested to send copies of these documents to the Data Coordinating Center. Since this study will use a central pharmacy, that pharmacy must also maintain adequate records and will also be monitored.

10 Regulatory Issues

10.1 Food and Drug Administration

This trial is being conducted under an Investigational New Drug application approved by the Food and Drug Administration (Investigational New Drug application #15371). The clinical investigator at each participating site will complete a Form FDA 1572, "Statement of Investigator."

10.2 Health Insurance Portability and Accountability Act

The abstracted data will include limited identifiers as defined by the Health Insurance Portability and Accountability Act, and specific contact information will be provided to research staff conducting follow up with parents. Abstracted data will be retained and archived at the Data Coordinating Center in accordance with record retention requires of the Food and Drug Administration and the NIH. Contact information will not be provided to the Data Coordinating Center (it will be provided directly to the central follow up research staff). For data analysis outside the Data Coordinating Center (e.g., when a public access database is made available), the Data Coordinating Center will create a completely de-identified analytical database for use by the study investigators, and for final archiving. All study sites have been or will be offered Business Associate

Agreements with the University of Utah. Copies of signed Business Associate Agreements are maintained at the Data Coordinating Center.

10.3 Inclusion of Women and Minorities

The gender, ethnic and racial composition of patients enrolled in all PECARN studies is a function of the underlying referral population at each PECARN site participating in this trial. There will be no exclusion of patients based on gender, race, or ethnicity.

10.4 ClinicalTrials.gov Requirements

This trial has been registered at ClinicalTrials.gov (NCT #01773967). The title is "Impact of Emergency Department Probiotic (LGG) Treatment of Pediatric Gastroenteritis."

10.5 Retention of Records

For federally funded studies subject to the Common Rule, records relating to the research conducted shall be retained for at least 3 years after completion of the research. Completion of the research for this protocol should be anticipated to include planned primary and secondary analyses, as well as subsequent derivative analyses. Completion of the research also entails completion of all publications relating to the research. All records shall be accessible for inspection and copying by authorized representatives of the regulatory authorities at reasonable times and in a reasonable manner [45 CFR §46.115(b)].

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Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis (Probiotics Study) PECARN Protocol Number 032

Pediatric Emergency Care Applied Research Network
National Institute for Child Health and Human Development (NICHD)

Protocol Version 3.00
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PROTOCOL TITLE:

Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis

Short Title: Probiotics Study
PECARN Protocol Number: 032

Lead Investigator and Author:
David Schnadower, M.D.
Washington University

Protocol Version: 3.00
Version Date: February 13, 2017

I confirm that I have read this protocol, I understand it, and I will conduct the study according to the protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and will adhere to the Ethical and Regulatory Considerations as stated. I confirm that if I or any of my staff are members of the Institutional Review Board, we will abstain from voting on this protocol, its future renewals, and its future amendments.

Principal Investigator Name: _____

Principal Investigator Signature: _____

Date: _____

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Abstract

Practice guidelines do not endorse routine use of probiotics in pediatric acute gastroenteritis. However, probiotic manufacturers aggressively purvey probiotics using health claims that have not been supported by rigorous research, and the world-wide market for probiotic products is equivalent to $32.6 billion per year. The Food and Drug Administration and the European Food Safety Authority remain concerned about probiotic value and safety.[1–3] Some institutions now recommend the routine use of probiotics based on potentially flawed or limited evidence,[4] and parents of patients with acute gastroenteritis often administer probiotics to their children without guidance from medical professionals.[5] Since the use of probiotics is increasing without adequate evidence to support its use in the United States, the study described in this protocol was designed and successfully submitted to the National Institute for Child Health and Human Development (NICHD) for funding.

The overall objective of this randomized, placebo-controlled, double-blind study is to determine if probiotic administration reduces the severity of acute gastroenteritis episodes in children aged 3 to 48 months. The probiotic agent that will be used in this trial is *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG).

Participants will be randomized to receive a five-day course of probiotic or placebo. The trial is anticipated to enroll 970 children, age 3-48 months, treated in participating emergency departments for acute gastroenteritis. We define acute gastroenteritis as the presence of three or more watery stools in a 24-hour period. Children with underlying illness that pose potential increased risk from probiotic therapy (e.g. indwelling vascular access line, structural heart disease, immunosuppressive therapy or known immunodeficiency, chronic gastrointestinal disease such as inflammatory bowel disease, or household contact with immunodeficiency or immunosuppressive therapy) will be excluded. The age range of 3-48 months was selected because this age group has the highest incidence of acute gastroenteritis and the greatest morbidity. Subjects will be recruited at the time of an emergency department visit. All eligible subjects who present for treatment when study personnel are available will be approached for participation. Those agreeing to participate will be randomized, using block randomization stratified by study site, to treatment groups. The study will be conducted at multiple sites within the Pediatric Emergency Care Applied Research Network (PECARN). We will also collect and freeze pre- and post-treatment bulk stool specimens at the lead study site to assess the mechanism of action of the specific probiotic agent, *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG).

1 Study Summary

This randomized, double-blind, placebo-controlled trial will quantify the benefits and potential side effects associated with probiotic administration in ambulatory children presenting to the emergency department with acute gastroenteritis. This will provide the first definitive evidence in the United States for, or against, using probiotic therapy for this condition. This study will use the most commonly employed probiotic agent (*Lactobacillus rhamnosus* GG, ATCC 53103 (LGG)) approved for use in the United States. The results of this multicenter study will guide the standard of care: if probiotic administration is associated with benefit, it offers an inexpensive, safe and easy way to administer treatment to reduce morbidity from acute gastroenteritis. If the trial does not demonstrate probiotic efficacy, healthcare, caregiver and societal resources may be refocused on alternative therapeutic interventions.

1.1 Hypotheses

The hypotheses of this study are:

1. In children with acute gastroenteritis, probiotic administration in the emergency department will be associated with a clinically-important decrease in the proportion of children suffering from moderate-severe disease, defined by a validated Modified Vesikari Score ≥ 9, compared to placebo.

2. In children with acute gastroenteritis, probiotic administration will not be associated with serious adverse events, and will have a similar rate of side effects (e.g. bloating, fever) as compared to placebo-treated children.

1.2 Specific Aims

This project has the following Specific Aims:

Specific Aim 1. Determine the clinical effectiveness of probiotic administration to reduce morbidity in children presenting to the emergency department with acute gastroenteritis.

Specific Aim 2. Determine the safety and side effect profiles of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) in children presenting to the emergency department with acute gastroenteritis.

1.3 Trial Endpoints

Primary Endpoints

- The **primary efficacy endpoint** of this study is the presence of moderate-severe acute gastroenteritis, as defined by a total post-enrollment Modified Vesikari Score ≥ 9 during the 2-week follow-up period.

- The **primary safety endpoint** of this study is the occurrence of invasive disease (including meningitis and bacteremia) from *Lactobacillus rhamnosus*.

Secondary Endpoints

1. **Diarrhea duration:** Time from randomization until the appearance of the last watery stool as reported during daily surveys.

2. **Vomiting duration:** Time from randomization until the last vomiting episode as reported during daily surveys. Vomiting duration is only evaluated in children who vomited ≥ 3 times during the 24 hours prior to the emergency department visit.

3. **Return visits:** Return visits for unscheduled care to a healthcare provider related to vomiting, diarrhea, dehydration, fever, or fluid refusal, within 2 weeks of the index visit. Scheduled visits (e.g. reassessment, vaccinations, unrelated issues) will not be included.

4. **Missed daycare:** Days of daycare missed by subjects who attend daycare.

5. **Missed work:** Days of work missed by caregivers who work outside of the home.

6. **Household transmission rate:** A household census will be obtained at the time of enrollment, and information about household contacts symptoms will be obtained during daily surveys to determine household transmission rate.

7. **Side Effects:** Occurrence of side effects such as weakness, bloating, gas, intestinal rumbling, diarrhea, blood in stool, abdominal pain, abdominal cramps, nausea, vomiting, loss of appetite, heartburn, constipation, skin rash, fever, nasal congestion, sore throat, cough, headache, muscle aches, chills, and diaper rash.

1.4 Subject Eligibility, Accrual and Study Duration

Eligible participants will be identified by on-site study staff. Inclusion criteria are:

1. Age 3-48 months (have not yet reached their fourth birthday); AND
2. Presence of 3 or more watery stools within 24 hours of screening; AND
3. Duration of vomiting or diarrhea less than 7 days; AND
4. Symptoms consistent with acute intestinal infectious process.

We will exclude subjects with any of the following:

1. Presence of an indwelling vascular access line; OR
2. Presence of structural heart disease excluding non-pathological heart murmurs; OR
3. Receiving immunosuppressive therapy or history of immunodeficiency; OR
4. Hematochezia in the preceding 48 hours; OR
5. Chronic gastrointestinal problems (e.g. short gut syndrome, inflammatory bowel disease); OR
6. Patients with known pancreatitis; OR
7. History of abdominal surgery; OR
8. Critically ill patients; OR
9. Family member with an indwelling vascular access line, or on immunosuppressive therapy, or with a known immunodeficiency; OR
10. Bilious emesis; OR
11. Probiotic use (supplement) in the preceding 2 weeks; OR
12. Oral or intravenous steroid use in the preceding six months; OR
13. Previously enrolled in this trial; OR
14. Allergy to lactobacillus or Microcrystalline Cellulose (MCC); OR
15. Allergy to erythromycin, clindamycin, AND β-lactam antibiotics (all); OR
16. Not available for daily follow-up while symptomatic; OR
17. Parent/guardian not speaking English or Spanish; OR
18. Under 6 months old AND premature (<37 weeks).

The trial is anticipated to enroll 970 subjects over four years, which will include four acute gastroenteritis seasons. An interim safety analysis will be prepared for Data Safety Monitoring Board (DSMB) review after the first 80 subjects (half of whom will be under 1 year of age) have been followed for 1 month. At the time of this review, the DSMB *may* alter the subsequent sample size based on overall event rates.

2 Rationale and Background

Acute gastroenteritis is a leading cause of malnutrition and death worldwide and affects millions of children in the United States each year.[6, 7] Acute gastroenteritis exerts high

impact burdens on children and their families: each episode causes on average 6-8 days of diarrhea, 2-4 days of vomiting, and 2-4 days of fever.[8–11] In two prospective studies conducted in 11 Canadian and 5 United States pediatric emergency departments, 433/683 (63%) children 3-48 months of age with acute gastroenteritis had moderate to-severe symptoms at presentation, resulting in substantial daycare and parental work absenteeism.[12, 13] The economic burden associated with such cases is considerable.[14] Unfortunately, current treatment options are limited to rehydration, symptomatic management and supportive care, prevention of severe dehydration and secondary infection control.[15–17]

Probiotic agents represent a novel approach to management of pediatric acute gastroenteritis. Probiotics are viable microbes that are generally considered to be safe and are relatively inexpensive, easily administered, and hypothesized to modulate disease processes.[18] They may also reduce symptoms (i.e. diarrhea, vomiting, fever)[19–22] and morbidity, thereby diminishing acute gastroenteritis-associated resource consumption (e.g. physician visits, hospitalization) and societal costs. Numerous studies report promising results in children with acute gastroenteritis treated with probiotics, but these studies have been inadequate to change clinical practice, because of flaws in methodology including small sample sizes, lack of probiotic quality control, and outcomes that are of minimal relevance to patients and their families.[19] Remarkably, few studies of probiotics have evaluated outpatients, a group representing >90% of acute gastroenteritis episodes in the United States.[23, 24] Moreover, only a single emergency department study of probiotic efficacy in acute gastroenteritis has been reported.[20]

To date, practice guidelines do not endorse routine use of probiotics in pediatric acute gastroenteritis.[5, 19, 25–27] However, current trends provide the motivations for the study described in this protocol. First, probiotic manufacturers aggressively purvey probiotics using health claims that have not been supported by rigorous research,[28–31] and the world-wide market for probiotic products is growing rapidly (projected growth 2009-2014 12.6%, now equivalent to $32.6 billion per year).[32] Second, the United States Food and Drug Administration and the European Food Safety Authority remain concerned about probiotic value and safety.[1–3] Third, some institutions now recommend the routine use of probiotics based on potentially flawed or limited evidence.[4] Fourth, parents of patients with acute gastroenteritis often administer probiotics to their children without guidance from medical professionals.[5]

2.1 Rationale for Probiotic Efficacy

Probiotics, defined as viable microbial preparations with beneficial effects on the health of the host,[33] are regarded as safe, and are generally affordable and convenient (i.e., as over-the-counter food supplements). Probiotics, and *Lactobacillus rhamnosus* GG, ATCC

53103 (LGG) in particular, promote colonization resistance and modulate immune responses in the host, and can be directly antimicrobial. Over 100 studies have studied how probiotics can improve immune function in humans.[34] At the mucosal surface, probiotics:

- compete with pathogenic bacteria for nutrients and adhesion binding sites;[35–38]
- produce antimicrobial substances;[37, 39, 40]
- provide nutrients to colonocytes;[41]
- alter epithelial gene expression;[42] and
- reduce intestinal permeability.[43]

Additional mechanisms of action include enhancing phagocyte[44] and natural killer cell activity,[45] and increasing fecal,[46] salivary,[47]and systemic[48] IgA levels.

Preliminary probiotic studies in humans, including over 56 trials in children with gastroenteritis (17 of which used some form of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) , the agent employed in this trial) suggest that probiotics may improve the course of acute gastroenteritis. Meta-analyses[19, 21, 49–51] report reduced mean duration of diarrhea (by 25 hours), reduced proportion of diarrhea lasting four days (risk ratio 0.41), and reduced stool frequency on Day 2 (mean difference 0.8).[19] This has prompted some physicians and institutions to recommend probiotics in children with acute gastroenteritis. Careful examination of the data, however, shows significant methodological flaws in these studies. For these reasons, experts and agencies have continued to recommend large, unbiased, well designed definitive trials to inform practice.[19, 26]

2.2 Limitations of Research to Date

Most trials of probiotics for acute gastroenteritis have been methodologically flawed.[19] Published studies have used small sample sizes and non-standardized treatment regimens, probiotic preparations with variable production quality and non-verified colony counts. Outcomes have been focused almost exclusively on diarrhea duration, and use inconsistent (or unreported) definitions,[19, 52] without considering the more global (and relevant) assessment of the illness (e.g. fever, vomiting, repeat emergency department visits, hospitalization). Because the outcomes sought are heterogeneous, and/or not validated, and/or of minimal importance to participants,[53] and have not addressed clinical and socioeconomic efficacy,[49, 50] the value of the existing data has been questioned.[54] Moreover, few studies address side effects and adverse events systematically.[1–3]

Although >90% of children with acute gastroenteritis are treated as outpatients,[23]

hospitalized children have been the main focus of most research in this area. Extrapolation to outpatients is problematic because hospitalized children are more likely to have rotavirus infections,[55] severe disease, and to benefit from probiotics.[21, 52, 56] Emergency department patients are ideal for outpatient probiotic trials, as their acute gastroenteritis disease severity represents the entire spectrum in a normal distribution,[13] offering distinct advantages over primary care populations. Emergency department patients are at risk for more severe disease, there is a greater potential for benefit because of the disease acuity and the large sample size, and disease spectrum will enable identification of subgroups most likely to benefit. Emergency departments are also a high venue of presentation for acute gastroenteritis, enabling uniform trial design and compliance compared to non-emergency department ambulatory settings.[24, 57–59] Only one emergency department study has been conducted previously, but the study was underpowered and did not explore side effects.[20]

2.3 Validation of the Modified Vesikari Score

The Vesikari Score, a composite measure of seven items first described in 1990,[60] has been used in many clinical trials for treating acute gastroenteritis, particularly those evaluating rotavirus vaccines in infants, and in large international epidemiological studies.[8, 61–65] The score incorporates features of acute gastroenteritis that are important to parents, children and physicians, including

- diarrhea duration
- diarrhea frequency,
- vomiting duration,
- vomiting frequency,
- maximum daily temperature,
- medical interventions required, and
- degree of dehydration.

In outpatient studies, the degree of dehydration, measured as weight loss, is problematic because it requires in-person re-evaluations, can vary based on recent voiding, defecation, and liquid intake, and also might reflect inadequate caloric intake or catabolism during acute illness. In fact, the assessment of dehydration using signs or scales designed based on the use of post-illness weight as a gold standard has been questioned.[66] Because of these limitations, modifications to the original Vesikari score have been tested.[67, 68] The modification replaces percent dehydration with the need for an unscheduled future healthcare visit within the two week follow-up period (Table 1 on the facing page). This

Points	0	1	2	3
Diarrhea duration	0	1-96 hrs	97-120 hrs	$\geq$ 121 hours
Max # of diarrheal stools/24 hrs	0	1-3	4-5	$\geq$ 6
Vomiting duration	0	1-24 hrs	25-48 hrs	$\geq$ 49 hours
Max # of vomiting episodes/24 hrs	0	1	2-4	$\geq$ 5
Max recorded fever	$\leq$ 37° C	37.1-38.4° C	38.5-38.9° C	$\geq$ 39° C
Unscheduled healthcare visit	0	—	Primary Care	Emergency Department
Treatment	None	Rehydration	Hospital Admission	—

Table 1: Modified Vesikari Score

modification is supported by evidence that the demand for professional medical care correlates with disease severity.[67] The Modified Vesikari Score has been validated in two multicenter studies[12, 13] conducted during the planning of the current trial described in this protocol.

3 Study Design and Data Collection

3.1 Study Design Overview

This is a double-blind randomized placebo controlled trial of probiotic therapy for acute gastroenteritis. Children 3 months of age to 48 months of age who present to a participating emergency department with acute gastroenteritis will be assessed for eligibility. Children for whom parents provide permission to participate in the trial will be randomized to receive probiotic therapy (*Lactobacillus rhamnosus* GG, ATCC 53103 (LGG)) or placebo. The trial will be analyzed as an intention-to-treat study.

3.2 Participant Screening and Consent

Potentially eligible patients will be identified and screened for eligibility. The treating physician or other study personnel will discuss the details of the study with the patient's caregivers to explain the study and obtain parental permission.

For patients who are actively screened in real time and meet all inclusion criteria, data will be recorded that include yes/no answers to each of the exclusion criteria. If a patient is ineligible at this point, no further data will be recorded, and the patient's parents will

not be approached for participation in the study. If a patient is eligible, the parents will be approached to request permission for their child to participate in the study. If parents are not approached for permission, the reason the parents were not approached will be recorded. If the parents decline permission to participate, the reason for refusal will be documented (if offered by the parent). For all eligible patients who are not randomized, study personnel will record whether the patient was prescribed probiotics. The recorded exclusion criteria, reasons for declining permission, and off-study probiotic use will enable the investigators to assess barriers to trial enrollment and identify potential biases in the study.

3.3 Baseline Data Collection

Trained study staff at each site will collect baseline demographic information (e.g. birthdate, gender, race, ethnicity) and relevant clinical variables, record the data on worksheets, and enter the data into the electronic data capture system provided by the Data Coordinating Center. Baseline clinical dehydration scale[69] and disease severity scores (Modified Vesikari Score)[13] will be assigned to enable baseline comparisons between treatment arms.

3.4 Follow Up Data Collection

Successful follow up will be maximized by obtaining multiple phone numbers for families and emergency contacts, scheduling calls and sending phone text reminders, allowing for electronic completion and centralizing all follow-up procedures at the lead institution (Washington University). Trained, experienced study staff will contact the family daily (including weekends) until both the diarrhea and vomiting have resolved and the treatment has been completed on day 5. Additional contact will be made on day 14 for outcome assessment, and months 1, 3, 6, 9 and 12 for long term safety outcomes. Study staff will use a standardized data collection form, and will inquire about ongoing symptoms, medical evaluations, treatments, child care, work absenteeism, and side effects. Detailed questioning will follow positive responses. A study diary will be provided to caregivers to use as a note-taking tool. Caregivers may also be provided an option of completing the follow-up collection electronically. Patient compliance with study drug administration will be assessed on day 5. To maximize compliance, caregivers will be reminded of the importance and method of administering the study drug. On day 14, the study site staff (not the central staff conducting the follow up) will perform a chart review for final data collection and identification of recorded adverse events during the 14 day study period. Adverse event reporting is described in detail in Section 8.3 on page 33.

Every effort will be made complete daily follow-up on the day necessary. However, if one or two days of follow-up contact is missed, data for those missed days may be collected on the next contact, if the caregiver used the home diaries or can recall the information. If all contact is missed but then the parent is contacted on day 14, follow-up data for the daily calls will be collected only if the parent diaries were used. For day 5 and 14, contact may be made within + 3 days. For month 1, 3, 6, 9, and 12 follow-up, contact may be made within ± 2 weeks.

4 Study Procedures

4.1 Randomization (Enrollment)

Randomization will be accomplished through the use of an online randomization service. Subjects will be randomized to receive either probiotic (*Lactobacillus rhamnosus* GG, ATCC 53103 (LGG)) or placebo. Equal allocation randomization tables will be provided by the Data Coordinating Center to the central research pharmacy. The central research pharmacy will prepare consecutively numbered study kits according to the randomization schedule. Study kits will be sent to the clinical sites. Randomization tables will be created at the Data Coordinating Center using permuted-block randomization stratified by clinical site and duration of symptoms. This will ensure that variations (e.g. site specific practice patterns, gastrointestinal patterns) are comparably distributed across treatment arms. The randomization number will be recorded in the database.

4.2 Study Drug Administration

Study Drug Description

Lactobacillus rhamnosus GG, ATCC 53103 (LGG) is supplied in a gelatin capsule containing 10^{10} colony forming units of *Lactobacillus rhamnosus*(75 mg). The capsule also contains 250 mg of microcrystalline cellulose (MCC – purified partially depolymerized cellulose), an inert ingredient. Placebo capsules contain only MCC (to a total of 325 mg). Each capsule is wrapped in double foil to protect it against harmful light, air, and moisture. Blister packs are labeled with the lot number. The probiotic and placebo capsules have active Drug Master Files at the Food and Drug Administration (BB-MF 2 #13668 and MF2 # 13646).

Initial Study Drug Administration

Clinical staff in the emergency department will administer the first dose of study drug by sprinkling the capsule's contents into 20-30 mL of room temperature non-carbonated liquid. Capsule contents may also be sprinkled into 1 tablespoon of applesauce as an alternative to liquid. Oral fluid therapy will be encouraged according to sites' existing clinical guidelines.[26] At discharge, parents will also be provided with clinical instructions concerning what and how much fluid to drink, criteria for returning to the emergency department or seeing their physician, and other standardized discharge instructions for acute gastroenteritis. Study research coordinators will provide the parents with instructions concerning study drug administration at home, completion of study forms, and a letter for their pediatrician that explains the study. The importance of administering all doses dispensed and the need to communicate with the study team on a daily basis until symptoms resolve will be stressed.

Home Study Drug Administration

All patients will consume one capsule twice a day for 5 days (total of 9 home doses). Patients will receive the medication mixed with 20-30 mL of a room temperature non-carbonated liquid (or 1 tablespoon of applesauce as an alternative to liquid) and ingested immediately to optimize probiotic viability. One extra dose/day will be provided (i.e. kits will contain 5 extra doses total of 15 capsules) to account for vomiting or wastage. The dose may be repeated once if vomiting occurs within 15 minutes of administration; this rarely happens more than once.[70] Oral fluid therapy will be encouraged according to established guidelines.[26]

Hospital Study Drug Administration

We estimate that only a small proportion (<5%) of enrolled subjects will be hospitalized. If that does occur, subjects will continue on the study protocol as described for home study drug administration, as done in previous studies.[71] Future unscheduled hospitalization at a non-study hospital site is even less common: none of the 274 patients enrolled in our pilot study were admitted to a different institution after evaluation.[12] To minimize the impact that such an event could have on this trial, caregivers will be provided with a letter that they will be instructed to share with their pediatrician and with the admitting physician. The letter will describe the study, the care-plan, and it will include site investigator contact information and the importance of adhering to the study protocol.

4.3 Discontinuation of Study Drug

Study drugs are sometimes discontinued because of intolerance or perception of a clinical adverse event from study drug administration. If any clinical abnormality, laboratory abnormality, intercurrent illness, new immunosuppressive therapy, or other condition occurs such that continued administration of study drug would not be in the best interest of the subject in the opinion of the investigator, study drug administration may be discontinued. When this occurs, regardless of the reason, the subject remains in the intention-to-treat population, all data collection should proceed, and long term follow up for adverse events is carried out. Discontinuation of study drug will be documented and will be reported in the closed session of the next scheduled Data Safety Monitoring Board meeting. Clinical staff and parents should *not* report the early discontinuation of study drug to research staff who are conducting follow up data collection. The Principal Investigator should *not* be contacted by the site investigator, as intentional discontinuation of study drug implies a safety outcome, and the Principal Investigator should be blind to all study outcomes. *Discontinuation of study drug is not a withdrawal from the study.*

If study subjects are hospitalized after discharge to home from the emergency department, this is not a reason for discontinuation of study drug. We estimate that only a small proportion (<5%) of enrolled children will be hospitalized after discharge to home from the emergency department. If that does occur, subjects should continue on the study protocol unless they have developed an exclusion criterion for study drug administration (e.g. critical illness, immunosuppression, etc.). To facilitate continuation of study drug administration during hospitalization, parents are provided with a letter for their pediatrician. The letter will describe the study, the care-plan, and it will include site investigator contact information and the importance of adhering to the study protocol. The parents will be instructed to share this letter with their pediatrician and with the admitting physician.

Breaking the blinding of the study drug should not be necessary, but an unblinding procedure will be available. This will require contacting the Medical Monitor at the Data Coordinating Center. However, unblinding is almost never necessary. The primary anticipated reason for a clinician requesting unblinding is the suspected occurrence of invasive infection (bacteremia/septicemia or meningitis), and the natural desire to know if the subject was receiving active *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG). The initial recommendation from the Medical Monitor will always be that the clinician should *assume* that the subject was receiving active drug, and include *Lactobacillus rhamnosus* in the differential diagnosis (and treatment coverage) of the suspected invasive infection. *Lactobacillus rhamnosus* is readily susceptible to common antibiotics, and the

differential diagnosis of subsequent invasive infection would include organisms other than *Lactobacillus rhamnosus*. Thus, future clinical treatment should not be dependent on unblinding information.

If the situation requires unblinding, the Medical Monitor and Data Coordinating Center staff will provide the information, and the unblinding will be documented. Study drug will be discontinued, *but the subject should not be withdrawn from the study!* All study procedures (except study drug administration) should be continued, as the subsequent clinical course of the subject is critical to the science of the trial. All unblinding events will be reported in the closed session of the next scheduled Data Safety Monitoring Board meeting. Clinical staff and parents should *not* report the unblinding to research staff who are conducting follow up data collection. The Principal Investigator should *not* be contacted by the site investigator, as intentional unblinding of a subject implies a safety outcome, and the Principal Investigator should be blind to all study outcomes.

4.4 Withdrawal from Study

Parents may completely withdraw their child from participation in this study at any time, including discontinuation of data collection. However, withdrawal from study is a completely different issue from discontinuation of study drug, and true withdrawal from study is an exceedingly rare event. If parents demand withdrawal of their child from the study, research staff will attempt to obtain adverse event information as required by the Food and Drug Administration (all adverse events for 30 days, monthly follow up for long term adverse events).

4.5 Stool Sample Testing

Stool sample swabs will be collected from all study subjects, if possible, for polymerase chain reaction (PCR) analysis. Once obtained, swabs will be frozen and sent to a central laboratory for analysis. A storage and shipping protocol will be provided in the study manual of operations. Samples will be tested with multiplex PCR. Sites will also be asked to collect an optional bulk sample of stool if one is available at the time of enrollment (i.e. fresh diaper, etc). This will be used for future studies.

In addition, at the lead site, Washington University, study research personnel will collect and freeze subjects' bulk stool specimens in the acute phase (within 24 hours of presentation) and 14 days after presentation using a previously tested bulk stool specimen collection protocol. Specimens may be collected in the emergency department or the

families will be given a specimen collection kit, gel packs and a metallic envelope. When the specimen is ready for collection, a courier will pick up the specimen and cool pack at the patient's home and deliver it to a logistics collection center at Washington University School of Medicine. This service is available 24 hours a day, year round. The stools will be stored as a part of a future project to assess the potential mechanisms of action of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) in acute gastroenteritis.

5 Data Analysis

The hypotheses of this study are:

1. In children with acute gastroenteritis, probiotic administration in the emergency department will be associated with a clinically-important decrease in the proportion of children suffering from moderate-severe disease, defined by a validated Modified Vesikari Score ≥ 9, compared to placebo.

2. In children with acute gastroenteritis, probiotic administration will not be associated with serious adverse events, and will have a similar rate of side effects (e.g. bloating, fever) as compared to placebo-treated children.

All analyses will be undertaken by the intention-to-treat (ITT) principle, except for adverse events, which will use the as-treated principle (compare the subjects based on the treatment regimen that they received). Patients who drop out or inadvertently crossover will be followed and included in the ITT analysis. All statistical tests of hypotheses will be two-sided. Baseline characteristics will be analyzed to determine if there is a need to adjust for differences between groups in exploratory analyses. Sensitivity analyses will be performed to assess the possibility and consequences of non-random loss to follow-up. The proportions of children experiencing an unscheduled healthcare visit or any potential adverse effect, as reported by the caregivers, will be compared between groups using the Mantel-Haenszel test, stratified by site. The analysis will evaluate the presence/absence of pre-specified side effects, as an aggregate outcome variable. A per-protocol analysis will be conducted to provide additional insight as non-compliance may result in an underestimation of the benefits of probiotics in the ITT analysis.[72]

5.1 Specific Aim Analyses

Specific Aim 1. Determine the clinical effectiveness of probiotic administration to reduce morbidity in children presenting to the emergency department with acute

gastroenteritis.

The **primary efficacy endpoint** of this study is the presence of moderate-severe acute gastroenteritis, as defined by a total post-enrollment Modified Vesikari Score ≥ 9 during the 2-week follow-up period. Each of the 7 items in the score is tabulated individually (maximum of 20 points); the sum of these individual variables represents the total Modified Vesikari Score. At the time of randomization a baseline Modified Vesikari Score will be assigned based on symptoms prior to randomization. This baseline score will serve as a covariate in a secondary analysis of the primary outcome. The post-enrollment Modified Vesikari Score which will be employed to determine the presence/absence of the primary efficacy outcome, is based only on symptoms that occur between randomization and day 14, the conclusion of the study period for this outcome. Only symptoms and outcomes that occur following randomization will be included in the post-enrollment score.

The post-enrollment score is calculated only once, on day 14. At that time, each of the seven variables will be assigned a score for the entire study period (from randomization to day 14). Each variable will be scored by 1 of 3 methods:

1. Worst 24 hour period - maximal number of episodes of vomiting in a 24 hour period, maximal number of episodes of diarrhea in a 24 hour period, and maximal temperature;
2. Total duration of symptoms, including the number of days on which any gastroenteritis-related symptom occurred;
3. Occurrence of a treatment outcome or unscheduled subsequent healthcare utilization. If the patient was admitted directly following their enrollment ED visit and that admission stay is longer than 48 hours, it will be included as treatment in the post enrollment MVS. If the patient's admission stay was less than 48 hours, it will not be included in any MVS calculation.

The primary efficacy endpoint (the presence of moderate or severe disease, as defined by a total Modified Vesikari Score ≥ 9 during the 2 week follow-up period) will be limited to symptoms and outcomes that occur after randomization. If a patient's symptoms of vomiting and diarrhea stop during the same 24 hours, and then recur, that is not included in the post-enrollment score since that will be considered a *new* illness. In the original score, severe disease was defined as ≥ 11[61, 62, 73–75] and moderate as ≥ 9.[76] In our derivation and validation pilot studies,[12, 13] construct validity was demonstrated and validated by using scores of ≥ 9 to define moderate and ≥ 11 to define severe disease. These cut-points were associated with significant increases in other measures of disease severity such as degree of dehydration, likelihood of admission and daycare ($p = 0.01$)

and parental work absenteeism (p <0.001).[12, 13]

The proportion of children with moderate-to-severe disease (i.e. Modified Vesikari Score ≥ 9), the primary outcome, will be analyzed by comparing proportions utilizing a Mantel-Haenszel test, stratified by participating center and by duration of symptoms at enrollment. Significance for this primary outcome measure will be set at 0.05. Secondary analyses of the primary outcome will use logistic regression methods to adjust for covariates that may be imbalanced between groups, (e.g. age, pre-enrollment Modified Vesikari Score, hydration assessment, need for hospitalization at index visit). We will also analyze the outcome using Modified Vesikari Score as a continuous variable through a stratified Wilcoxon rank-sum test and compare the results with the primary analysis.

Specific Aim 2. Determine the safety and side effect profiles of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) in children presenting to the emergency department with acute gastroenteritis.

The **primary safety endpoint** of this study is the occurrence of invasive disease (including meningitis and bacteremia) from *Lactobacillus rhamnosus*. The secondary safety outcome will be the presence of any adverse events. For more information on adverse events, see Section 8.3 on page 33. Adverse events (including serious) will be tabulated by study arm for DSMB reporting and for final analysis of the safety of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) in this setting. Adverse events will also be coded using the MedDRA vocabulary.

Side Effects: Occurrence of side effects such as weakness, bloating, gas, intestinal rumbling, diarrhea, blood in stool, abdominal pain, abdominal cramps, nausea, vomiting, loss of appetite, heartburn, constipation, skin rash, fever, nasal congestion, sore throat, cough, headache, muscle aches, chills, and diaper rash. These will be tabulated and compared by study arm.

5.2 Secondary Study Outcomes

Secondary outcomes will include three of the individual components of the Modified Vesikari Score considered independently and one additional measure of burden of illness. Specifically, they are:

1. **Diarrhea duration:** Time from randomization until the appearance of the last watery stool as reported during daily surveys.

2. **Vomiting duration:** Time from randomization until the last vomiting episode as reported during daily surveys. Vomiting duration is only evaluated in children who vomited ≥ 3 times during the 24 hours prior to the emergency department visit.

3. **Return visits:** Return visits for unscheduled care to a healthcare provider related to vomiting, diarrhea, dehydration, fever, or fluid refusal, within 2 weeks of the index visit. Scheduled visits (e.g. reassessment, vaccinations, unrelated issues) will not be included.

4. **Missed daycare:** Days of daycare missed by subjects who attend daycare.

5. **Missed work:** Days of work missed by caregivers who work outside of the home.

6. **Household transmission rate:** A household census will be obtained at the time of enrollment, and information about household contacts symptoms will be obtained during daily surveys to determine household transmission rate.

The overall significance level for statistical tests on the secondary outcomes will be set at 0.05. Holm's method will be used to adjust for multiple comparisons.[77] The continuous variables of duration of diarrhea and duration of vomiting (measured in hours and analyzed with a Van Elteren test),[78] will be stratified by clinical center. Similarly, the number of days the child is absent from daycare and the caregiver is absent from work will be analyzed with a Van Elteren test, stratified by clinical center and etiology.

Dichotomous outcomes to be evaluated include emergency department acute gastroenteritis-related revisits, intravenous rehydration, and hospitalization. Additional analyses involving these outcomes will include linear and logistic regression models that adjust for possible effects of baseline characteristics.

5.3 Sample Size Calculations and Statistical Power

The primary analysis will be performed on a binary outcome: development of moderate-to-severe disease. The power of this analysis is based on the proportion of patients with moderate-to-severe disease. Our pilot data[12, 13] indicate that 25% of patients will have moderate to severe disease during the course of their illness. Furthermore expert surveys indicated that an absolute risk reduction of 10% would constitute a minimal clinically-important difference (MCID). Therefore our sample size calculation assumed a 25% event rate in the control group for which we desire to detect an absolute beneficial treatment effect of 10% with 90% power. Using a two-sided type I error (α) of 0.05 and the hypothesized proportions yields a required total sample size of 670 patients.[79] Our expected power, should we find different event rates in our 2 groups, is displayed in Table 2 on the next page.

Based on previous work by our group,[70, 71, 80] we assumed a 10% loss to follow up ($670/0.90 = 744$), 5% drop out, and 3% drop in (caregivers who buy a probiotic agent

Outcome Control	Outcome Intervention	% Difference	Power
0.30	0.21	9%	0.76
0.30	0.20	10%	0.85
0.25	0.15	10%	0.90
0.25	0.16	9%	0.82
0.25	0.17	8%	0.72
0.20	0.10	10%	0.95
0.20	0.12	8%	0.81
0.20	0.13	7%	0.69

Table 2: Power Analysis

to administer to their child) rate $(744/(0.92)^2 = 879)$. Adjustment for O'Brien-Fleming monitoring boundaries requires a further 2% increase. Thus, the total number randomized (final sample size) will be 897.

5.3.1 Enrichment Design

The above study design and power analysis are based on the assumption of homogeneous treatment effect. We may also incorporate an enrichment design[81, 82] to restore the statistical power in case the presence of a subpopulation with a substantially low treatment effect is identified. We are particularly interested in two potential subpopulations: participants with <2 days of symptoms and those with $\geq$ 2 days of symptoms. Based on our pilot data, each subpopulation accounts for approximately 50% of the total population. The decision for enrollment modification will be made at the first interim analysis for efficacy. Specifically, three statistics (based on a normal approximation of binomial distribution, or z-statistics) will be calculated to compare the primary efficacy endpoint between treatment and control groups for subjects in the total population and the two subpopulations, respectively. If the z-statistic from a subpopulation is <0.3 and also smaller than that in the total population, subjects from this subpopulation will no longer be considered in the subsequent enrollment. All subjects, regardless of symptom duration will be included in the final analyses. Our simulation studies have shown that such an enrichment design can increase the power considerably when the treatment effects are different across subpopulations, while it will have little impact on power when the treatment effects are similar.

5.3.2 Sub-group Analyses

The presence of a Modified Vesikari Score ≥ 9 will be analyzed by age <1 year, duration of symptoms, breast-feeding status, antibiotic usage, rotavirus and norovirus positivity. A subgroup effect will be considered significant if the interaction between subgroup and treatment in a logistic regression model is significant at a Bonferroni-corrected level.

5.4 Recruitment Estimates and Attrition

5.4.1 Recruitment

To estimate the number of subjects with acute gastroenteritis of eligible ages that would meet all inclusion criteria and be enrolled annually, we obtained discharge diagnoses from all study sites for 2005-2011. In 2011, the study sites evaluated 14,048 potentially eligible subjects (patients 3-48 months with ICD9 codes for diarrhea) out of 507,039 total emergency department visits (2.8%). In the past 7 years the lowest proportion of such patients per total emergency department volume was 2.3% (2008). Therefore, using 2011 emergency department volumes and lowest proportion of patients with diarrhea our conservative estimate for eligible patients would be 11,150 patients 3-48 months with diarrhea per year (or 30 patients per day network wide). Our best estimate is that 4.5% of children with acute gastroenteritis aged 3-48 months will be enrolled. We plan to enroll 225 patients/year and 970 subjects over 4 acute gastroenteritis seasons.

5.4.2 Potential for Bias

Reporting bias will be minimized by adhering to CONSORT recommendations including the use of third-party assignment.[83] Placebo capsules and active drug will be provided by I-Health Inc. The total weight of all capsules is 325 mg. The probiotic and placebo capsules and powder are identical in appearance, taste, texture, and odor. Participants, families, healthcare providers, data collectors, outcome adjudicators, and data analysts will be blinded as to intervention arm, thereby preventing bias in outcome assessment. Two DCC statisticians will be partially unblinded (with knowledge of group assignments, but not group identities) in order to present interim results to the DSMB. An intention-to-treat analysis will be performed to minimize bias associated with poor compliance and non-random loss of participants.[84] Co-interventions (e.g. antiemetic administration, intravenous rehydration) and other potential sources of confounding will be recorded. Our use of a published validated score as an outcome measure will protect against the introduction of bias in the assessment of treatment effects.[85]

5.4.3 Compliance

Noncompliance with probiotics is rarely reported in previous studies, and not expected to be problematic in this cohort.[86, 87] Participant withdrawal has been related mostly to the primary illness.[19] In the Canadian pilot study, compliance was 91%. We will track compliance by obtaining information from parents during the day 5 follow-up contact. If there are any unused capsules, we will request their return (a pre-stamped, pre-addressed envelope will be provided).

5.4.4 Loss to Follow-up

Our previous emergency department pediatric acute gastroenteritis research studies achieved telephone follow-up rates of 98% on day 3 and 96% on day 7.[70] Similar success has been documented in other PECARN multicenter studies (91%).[80] We estimate a 10% loss to follow-up. If daily contact does not occur, we will collect data from missed days on subsequent days when caregivers are contacted. The use of patient diaries (paper and/or electronic) and chart review will supplement parent contact. Based on our pilot trial we have devised the following strategies to maximize follow-up: all follow-up procedures will be centralized at the lead institution and conducted by experienced study staff; we will obtain multiple phone numbers from caregivers as well as emergency contacts, and we will schedule calls and send reminders prior to the call if preferred. Finally, electronic diary filing and transmission may be available for interested families.

5.4.5 Compensation

Financial compensation may be provided to compensate for parent's time completing follow-up. This compensation must be approved by each site's Institutional Review Board.

6 Data Management

6.1 Clinical Sites

Study data will be recorded on paper work forms or by direct data entry into an electronic data capture (EDC) system. If paper work forms are used they will be retained at the clinical site. The data from the work forms will then be entered into the EDC system provided by the Data Coordinating Center at the University of Utah School of Medicine.

The clinical investigator at each participating site will complete a Form FDA 1572, Statement of Investigator. That individual is responsible for all aspects of study imple-

mentation, including administration of study drug, collection of accurate study data, and correct entry of the data into the EDC. These tasks may be specifically delegated to other individuals at the clinical site, but the clinical investigator is responsible to supervise all aspects of the study, and is responsible to assure that all staff involved in this study are adequately trained to perform the delegated tasks. All local research records will remain in a locked file in a secure room unless being used by research staff, and all computerized information will be maintained on password protected computers.

6.2 PECARN Data Coordinating Center (Utah)

In addition to locally secured, identifiable information, partially identifiable information for all sites will be maintained at the PECARN Data Coordinating Center, located at the University of Utah in Salt Lake City, Utah. The Data Coordinating Center has a state-of-the-art computer infrastructure with a dedicated server room with a fire suppression system, air conditioning, cooling system and separate air filtering. The server facility is locked separately from the remainder of the Data Coordinating Center and access to the building is monitored by security personnel year round. The Data Coordinating Center coordinates its network infrastructure and security with the Health Sciences Campus (HSC) information systems at the University of Utah. This provides the Data Coordinating Center with effective firewall hardware, automatic network intrusion detection, and the expertise of dedicated security experts working at the University.

Network equipment includes four high-speed switches. User authentication is centralized with two Windows 2008 domain servers. Communication over public networks is encrypted with virtual point-to-point sessions using SSL or VPN technologies, both of which provide at least 128 bit encryption. OpenClinica (Web-based clinical studies data management system), eRoom^{TM} (Web-based collaborative workspace) and other web applications use the SSL protocol to transmit data securely over the Internet. Direct access to Data Coordinating Center machines is only available while physically located inside the Data Coordinating Center offices, or via a VPN client. All network traffic is monitored for intrusion attempts, security scans are regularly run against our servers, and IT staff are notified of intrusion alerts.

Production servers running mission critical applications are clustered and configured for failover events. Servers are backed up with encryption through a dedicated backup server that connects across an internal 10 gigabit network to a tape drive. Storage area networking (SAN) applications, clusters, and switch-to-switch links are also on a 10 gigabit network. Incremental backups occur hourly during the week. Incremental backups

also are performed nightly with full system backups occurring every week. Tapes are stored in a fireproof safe inside the data center facility, and full backups are taken to an off-site commercial storage facility. Security is maintained with Windows 2008 user/group domain-level security.

Users are required to change their passwords every 90 days, and workstations time out after 5 minutes of inactivity. All files are protected at group and user levels; database security is handled in a similar manner with group level access to databases, tables, and views in Microsoft SQL Server. All portable computers are whole-disk-encrypted.

6.3 Data Confidentiality

The PI and other research personnel have all completed training and received certification in Human Subjects Research Protection and HIPAA. All project staff hired will also successfully complete this training prior to engaging in any research or treatment with study participants and renew this training as required by their institution.

The investigators and staff of the Data Coordinating Center are fully committed to the security and confidentiality of all data collected for PECARN studies. All Data Coordinating Center personnel at the University of Utah have signed confidentiality agreements concerning all data encountered in the center. Violation of these agreements may result in termination from employment at the University of Utah. In addition, all personnel involved with data coordinating center data systems have received Human Subjects Protection and HIPAA education.

The staff, reviewers and investigators involved with this study will be required to sign agreements from the Data Coordinating Center that relate to maintenance of passwords, information system security, and data confidentiality.

6.4 Data Quality Management and Monitoring

The Data Coordinating Center monitors PECARN studies on behalf of the investigators and the funding agency. The purposes of monitoring include demonstration of adherence to human subjects protection requirements and assurance of high quality study data. Monitoring is usually done remotely and may also involve physical site monitoring visits. Site monitoring is described in more detail in Section 9.2.

6.5 Record Access

The medical record and study files (including informed consent, permission, and assent documents) must be made available to authorized representatives of the Data Coordinating Center, upon request, for source verification of study documentation. In addition, medical information and data generated by this study must be available for inspection upon request by representatives of the the National Institutes of Health, Food and Drug Administration, and the Institutional Review Board (IRB) for each study site.

7 Protection of Human Subjects

7.1 Institutional Review Board (IRB) Approval

The Data Coordinating Center and each clinical center must obtain approval from their respective IRB prior to participating in the study. The Data Coordinating Center will track IRB approval status at all participating centers and will not permit subject enrollment without documentation of initial IRB approval and maintenance of that approval throughout subsequent years of the project.

7.2 Informed Consent

This protocol requires that parents or other legally empowered guardians sign a parental permission form. The parent or legal guardian will be informed about the objectives of the study and the potential risks. Parents or legal guardians of eligible children with symptoms of gastroenteritis will be approached to provide permission for their child's participation in the study. Parental or guardian permission will be obtained prior to initiation of study activities. Documentation of parental permission will be maintained at the study site. As the maximum eligible age is 48 months, child assent is not applicable.

7.3 Potential Risks

Lactobacilli are ubiquitous in the human diet and are a large part of the over 1 trillion live bacteria that reside in the gastrointestinal tract of healthy individuals. Overall, *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) is well tolerated in the pediatric patient population. Infections (bacteremia, endocarditis, pneumonia, deep abdominal abscesses) have been reported in sick neonates, severely debilitated and immune-compromised individuals given probiotics. The use of probiotics in these individuals continues to be controversial; however prospective studies have been conducted in adults and children

with HIV as well as preterm neonates, with no reported systemic infections. Finally, there are 7 case reports of invasive disease after administration of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG). These occurred in patients in intensive care units, in patients with central venous catheters, or in patients who are on immunosuppressive therapy, have short gut syndrome, or are at risk for endocarditis. These risk factors are exclusion criteria from our study population.

The clinical risks of invasive infection from *Lactobacillus rhamnosus* are related to receiving *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG). There are risks associated with acute gastroenteritis that are not related to study participation, including dehydration, electrolyte abnormalities, systemic infection and co-infection, and hospital admission. All subjects will be clinically treated for acute gastroenteritis in accordance with local site treatment protocols. There may be other unknown risks of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG) in children with acute gastroenteritis.

Loss of confidentially is always a risk in a study, but safeguards are in place to protect against this.

7.4 Protections Against Potential Risks

Several steps will be taken to minimize the risks of participation in the study. All of the participating clinical centers are tertiary pediatric hospitals with highly trained pediatric staff. Subjects will be closely followed after discharge. Families will be contacted daily until both the diarrhea and vomiting have resolved and the treatment has been completed. Additional contact will be made on day 14, and months 1, 3, 6, 9 and 12 for long term safety outcomes. Parents or legally authorized representatives will also receive specific instructions as to when to see a health care provider. If an adverse event is reported at the time of follow-up, research personnel will refer subjects for appropriate medical care, when applicable.

Loss of confidentiality will be mitigated by the use of the PECARN Data Coordinating Center which has a highly secure IT infrastructure, and by the existence of trained research staff at participating sites. Data security is described in Section 6.

7.5 Potential Benefits

Subjects participating in the study may benefit directly by experiencing less severe disease, shorter duration of symptoms, and decreased need for further health care utilization.

There may also be reduced symptoms (i.e. diarrhea, vomiting, fever) and morbidity, thereby diminishing acute gastroenteritis-associated resource consumption (e.g. physician visits, hospitalization) and societal costs. Additional benefits in the study include closer monitoring of symptoms and potentially earlier recognition of any worsening of acute gastroenteritis due to the frequency of telephone follow up and communication with the family. The knowledge gained in this study may lead to improved treatment options for gastroenteritis for other children and lead to new therapeutic options for future patients. Subjects and their families may therefore benefit indirectly by participating in research with the potential to provide subsequent benefit to others. Finally, future analysis of bulk stool specimens will enable a better understanding of potential mechanisms of action of *Lactobacillus rhamnosus* GG, ATCC 53103 (LGG), which will likely provide benefit to future patients.

8 Data and Safety Monitoring Plan

8.1 Data Safety Monitoring Board (DSMB)

This study will have a Data Safety Monitoring Board (DSMB) appointed in accordance with instructions from the NICHD program officer. The DSMB will have a charter, will approve the protocol prior to implementation, and will review interim analyses for safety and efficacy.

The purpose of the DSMB is to advise the Federal funding agency (NICHD), the study Principal Investigator (Dr. Schnadower), and the PECARN Steering Committee regarding the continuing safety of study subjects and the continuing validity and scientific merit of the study. The DSMB is responsible for monitoring accrual of study subjects, adherence to the study protocol, assessments of data quality, performance of individual clinical sites, review of serious adverse events and other subject safety issues, and review of formal interim statistical analyses of treatment efficacy.

The Data Coordinating Center will send reports relating to these topics to DSMB members prior to each DSMB meeting. The Data Coordinating Center will staff DSMB meetings. The production and approval of DSMB minutes will be done in accordance with requirements of the NICHD. Each DSMB meeting will have a summary recommendation that will be provided to each participating clinical site for submission to the local Institutional Review Board (IRB). More detailed information from the DSMB meetings will not be routinely provided for local IRB submission.

8.2 Frequency of Interim Analysis:

The Data Safety Monitoring Board (DSMB) will meet after 80 patients (safety end points for 1 month), approximately 350 patients (end of 2nd year of enrollment) and 620 patients (end of 3rd year of enrollment) to review enrollment, study procedures, loss to follow-up, drop-in rate, and interim safety and efficacy results. The analyses will test the hypothesis that the probability of developing moderate-to-severe acute gastroenteritis in the probiotic arm is equal to that in the placebo arm. An analysis will also be conducted after 20 subjects under 6 months of age have been enrolled. The DSMB is to review interim data and make recommendations regarding continuation or modification of the study based on safety in this age group. Conservative O'Brien-Fleming monitoring boundaries, implemented using the Lan-DeMets α-spending function approach, will be used as guidelines for early stopping for efficacy.

8.3 Adverse Event Reporting

8.3.1 Definitions, Relatedness, Severity and Expectedness

Definition: An adverse event (AE) is any untoward medical occurrence experienced by a subject. An event constitutes a disease, a set of related signs or symptoms, or a single sign or symptom. On each study day, the site investigators will evaluate adverse events. Study staff will obtain information on symptoms and adverse events on scheduled follow up calls. Adverse events not previously documented in the study will be recorded on the adverse event record form. The nature of each experience, date and time (where appropriate) of onset, outcome, course, and relationship to treatment should be established.

Side Effects: Occurrence of side effects such as weakness, bloating, gas, intestinal rumbling, diarrhea, blood in stool, abdominal pain, abdominal cramps, nausea, vomiting, loss of appetite, heartburn, constipation, skin rash, fever, nasal congestion, sore throat, cough, headache, muscle aches, chills, and diaper rash. Study staff will obtain information about side effects on scheduled follow up contacts.

Relatedness: The suspected relationship between study interventions and any adverse event will be determined by the site investigator using the following criteria. *Relatedness may **not** be assessed by a research coordinator, and must be assessed by an investigator.*

Not Related: The event is clearly related to other factors, such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Possibly Related: The event follows compatible temporal sequence from the time of beginning the assigned study intervention, but could have been produced by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Probably Related: The event follows a reasonable temporal sequence from the time of beginning the assigned study intervention, and *cannot be reasonably explained* by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Some toxicities will be difficult to distinguish from abdominal symptoms related to acute gastroenteritis (such as bloating, abdominal pain, diarrhea, fever and diaper rash), and only at the time of analysis will we be able to determine whether these signs and symptoms are different between the groups.

Seriousness: The severity of clinical adverse events and laboratory abnormalities will be recorded by the site investigator and categorized. A serious adverse event (SAE) is an adverse event that:

- results in death; or
- is life-threatening (the patient was, in the view of the site investigator, in immediate danger of death from the event as it occurred); or
- requires inpatient hospitalization or prolongs an existing hospitalization; or
- results in persistent or significant disability or incapacity; or
- results in congenital anomaly/birth defect; or
- any other event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Expectedness of the Event: All adverse events, including serious adverse events, will be evaluated as to whether their occurrence was expected or unexpected. An adverse event is considered expected if it is known to be associated with acute gastroenteritis (acute gastroenteritis), other underlying medical conditions of the subject, is directly related to study outcome, or is otherwise mentioned in the protocol, informed consent, investigator brochure, or other study documents. Expected complications of acute gastroenteritis include abnormal taste, malaise, abdominal distention, foul smelling stools, dehydration, electrolyte and other laboratory abnormalities, other symptoms associated with viral syndromes, systemic infection and co-infection, seizures, and hospital admission.

Treatment or Action Taken: For each adverse event, the site investigator will record whether an intervention was required:

- Intervention: Surgery or procedure
- Other Treatment: Medication initiation, change, or discontinuation
- None: No action taken

Outcome of Event: Finally, the site investigator will record the clinical outcome of each adverse event as follows:

- Death
- Recovered and the patient returned to baseline status
- Recovered with permanent sequelae
- Symptoms continue

8.3.2 Time Period for Adverse Events

For purposes of this study, adverse events occur following randomization through 30 days after the last study drug dose will be recorded. Serious adverse events, unexpected medically attended events, and new onset chronic illnesses will be recorded from randomization through twelve months after the last study dose. Specifically, events that occur following parental permission to participate in the study, but prior to actual randomization, are *not* adverse events. These should be recorded as baseline conditions.

8.3.3 Data Collection Procedures for Adverse Events

After patient randomization, all adverse events (including serious adverse events), whether anticipated or unanticipated, will be recorded according to the date of first occurrence, severity, and their duration, as well as any treatment prescribed. Any medical condition present at the time of randomization, recorded in the patient's baseline history at study entry, which remains unchanged or improves, will not be recorded as an adverse event at subsequent evaluations. However, worsening of a medical condition that was present at the time of randomization will be considered a new adverse event and reported.

Abnormal laboratory values that are clinically significant will be recorded as adverse events and the site investigator will assess the severity and relationship to the study. Laboratory values that are abnormal at the time of randomization and that do not worsen

will not be recorded as adverse events.

Adverse events will be coded using the MedDRA coding vocabulary. Coding will be done centrally at the Data Coordinating Center because this requires specific training.

8.3.4 Unanticipated Problems (UP)

Unanticipated problems (UP) are defined as incidents, experiences, or outcomes that are unexpected, related to participation in the study, and suggest that the research places subjects at a greater risk of harm than was previously known or recognized. The site investigator will report unanticipated problems to the Data Coordinating Center within 24 hours. A detailed completed report will be required to be sent to the Data Coordinating Center within 3 working days of the event. After receipt of the complete report, the Data Coordinating Center will report these unanticipated problems to the NICHD Program Official or Project Officer in an expedited manner (within 24 hours). In accordance with local IRB requirements, the site investigator may be required to report such unanticipated problems to the IRB in addition to notifying the Data Coordinating Center. In the event that the medical monitor believes that such an event warrants emergent suspension of enrollment in the trial, and NICHD staff cannot be reached expeditiously, the Data Coordinating Center will notify the study investigator (Dr.Schnadower) and all site investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the NICHD staff after discussion with the DSMB.

8.3.5 Monitoring Serious Adverse Events

The Principal Investigator of the Data Coordinating Center (Dr. Dean) will act as the medical monitor for this study. If Dr. Dean is unavailable, a qualified physician will be designated to fulfill this function. Site investigators and/or research coordinators will report serious adverse events to the Data Coordinating Center within 24 hours. A detailed completed report will be required to be sent to the Data Coordinating Center within 3 working days of the event, and the medical monitor will assess all serious adverse events reported from site investigators.

For each of these serious adverse events, the site investigator will provide sufficient medical history and clinical details for a safety assessment to be made with regard to continuation of the trial. The medical monitor will sign each SAE report after review. All SAE reports will be retained at the Data Coordinating Center, and all SAE reports will be available for review by DSMB members and NICHD staff.

In the unlikely event that the medical monitor believes an unexpected and study-related SAE warrants emergent cessation of enrollment in the trial, NICHD staff and the

DSMB chairperson will be immediately consulted. If these individuals concur with the judgment of the medical monitor, or if the NICHD staff and the DSMB chairperson cannot be reached expeditiously, the Data Coordinating Center will notify the study investigator (Dr.Schnadower) and all site investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the NICHD staff after discussion with the DSMB.

In accordance with local IRB requirements, the site investigator may be required to report such events to the IRB in addition to notifying the Data Coordinating Center.

After notification of the NICHD Program Official or Project Officer, and the DSMB chairperson, of *serious, unexpected, and study-related* adverse events or unanticipated problems (UP), decisions will be made whether to continue the study without change, and whether to convene the entire DSMB for an emergent meeting. If a decision is made to suspend enrollment in the trial, this will be reported to the study investigator (Dr. Schnadower) and all clinical investigators, who will be instructed to report this to their local IRB.

The DSMB will review all adverse events (not necessarily serious, unexpected, and study-related) during scheduled DSMB meetings. The Data Coordinating Center will prepare a Summary Report of Adverse Events for the DSMB meetings, classified with the MedDRA coding system.

8.3.6 Follow-up of Serious, Unexpected and Related Adverse Events

All serious, unexpected and related adverse events, that are unresolved at the time of the patient's termination from the study or discharge from the hospital, will be followed by the Clinical Center investigators until the events are resolved, subject is lost to follow-up, the adverse event is otherwise explained or has stabilized, or 12 months have passed from the time of last study dose.

9 Study Training and Monitoring

9.1 Study Training

A formal training program for investigators and research staff will be held prior to the start of enrollment. The training program will cover regulatory topics and Good Clinical Practice. The training will also provide in depth explanations regarding study procedures, clinical care, adverse event reporting, data entry procedures, quality assurance, site monitoring, and the informed consent process. A manual of operations will be provided to each Clinical Center investigator prior to the start of enrollment. The manual will

detail specific information about the study procedures, regulatory information, safety reporting, and other necessary information. Updates and revisions to the manual will be made available electronically. The Data Coordinating Center, in collaboration with the study investigator (Dr. Schnadower), will be the main contact for study questions.

9.2 Study Monitoring

The investigators recognize the importance of ensuring data of excellent quality. Site monitoring is critical to this process. Site monitoring has been a very effective tool for maintaining data quality in previous PECARN studies, and we will utilize this process to ensure excellent quality data in the proposed study. Our site monitoring plan is designed to identify problems with sites and methods for handling problems that arise. Site monitors must be provided with full access to study materials and the medical records for study subjects. If the medical records are in electronic form, the clinical investigator or an authorized individual must provide any assistance necessary to facilitate the site monitor's review of data in the electronic medical record.

9.2.1 Site Monitoring Plan

A supplemental study-specific monitoring plan, separate from the protocol will be completed which outlines specific criteria for monitoring. This plan will include the number of planned site visits, criteria for focused visits, or additional visits, a plan for chart review and a follow up plan for non-compliant sites. The monitoring plan also describes the type of monitoring that will take place (e.g. sample of all subjects within a site; key data or all data), the schedule of visits, how they are reported and a time frame to resolve any issues found. Remote site monitoring schedules will be determined by the Data Coordinating Center in coordination with the study principal investigator.

9.2.2 Clinical Site Monitoring

Site monitoring visits will be performed by a trained site monitor during the study period to ensure regulatory compliance, patient safety, and to monitor the quality of data collected. Essential document binders, regulatory documents and data collection forms may be reviewed. Interim visits will take place depending on grant budget, site enrollment, and compliance issues identified. The site monitor will provide each site with a written report, and sites will be required to follow up on any deficiencies. It is anticipated that the study monitoring visits for this protocol will consist of a site initiation visit (prior to patient enrollment), interim visits, and a close out visit. The site initiation may take place as group training made up of site investigators and research assistants.

9.2.3 Remote Monitoring

The Data Coordinating Center may supplement on-site monitoring with remote monitoring activities. Remote monitoring involves detailed review of the data entered by the Clinical Center and consultations with the Clinical Center investigator and/or research coordinator to review safety and data quality. This may require uploading de-identified copies of specific parts of the medical record, patient study file, regulatory documentation, or other source documents to the Data Coordinating Center staff, who review those materials against the data recorded in the electronic data capture system. This helps assure protocol compliance and accurate data collection. The Data Coordinating Center may conduct more remote monitoring activities early in the trial to assure protocol compliance and identify any training issues that may exist. Remote monitoring the documents will be retained in accordance with federal requirements. Safety of subjects will be monitored and ensured in accordance with the Data and Safety Monitoring Board (DSMB) plan.

9.2.4 Pharmacy Monitoring

The Clinical Center pharmacy must maintain adequate records of all dispensed study drug. Each pharmacy may be monitored, either in person or remotely, and may be requested to send copies of these documents to the Data Coordinating Center. Since this study will use a central pharmacy, that pharmacy must also maintain adequate records and may also be monitored.

10 Regulatory Issues

10.1 Food and Drug Administration

This trial is being conducted under an Investigational New Drug application approved by the Food and Drug Administration (Investigational New Drug application #15371). The clinical investigator at each participating site will complete a Form FDA 1572, "Statement of Investigator."

10.2 Health Insurance Portability and Accountability Act

The abstracted data will include limited identifiers as defined by the Health Insurance Portability and Accountability Act, and specific contact information will be provided to research staff conducting follow up with parents. Abstracted data will be retained and archived at the Data Coordinating Center in accordance with record retention requires of the Food and Drug Administration and the NIH. Contact information will not be

provided to the Data Coordinating Center (it will be provided directly to the central follow up research staff). For data analysis outside the Data Coordinating Center (e.g., when a public access database is made available), the Data Coordinating Center will create a completely de-identified analytical database for use by the study investigators, and for final archiving. All study sites have been or will be offered Business Associate Agreements with the University of Utah. Copies of signed Business Associate Agreements are maintained at the Data Coordinating Center.

10.3 Inclusion of Women and Minorities

The gender, ethnic and racial composition of patients enrolled in all PECARN studies is a function of the underlying referral population at each PECARN site participating in this trial. There will be no exclusion of patients based on gender, race, or ethnicity.

10.4 ClinicalTrials.gov Requirements

This trial has been registered at ClinicalTrials.gov (NCT #01773967). The title is "Impact of Emergency Department Probiotic (LGG) Treatment of Pediatric Gastroenteritis."

10.5 Retention of Records

For federally funded studies subject to the Common Rule, records relating to the research conducted shall be retained for at least 3 years after completion of the research. Completion of the research for this protocol should be anticipated to include planned primary and secondary analyses, as well as subsequent derivative analyses. Completion of the research also entails completion of all publications relating to the research. All records shall be accessible for inspection and copying by authorized representatives of the regulatory authorities at reasonable times and in a reasonable manner [45 CFR §46.115(b)].

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Lactobacillus Rhamnosus GG vs. Placebo for Pediatric Acute Gastroenteritis

Summary of Protocol Changes (from version 1.0- January 2014- to V2.0 – April 2014- to V 3.0 – February 2017)

A) Changes made in April 2014 (V1.0 to V2.0)

1. Added and clarified exclusion criteria (history of abdominal surgery, oral or intravenous steroid use in the past 6 months, under 6 months and premature (page 11)
2. Clarification of adverse events section separating adverse from side effects (page 10 and 42) and added list of side effects (pages 26 and 39)
3. Added question about whether non-randomized eligible patients were prescribed probiotics (page 16)
4. Clarification of product preparation and administration (pages 19-20)
5. Clarification of wording related to PI and Medical Monitor at the Data Coordinating Center (page 21)
6. Clarification regarding stool sample collection (page 22)
7. Clarified how compliance questions should be asked (page 31)
8. Clarification of data collection methods to include direct data capture (page 32)
9. Clarification of wording related to pharmacy monitoring (page 47)

B) Changes made in February 2017 (V2.0 to V3.0)

1. Increased sample size from 900 to 970 following discovery that up to 36 patients were potentially exposed to lower probiotic dosing (pages 8, 11 and 26)

Statistical Analysis Plan

Protocol Title (Number): Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis (Probiotics Study) (PECARN Protocol # 032)

Protocol Version and Date: 1.00; January 24, 2014

SAP Author: T. Charles Casper, Ph.D.

SAP Version and Date: 1.0; January 16, 2015

Changes from last version:

SAP Version 1 Date: January 16, 2015

CONFIDENTIAL

Approvals:

Approved By:

Name	Title	Date

Name	Title	Date

Contents

Abbreviations

Abbreviation	Definition
DCC	Data Coordinating Center
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
ITT	Intent-To-Treat
MCID	Minimal Clinically-Important Difference
MVS	Modified Vesikari Score
PECARN	Pediatric Emergency Care Applied Research Network
PP	Per-Protocol
(S)AE	(Serious) Adverse Event
SAFETY	Safety Population
SAP	Statistical Analysis Plan

1 Preface

1.1 Purpose of SAP

This Statistical Analysis Plan (SAP) describes the planned analysis and reporting for the PECARN Protocol: Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis (Probiotics Study).

The purpose of this study is to determine if probiotic administration reduces the severity of acute gastroenteritis episodes in children aged 3 to 48 months. The study is a randomized, placebo-controlled trial with a parallel-group design.

The structure and content of this SAP provides sufficient detail to meet the requirements and standards set by the PECARN Data Coordinating Center (DCC). All work planned and reported for this SAP will follow guidelines for statistical practice published by the American Statistical Association [2].

1.2 Auxiliary/Other Documents

The following documents were reviewed in preparation of this SAP:

- PECARN Protocol: Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis (Probiotics Study).
- Case Report Forms (CRFs) for the Probiotics protocol

The reader of this SAP is encouraged to read the protocol for details on the conduct of this study, and the operational aspects of clinical assessments.

The purpose of this SAP is to outline the planned analyses to be completed for the Probiotics trial. The planned analyses identified in this SAP will be included in future study abstracts and manuscripts. Also, exploratory analyses not necessarily identified in this SAP may be performed. Any post hoc, or unplanned, analyses not explicitly identified in this SAP will be clearly identified as such in any published reports from this study.

It is possible that, due to updates or identification of errors in specific statistical software discussed in this SAP, the exact technical specifications for carrying out a given analysis may be modified. This is considered acceptable as long as the original, prespecified statistical analysis approach is completely followed in the revised technical specifications.

2 Study Objectives and Outcomes

2.1 Study Objectives

2.1.1 Primary Objectives

The primary objectives of the Probiotics trial are:

1. to test the hypothesis that, in children with acute gastroenteritis, probiotic administration in the emergency department will decrease the likelihood of moderate-severe disease, defined by a validated Modified Vesikari Score (MVS) $\geq$9, compared to placebo.

2. to test the hypothesis that, in children with acute gastroenteritis, probiotic administration will not be associated with serious adverse events, and will have a similar rate of side effects (e.g., bloating, fever, abdominal distention) as compared to placebo-treated children.

2.2 Study Outcomes

2.2.1 Primary Outcome

The primary outcome is the presence of moderate-severe acute gastroenteritis, as defined by a total post-enrollment Modified Vesikari Score (MVS) $\geq$9 during the 2-week follow-up period.

2.2.2 Safety Outcomes

Safety outcomes are:

1. The occurrence of invasive disease (including meningitis and bacteremia) from Lactobacillus rhamnosus.

2. Occurrence of side effects: bloating, gas, intestinal rumbling, diarrhea, blood in stool, abdominal pain, abdominal cramps, nausea, vomiting, loss of appetite, heartburn, constipation, skin rash, diaper rash, fever, nasal congestion, runny nose, sore throat, cough, headache, muscle aches, chills, weakness.

2.2.3 Secondary Outcomes

Secondary outcomes are:

1. Time to last watery stool as reported during daily surveys.

2. Time to last vomiting episode. Vomiting duration is only evaluated in children who vomited $\geq$3 times during the 24 hours prior to the emergency department visit.

3. Return visits for unscheduled care to a healthcare provider related to vomiting, diarrhea, dehydration, fever, or fluid refusal, within 2 weeks of the index visit.

4. Days of daycare missed by subjects who attend daycare.

5. Days of work missed by caregivers who work outside of the home.

6. Household transmission rate.

In addition to the primary analysis, MVS will be analyzed in secondary analyses for confirmatory purposes as:

1. a numeric variable (i.e., actual MVS, rather than dichotomized)

2.2.4 Tertiary Outcomes

Tertiary outcomes are:

1. Emergency department acute gastroenteritis-related visits
2. Intravenous rehydration
3. Hospitalization
4. Total number of diarrheal stools
5. Total number of vomiting episodes
6. Adherence to oral medication (defined as $\geq$ 7 out of 10 doses)

In the event that efficacy of the probiotic is demonstrated, cost will also be analyzed (cost-effectiveness).

2.3 Covariates

Although randomization should result in approximate balance between treatment groups with respect to critical baseline variables affecting the outcomes, additional exploratory analyses will be conducted to assess the effects of treatment after adjusting for baseline covariates in models for each outcome. The covariates considered are:

- baseline MVS (actual score),
- duration of symptoms prior to enrollment (<48 hours vs. 48 hours or more),
- age,
- gender,
- clinical center,
- breast-feeding status,
- antibiotic useage during the 14 days prior to ED visit,

- infective agent at baseline (e.g., norovirus, rotavirus),
- hydration assessment (Gorelick dehydration score),
- need for hospitalization from initial ED visit,

3 Study Design and Methods

3.1 Overall Study Design

The Probiotics trial has a parallel group design with a placebo control. We will not describe specifics about the active agent or placebo here, as these are contained in the protocol. We will refer to the two trial arms as Active and Placebo. Study participants will be randomized to Active or Placebo, each having equal allocation. Treatment with the assigned therapy is to commence immediately following randomization. The primary analysis will be performed on an intention-to-treat basis. We will also use an enrichment design, possibly modifying the eligibility criteria of the trial based on interim results.

3.2 Randomization and Blinding

3.2.1 Method of Treatment Assignment

Randomization will be balanced among the study arms. Randomization will be stratified by clinical center and by duration of symptoms. Permuted blocks of lengths 2, 4, and 6 will be used for randomization. This trial will use a web-based system for randomization provided by randomize.net. Randomization/kit numbers will be provided in a scrambled order and separated into arms (A and B).

Delivery of Randomization Randomizations will be delivered to the clinical centers using a web-based system administered by randomize.net. This system will use each enrolled patient's clinical center and duration of symptoms (<48 hours vs. 48 hours or more) to deliver the next assigned treatment.

In this trial, there will be no "emergency backup" randomization, as we expect to be able to capture a sufficient number of patients.

3.2.2 Blinding

The Probiotics trial will be performed in a double-blind fashion. All study personnel, including investigators and research coordinators, shall be blinded to assigned treatment arm for each enrolled subject. Of necessity, biostatisticians involved in presenting interim analyses

to the DSMB will be aware which subjects have received "Treatment A" and which have received "Treatment B", but they will not be aware of the identity of the two arms.

Unblinded personnel in this study will include: the central research pharmacist and, possibly, a pharmacy monitor contracted by the DCC expressly for the purpose of ensuring assigned treatments have been correctly prepared and delivered in this study. The DSMB may request to be unblinded to treatment assignment at any time.

3.3 Sample Size and Power Determination

The primary outcome is binary: whether total post-enrollment Modified Vesikari Score (MVS) is $\geq$9 during the 2-week follow-up period. The difference between arms will be tested at a 0.05 level. The null and alternative hypotheses are, respectively,

$$H_0 : p_A = p_P \qquad \text{and} \qquad H_1 : p_A \neq p_P,$$

where p_A and p_P are, respectively, the true probabilities of MVS$\geq$9 on the Active drug and on Placebo.

Our pilot data indicate that 25% of patients will have moderate to severe disease (MVS$\geq$9) during the course of their illness. Furthermore, expert surveys indicated that an absolute risk reduction of 10% would constitute a minimal clinically-important difference (MCID). Therefore, our sample size calculation assumed a 25% event rate in the control group for which we desire to detect an absolute beneficial treatment effect of 10% with 90% power (0.1 Type II error rate). Using a two-sided type I error (α) of 0.05 and the hypothesized proportions yields a required total sample size of 670 patients. Our expected power, should we find different event rates in our 2 groups, is displayed in the following table.

Placebo	Active	% Difference	Power
0.30	0.21	9%	0.76
0.30	0.20	10%	0.85
0.25	0.15	10%	0.90
0.25	0.16	9%	0.82
0.25	0.17	8%	0.72
0.20	0.10	10%	0.95
0.20	0.12	8%	0.81
0.20	0.13	7%	0.69

Based on previous work by our group, we assumed 10% loss to follow-up ($670/0.90 = 744$), 5% drop-out, and 3% drop-in (caregivers who buy a probiotic agent to administer to their child) rate ($744/(0.92)^2 = 879$). Adjustment for O'Brien-Fleming monitoring boundaries requires a further 2% increase. Thus, the total number randomized (final target sample size) will be 900.

4 Study Subjects and Analysis Populations

4.1 Analysis Populations

4.1.1 Screening Population

The screening population (SCREEN) includes all patients who are screened for eligibility into the trial, regardless of randomization into the trial or treatment status. This population represents all patients who meet inclusion criteria outlined in the study protocol and who are screened in real-time by study staff at the site. This population will be used for reporting of study flow per CONSORT guidelines.

4.1.2 Intention-to-Treat Population

The Intention-to-Treat (ITT) population includes all subjects who are randomized into the trial, regardless of adherence to the protocol, including, for example, subjects who receive no study drug. The ITT population will be used for the primary efficacy analyses in the study, as well as for main efficacy analyses of secondary and tertiary outcomes. All analyses using the ITT population will be based on each subject's assigned treatment arm, regardless of treatment actually received.

4.1.3 Per-Protocol Efficacy Population

The Per-Protocol (PP) efficacy population includes all subjects in the ITT population who are verified to meet all study inclusion and exclusion criteria, who receive study drug according to their assigned study arm (defined as $\geq$ 7 out of 10 doses). This population will be used to examine whether results seen in the ITT population are maintained in the population adhering to the protocol.

4.1.4 Safety Population

The safety population (SAFETY) includes all subjects who receive any study drug. Reporting of results based on this population will be summarized according to treatment received. This population will be used for analysis of adverse events and (in addition to ITT) to examine safety outcomes.

4.2 Study Subjects

4.2.1 Inclusion and Exclusion Criteria

To be included in the study, patients:

- are between 3 and 48 months of age; AND

- have had 3 or more watery stools within 24 hours prior to screening; AND
- have had vomiting or diarrhea less than 7 days; AND
- have symptoms consistent with acute intestinal infectious process.

The following patients will be excluded from the study:

- patients with an indwelling vascular access line; OR
- patients with structural heart disease excluding non-pathological heart murmurs; OR
- patients receiving immunosuppressive therapy or having history of immunodeficiency; OR
- patients with hematochezia in the preceding 48 hours; OR
- patients with chronic gastrointestinal problems (e.g., short gut syndrome, inflammatory bowel disease); OR
- patients with known pancreatitis; OR
- critically ill patients; OR
- patients having a family member with an indwelling vascular access line, or on immunosuppressive therapy, or with a known immunodeficiency; OR
- patients with bilious emesis; OR
- patients who have used probiotics (supplement) in the preceding 2 weeks; OR
- patients previously enrolled in this trial; OR
- patients who are allergic to lactobacillus or Microcrystalline Cellulose (MCC); OR
- patients who are allergic to erythromycin, clindamycin, AND β-lactam antibiotics (all); OR
- patients who will not be available for daily follow-up while symptomatic; OR
- patients whose parent/guardian do not speak English or Spanish; OR
- patients under 6 months of age AND premature (<37 weeks).

4.3 Enrichment Design

The study design and power analysis described are based on the assumption of homogeneous treatment effect. We may also incorporate an enrichment design to restore the statistical power in case the presence of a subpopulation with a substantially low treatment effect is identified [1]. We are particularly interested in two potential subpopulations: participants with <48 hours of symptoms prior to presentation and those with 48 hours or more of symptoms. Based on pilot data, each subpopulation accounts for approximately 50% of the total population. The decision for enrollment modification will be made at the first interim analysis for efficacy. Specifically, three Mantel-Haenszel z-statistics will be calculated to compare the primary efficacy outcome between Active and Placebo groups for subjects in the total population and the two subpopulations, respectively. Denote these z-statistics as $Z_{0,1}$, $Z_{<48,1}$, and $Z_{>48,1}$. Note that a positive z-statistic sign will be used to indicate superiority of probiotic relative to placebo.

First, $Z_{0,1}$ will be compared to the O'Brien-Fleming boundary to determine whether the trial should be discontinued for efficacy (as described in Section 6.2). If the boundary is not crossed, or if the DSMB will otherwise recommend continuation of the trial, all three z-statistics will be considered. If $Z_{0,1}$ is larger than both $Z_{<48,1}$ and $Z_{>48,1}$, or if both $Z_{<48,1}$ and $Z_{>48,1}$ are >0.3, accrual will continue as before. Otherwise, future patients will be recruited only from the subpopulation with the larger z-statistic. All subjects, regardless of symptom duration will be included in the final analyses. At the second interim analysis and the final analysis (or any additional unplanned interim analysis), the following method will be used to test for efficacy.

Let Z_2 be the Mantel-Haenszel z-statistic that is based only on subjects enrolled since the first interim analysis. Then, the final test statistic to be used is a weighted sum of the statistics from each stage:

$$Z = \left(\frac{n_1}{n_1 + n_2}\right) Z_{0,1} + \left(\frac{n_2}{n_1 + n_2}\right) Z_2,$$

where n_1 and n_2 are, respectively, the number of subects enrolled prior to and following the first interim analysis. Note that, if no enrichment is performed (i.e., accrual from the full population), Z is asymptotically equivalent to the usual z-statistic ignoring the stages. This final Z value will be compared to the quantiles of the standard normal distribution corresponding to the interim or final boundaries described in Section 6.2. A significant result will indicate efficacy in the population that was enrolled following the first interim analysis.

Simulations were performed to evaluate the performance of this design. Additional assumptions/considerations:

- The (untreated) outcome rate in the subpopulation with greater than 2 days of symptoms is 20%, while it is 30% in the other subpopulation.

- Each subpopulation accounts for 50% of the overall population.
- The normal approximation to the binomial distribution was used, rather than a chi-square or Mantel-Haenszel test.
- The interim analyses occur at 35% and 70% of target patient enrollment.

Several different treatment effect scenarios were simulated. The results show that the enrichment design will increase power when the treatment effect differs by subpopulation, but will have little impact on power if the treatment effect is the same in both subpopulations. One slight disadvantage is that, depending on outcome rates, the enrichment design will only accrue a subpopulation after the first interim analysis with probability 0.05–0.35 when, in fact, there is a homogeneous treatment effect.

5 General Analysis Issues

5.1 Analysis Software

Analysis will be performed using SAS® Software version 9.3 or later whenever possible. Other software packages, including R and StatXact®, may be used for particular specialized procedures.

5.2 Methods for Withdrawals, Missing Data, and Outliers

Per the intention-to-treat principle, subjects who withdraw from the study or are lost to follow-up will have all available data used in the analysis. In the event that a substantial number of subjects are withdrawn or lost to follow-up, baseline characteristics and available information on hospital course will be reviewed and compared to subjects not withdrawn or lost, to assess empirically if these subjects differ from those remaining in the study for the scheduled treatment and follow-up time.

Data from the follow-up surveys will be collected in such a way that data for a particular day should be complete. Information may be missing, however, if an entire day is not completed. For cases where the information needed to derive the primary outcome is incomplete, multiple imputation methods will be used. This is described in more detail in Section 7.2.

Outliers will be reviewed for validity. Outliers that are valid, for example, high number of vomiting episodes, will be included in all primary reports from this trial.

5.3 Multicenter Studies

The randomization sequences will be stratified by clinical center, to assure approximate balance of sites between study arms at all times. The primary analysis, and other analyses

will be stratified by clinical center in order to account for possible baseline differences between centers.

5.4 Multiple Comparisons

The secondary outcomes will be subject to adjustment of significance level for multiple comparisons. Diarrhea duration, vomiting duration, return visits, missed daycare, missed work, and household transmission rate will be subject to Holm's stepdown procedure. Specifically, the smallest of the six p-values will be compared to a significance level of 0.05/6. If significance is reached, the next-smallest p-value will be compared to 0.05/5, and so on. The final p-value of the six will be compared to 0.05, assuming that all others are significant. Confirmatory and tertiary outcome results will not be adjusted for multiple comparisons, as these are more exploratory in nature.

5.5 Planned Subgroups, Interactions, and Covariates

There are three subgroup factors prespecified for formal analysis in this trial:

1. Age (under 1 year versus 1 and older)
2. Antibiotic useage during the 14 days prior to ED visit,
3. Infectious agent: virus, bacteria, parasite, or other/unidentified.

We will also consider the factor used to randomize, duration of symptoms (<48 hours vs. 48 hours or more), in subgroup analyses.

5.6 Derived and Computed Variables

All derived and computed variables will be outlined in the analysis dataset specifications for this study. These datasets are independently programmed by two statisticians. The SAS COMPARE procedure will be used to verify that the dual programmed analysis datasets are identical for each variable and each observation.

5.7 Independent Review

All statistical analyses for primary reporting of trial results will be independently verified through dual programming. Two statisticians will each program all datasets and analyses for the DSMB reports and the primary manuscript(s) and the results will be compared. This process will begin at the analysis design stage and will continue through writing of abstracts and manuscripts.

6 Overview of Planned Analyses

6.1 Schedule of Interim Analyses

This study has two planned *formal* interim analyses. There will be additional meetings for review of the protocol, safety of the first 80 patients, and other meetings, as necessary. Formal interim analyses will examine treatment efficacy as well as patient safety. These analyses will be reviewed by the DSMB. The DSMB will, at their discretion, be able to request analyses additional to those described in this SAP.

The total target enrollment for this study is 900 subjects. We estimate that this will take 4 years. The first analysis (safety) will take place after enrollment of 80 subjects (40 of these $<$1 year old and 40 $\geq$1). The first formal efficacy interim analysis will take place after the second year of enrollment, after approximately 350 subjects have been enrolled. The second formal efficacy interim analysis will be performed after approximately 620 subjects have been enrolled. The DSMB is to review interim data and make recommendations regarding continuation or modification of the study.

6.2 Stopping Rules for Interim Analyses

Two-sided O'Brien-Fleming boundaries, implemented using the alpha-spending function approach will be used. Specifically, if the analyses are performed exactly when planned, the significance levels that will be used at interim analyses are 0.00065 and 0.0136. If the trial reaches the anticipated total sample size, a significance level of 0.0457 will be used for the final test. These boundaries assure that the total Type I error rate will be less than or equal to 0.05.

6.3 Blinding in the Interim Analyses

Data center biostatisticians involved in this study will be partially blinded, aware of results by treatment arm, but not of arm identities. Other data center personnel will be blinded to treatment assignments, but data center study team for this trial will have access to individual safety and efficacy data. Personnel at the clinical centers will be blinded to aggregate safety and efficacy data until the time of final analysis or until the decision is made to unblind all investigators to study results.

All by-treatment interim analyses will refer to arms as "A" and "B" throughout the report presented to the DSMB. The DSMB will have the option of being unblinded to treatment arm identity at any time.

6.4 Schedule of Final Analyses

All final, planned analyses identified in the protocol and in this SAP will be performed only after all randomized patients have completed the protocol and the results of all significant queries have been resolved. Any *post hoc*, exploratory analyses completed to support planned study analyses, which were not identified in this SAP, will be documented and reported as such in all study publications.

7 Planned Analyses with Procedures for Completion

7.1 Analysis of Demographic and Other Pre-Treatment Characteristics

Publication of the primary results will include reporting of key baseline characteristics overall, and by assigned treatment arm. These will include, but are not limited to

- gender
- race
- age
- baseline MVS
- duration of symptoms prior to enrollment (<48 hours vs. 48 hours or more)
- breast-feeding status
- antibiotic useage during the 14 days prior to ED visit
- infective agent at baseline (e.g., norovirus, rotavirus)
- hydration assessment (Gorelick dehydration score)
- need for hospitalization from initial ED visit

7.2 Analysis of Primary Outcome

The primary outcome for the study is a post-enrollment (2-week) modified Vesikari Score (MVS), defined as MVS ≥ 9. The scientific hypotheses regarding the primary outcome are described in Section 3.3.

The post-enrollment score is calculated based on the elements of the score collected Day 1 through Day 14, or when symptoms resolve. Resolution of symptoms is defined as the first

Points	**0**	**1**	**2**	**3**
Diarrhea duration	0	1–96 hrs	97–120 hrs	$\geq$ 121 hrs
Max # of diarrheal stools/24 hrs	0	1–3	4–5	$\geq$ 6
Vomiting duration	0	1–24 hrs	25–48 hrs	$\geq$ 49 hrs
Max # of vomiting episodes/24 hrs	0	1	2–4	$\geq$ 5
Max recorded fever	$\leq$ 37°C	37.1–38.4°C	38.5–38.9°C	$\geq$ 39°C
Unscheduled healthcare visit	0	—	Primary Care	Emergency Department
Treatment	None	Rehydration	Hospital Admission	—

Table 1: Modified Vesikari Score

daily survey with absence of both diarrhea and vomiting. Each of the seven components will be assigned a score for the entire study period (from randomization to day 14). Scoring of each component is described in Table 1.

The components will be calculated by the following rules:

1. **Maximum number of episodes of vomiting in a 24-hour period.** The number of episodes of vomiting is not collected precisely in 24-hour blocks. However, this component will be defined as the maximum number of vomiting episodes reported on a single daily follow-up survey.

2. **Maximum number of episodes of diarrhea in a 24-hour period.** This will use the same procedure as the previous component.

3. **Maximum temperature.** This will also use the same procedure as the two above.

4. **Diarrhea duration.** The start time for this component is randomization time. If diarrhea is recorded only on consecutive daily surveys, the end time is the time of the last recorded diarrhea episode. On the other hand, if diarrhea is absent on any daily survey and then present on a later daily survey (but vomiting must be present on the daily surveys between, otherwise it will not be considered the same disease), then the component will be calculated as time from randomization through the last diarrhea episode prior to the break in diarrhea *plus* the time from the start (noon) of the next daily survey where diarrhea is reported until the time of the last diarrhea episode. If there is yet another break in diarrhea, the procedure is repeated. If multiple end times are recorded for a subject, the first valid time will be used.

5. **Vomiting duration.** This component will be calculated using exactly the same procedure described for diarrhea when vomiting is present at randomization. If vomiting

is not present at randomization and never occurs, vomiting duration will be 0. If vomiting starts after randomization, the start time will be the start time (noon) of the first daily survey where vomiting is present.

6. **Unscheduled healthcare visit.** Presence of an unscheduled healthcare visit will be collected on the survey and will be classified as "none", "primary care", or "emergency department".

7. **Treatment.** Treatment will be classified as "none", "rehydration", or "hospital admission". If the patient is admitted directly following their enrollment ED visit and that admission stay is longer than 48 hours, it will be counted as treatment in the post enrollment MVS. If the patient's enrollment admission stay was less than 48 hours, it will not be counted as "treatment". The same logic will be used for "rehydration" during the enrollment ED visit (and admission stay, if admitted).

This outcome will be tabulated by treatment for each stratum (clinical center and duration of symptoms). A Mantel-Haenszel statistic, assessing the effect of treatment on the primary outcome controlling for strata, will be calculated to test the primary hypothesis. Summary statistics for the effect sizes will be given. For dichotomous outomes, Mantel-Haenszel risk ratios will be reported to the DSMB at interim analyses. Choice of final summary statistic reported (e.g., risk difference, risk ratio, or odds ratio) will be based, in part, by which statistic is least variable across subgroups (i.e., shows the smallest degree of interaction across subgroups).

It is expected that, for both the final analyses as well as interim analyses, the asymptotic version of the Mantel-Haenszel test will be appropriate. This analysis will be performed using SAS PROC FREQ. In the case of low outcome counts, which may occur within strata at early interim analyses, the Mantel-Fleiss criterion will be assessed. If the criterion has a value less than 5, the exact version of the Mantel-Haenszel test will be used and the two-sided p-value will be defined as the sum of all probabilities of test statistics with point probabilities less than or equal to that of the observed value.

In the event any patients were randomized within an incorrect stratum (i.e., wrong duration of symptoms) due to misspecification at time of randomization, the actual rather than assigned category will be used in the above analysis.

7.2.1 Multiple Imputation

When the primary outcome is unable to be derived due to missing data, multiple imputation will be used. This method will be applied to the components of the overall MVS: Diarrhea duration, Maximum number of diarrheal stools/24 hours, Vomiting duration, Maximum number of vomiting episodes/24 hours, Maximum fever, Unscheduled ED visit, Unscheduled Primary Care Visit, Rehydration, and Hospital Admission. In the case where no MVS

outcome data are available, the subject will not be included in imputations and the outcomes will remain missing.

Sequential Regression Multivariate Imputation Models We will use logistic regression methods to model unscheduled ED visit, Unscheduled Primary Care Visit, Rehydration, and Hospital Admission. We will use Poisson or linear regression methods to model Diarrhea duration, Maximum number of diarrheal stools/24 hours, Vomiting duration, Maximum number of vomiting episodes/24 hours, and Maximum fever. For all models, we will use forward stepwise variable selection to select predictors.

Variables to include in imputation models *Stratum*: Stratify models using treatment assigned
Minimum and maximum possible values will be computed and used as bounds for imputing MVS components: Diarrhea duration, Maximum number of diarrheal stools/24 hours, Vomiting duration, Maximum number of vomiting episodes/24 hours, Maximum fever
General model variables: age, gender, breast-feeding status, hospital/site
Baseline model variables: duration of symptoms prior to enrollment, diarrhea duration, maximum number of diarrheal stools/24 hours, vomiting duration, maximum number of vomiting episodes/24 hours, maximum fever, dehydration status, antibiotics use, Number in household, number infected in household
Post-randomization model variables: Time to symptom resolution, ED disposition, primary care visits, Emergency department visits, hospital admissions, daycare days missed, work days missed, antibiotic use, invasive LGG disease, bloating, gas, intestinal rumbling, blood in stool, abdominal cramps, nausea, loss of appetite, heartburn, constipation, skin rash, muscle aches, sore throat, cough, headache, chills, weakness, runny nose, number infected in household
Minimum and maximum possible values will also be used for imputing the following: Daycare Days Missed, Work Days Missed, number infected in household

Number of imputations We will impute and analyze 10 datasets. We will increase that number if the efficiency of the imputed datasets is less than 0.99.

7.2.2 Additional Analyses of Primary Outcome

A secondary analysis of the primary outcome will test for treatment effect on the primary outcome using a logistic regression model with baseline MVS as a covariate. We will also analyze the MVS outcome as a continuous variable. For this analysis, treatment groups will be compared using a Van Elteren test, stratified by center and duration of symptoms.

We will perform further analyses of the MVS score outcomes by assessing the treatment effect after adjustment for covariates. The covariates that will be included have been de-

scribed previously. A logistic regression model will be used for the indicator outcome of moderate-severe disease (MVS $\geq$9). Linear models will be used for the continuous MVS score. These models will be evaluated and adjustments will be made if any assumptions appear to be seriously violated. For example, a distribution other than the normal distribution may be used for the MVS score.

7.3 Analysis of Secondary Outcomes

7.3.1 Duration of Symptoms, Missed Daycare and Missed Work

Duration of diarrhea, duration of vomiting (components of the MVS), days of missed daycare, and hours of missed work will be analyzed in a similar manner. Groups will be compared using the Van Elteren test, stratified by center and duration of symptoms (pre-enrollment). Analysis of duration of vomiting, missed daycare, and days of missed work will be restricted to the populations in which the measures are applicable. Additional analyses involving these outcomes will include linear regression models that adjust for possible effects of baseline characteristics.

7.3.2 Return Visits

Return visits will be assessed as to whether or not they are related to vomiting, diarrhea, dehydration, fever, or fluid refusal. If any such visit occurs within 2 weeks of the index visit, it will be counted for this outcome. The outcome will be compared between treatment groups using the Mantel-Haenszel test, stratified by clinical center and duration of symptoms.

7.4 Safety Outcomes

7.4.1 Side-Effects

The occurence of any side-effect (listed previously) will be analyzed as a dichotomous variable for two time windows: the initial disease window (through day 14, or until resolution of symptoms) and through day 35 (which includes the initial window). The first of these two windows will not count diarrhea, vomiting, or fever, as these will likely be experienced by a vast majority of subjects and are part of the primary and secondary outcomes. The latter window will only count diarrhea, vomiting, or fever that occur after the initial disease window.

The outcome will be compared between treatment groups using a Mantel-Haenszel test, stratified by center and duration of symptoms prior to randomization.

7.4.2 Adverse Events

Adverse events (AEs) will be recorded from the time of randomization through 35 days. Symptoms/side-effects described previously will generally not be counted as AEs, as they will be reported separately. Serious adverse events (SAEs), unexpected medically attended events, and new chronic illnesses will be reported from randomization through one year after last study dose. Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 14.0.

All Adverse Events Summaries of incidence rates (frequencies and percentages), intensity, and relationship to study of individual AEs by System Organ Class and Preferred Term (MedDRA) will be prepared. All AEs beginning after randomization but before discharge will be included. Basic summaries by assigned groups will be prepared. The DSMB may request to see more detailed tables. The occurrence of any adverse event will be considered as a dichotomous outcome and will be compared between groups using a Mantel-Haenszel test, stratified by clinical center and duration of symptoms.

Serious Adverse Events/Deaths SAEs/Deaths will be reported separately in a similar fashion to the more general AE reports. In addition, narratives will be available for each event.

7.5 Analysis of Tertiary Outcomes

Tertiary outcomes will be analyzed in a manner similar to the primary and secondary outcomes. Dichotomous outcomes will be compared between groups using the Mantel-Haenszel test, while continuous outcomes will be compared using the Van Elteren test. Both types of tests will be stratified by the randomization stratification factors: clinical center and duration of symptoms.

7.6 Analysis of Subgroups

There are 3 subgroup factors prespecified for formal analysis in this trial:

1. Age (under 1 year versus 1 and older)
2. Antibiotic useage during the 14 days prior to ED visit,
3. Infectious agent: virus, bacteria, parasite, or other/unidentified.

Rates of the primary study outcome will be reported by treatment arm for all prespecified subgroups. Secondary outcomes will also be reported by arm for all subgroups.

A "subgroup" effect will be declared to be significant only if the interaction between assigned treatment and the subgroup factor is significant in the appropriate statistical model testing for each particular interaction, at a significance level of $0.05/3 = 0.017$. These results must still be viewed with caution, given the number of outcomes. For the primary outcome, and other binary outcomes, logistic regression models will be used with a main effect for treatment, a main effect for the subgroup variable of interest, and an interaction between the subgroup variable of interest and the treatment. Interactions for continuous outcomes will be evaluated in a similar manner, using linear regression models.

References

[1] M Rosenblum and MJ Van Der Laan. Optimizing randomized trial designs to distinguish which subpopulations benefit from treatment. *Biometrika*, 98:845–860, 2011.

[2] The American Statistical Association, Committee on Professional Ethics. Ethical guidelines for statistical practice, August 1999.

Statistical Analysis Plan

Protocol Title (Number): Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis (Probiotics Study) (PECARN Protocol # 032)

Protocol Version and Date: 1.00; January 24, 2014

SAP Author: T. Charles Casper, Ph.D.

SAP Version and Date: 1.2; April 6, 2017

Changes from last version:

- Section 7.2: A sensitivity analysis was added. This will look at the primary results after excluding subjects with daily surveys that were completed more than 14 days after the corresponding survey day.

SAP Version 1 Date: January 16, 2015

CONFIDENTIAL

Approvals:

Approved By:

Name	Title	Date

Name	Title	Date

Contents

Abbreviations

Abbreviation	Definition
DCC	Data Coordinating Center
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
ITT	Intent-To-Treat
MCID	Minimal Clinically-Important Difference
MVS	Modified Vesikari Score
PECARN	Pediatric Emergency Care Applied Research Network
PP	Per-Protocol
(S)AE	(Serious) Adverse Event
SAFETY	Safety Population
SAP	Statistical Analysis Plan

1 Preface

1.1 Purpose of SAP

This Statistical Analysis Plan (SAP) describes the planned analysis and reporting for the PECARN Protocol: Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis (Probiotics Study).

The purpose of this study is to determine if probiotic administration reduces the severity of acute gastroenteritis episodes in children aged 3 to 48 months. The study is a randomized, placebo-controlled trial with a parallel-group design.

The structure and content of this SAP provides sufficient detail to meet the requirements and standards set by the PECARN Data Coordinating Center (DCC). All work planned and reported for this SAP will follow guidelines for statistical practice published by the American Statistical Association [2].

1.2 Auxiliary/Other Documents

The following documents were reviewed in preparation of this SAP:

- PECARN Protocol: Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis (Probiotics Study).
- Case Report Forms (CRFs) for the Probiotics protocol

The reader of this SAP is encouraged to read the protocol for details on the conduct of this study, and the operational aspects of clinical assessments.

The purpose of this SAP is to outline the planned analyses to be completed for the Probiotics trial. The planned analyses identified in this SAP will be included in future study abstracts and manuscripts. Also, exploratory analyses not necessarily identified in this SAP may be performed. Any post hoc, or unplanned, analyses not explicitly identified in this SAP will be clearly identified as such in any published reports from this study.

It is possible that, due to updates or identification of errors in specific statistical software discussed in this SAP, the exact technical specifications for carrying out a given analysis may be modified. This is considered acceptable as long as the original, prespecified statistical analysis approach is completely followed in the revised technical specifications.

2 Study Objectives and Outcomes

2.1 Study Objectives

2.1.1 Primary Objectives

The primary objectives of the Probiotics trial are:

1. to test the hypothesis that, in children with acute gastroenteritis, probiotic administration in the emergency department will decrease the likelihood of moderate-severe disease, defined by a validated Modified Vesikari Score (MVS) $\geq$9, compared to placebo.

2. to test the hypothesis that, in children with acute gastroenteritis, probiotic administration will not be associated with serious adverse events, and will have a similar rate of side effects (e.g., bloating, fever, abdominal distention) as compared to placebo-treated children.

2.2 Study Outcomes

2.2.1 Primary Outcome

The primary outcome is the presence of moderate-severe acute gastroenteritis, as defined by a total post-enrollment Modified Vesikari Score (MVS) $\geq$9 during the 2-week follow-up period.

2.2.2 Safety Outcomes

Safety outcomes are:

1. The occurrence of invasive disease (including meningitis and bacteremia) from Lactobacillus rhamnosus.

2. Occurrence of side effects: bloating, gas, intestinal rumbling, diarrhea, blood in stool, abdominal pain, abdominal cramps, nausea, vomiting, loss of appetite, heartburn, constipation, skin rash, diaper rash, fever, nasal congestion, runny nose, sore throat, cough, headache, muscle aches, chills, weakness.

2.2.3 Secondary Outcomes

Secondary outcomes are:

1. Time to last watery stool as reported during daily surveys.

2. Time to last vomiting episode. Vomiting duration is only evaluated in children who vomited $\geq$3 times during the 24 hours prior to the emergency department visit.

3. Return visits for unscheduled care to a healthcare provider related to vomiting, diarrhea, dehydration, fever, or fluid refusal, within 2 weeks of the index visit.

4. Days of daycare missed by subjects who attend daycare.

5. Days of work missed by caregivers who work outside of the home.

6. Household transmission rate.

In addition to the primary analysis, MVS will be analyzed in secondary analyses for confirmatory purposes as:

1. a numeric variable (i.e., actual MVS, rather than dichotomized)

2.2.4 Tertiary Outcomes

Tertiary outcomes are:

1. Emergency department acute gastroenteritis-related visits
2. Intravenous rehydration
3. Hospitalization
4. Total number of diarrheal stools
5. Total number of vomiting episodes
6. Adherence to oral medication (defined as $\geq$ 7 out of 10 doses)

In the event that efficacy of the probiotic is demonstrated, cost will also be analyzed (cost-effectiveness).

2.3 Covariates

Although randomization should result in approximate balance between treatment groups with respect to critical baseline variables affecting the outcomes, additional exploratory analyses will be conducted to assess the effects of treatment after adjusting for baseline covariates in models for each outcome. The covariates considered are:

- baseline MVS (actual score),
- duration of symptoms prior to enrollment (<48 hours vs. 48 hours or more),
- age,
- gender,
- clinical center,
- breast-feeding status,
- antibiotic useage during the 14 days prior to ED visit,

- infective agent at baseline (e.g., norovirus, rotavirus),
- hydration assessment (Gorelick dehydration score),
- need for hospitalization from initial ED visit,

3 Study Design and Methods

3.1 Overall Study Design

The Probiotics trial has a parallel group design with a placebo control. We will not describe specifics about the active agent or placebo here, as these are contained in the protocol. We will refer to the two trial arms as Active and Placebo. Study participants will be randomized to Active or Placebo, each having equal allocation. Treatment with the assigned therapy is to commence immediately following randomization. The primary analysis will be performed on an intention-to-treat basis. We will also use an enrichment design, possibly modifying the eligibility criteria of the trial based on interim results.

3.2 Randomization and Blinding

3.2.1 Method of Treatment Assignment

Randomization will be balanced among the study arms. Randomization will be stratified by clinical center and by duration of symptoms. Permuted blocks of lengths 2, 4, and 6 will be used for randomization. This trial will use a web-based system for randomization provided by randomize.net. Randomization/kit numbers will be provided in a scrambled order and separated into arms (A and B).

Delivery of Randomization Randomizations will be delivered to the clinical centers using a web-based system administered by randomize.net. This system will use each enrolled patient's clinical center and duration of symptoms (<48 hours vs. 48 hours or more) to deliver the next assigned treatment.

In this trial, there will be no "emergency backup" randomization, as we expect to be able to capture a sufficient number of patients.

3.2.2 Blinding

The Probiotics trial will be performed in a double-blind fashion. All study personnel, including investigators and research coordinators, shall be blinded to assigned treatment arm for each enrolled subject. Of necessity, biostatisticians involved in presenting interim analyses

to the DSMB will be aware which subjects have received "Treatment A" and which have received "Treatment B", but they will not be aware of the identity of the two arms.

Unblinded personnel in this study will include: the central research pharmacist and, possibly, a pharmacy monitor contracted by the DCC expressly for the purpose of ensuring assigned treatments have been correctly prepared and delivered in this study. The DSMB may request to be unblinded to treatment assignment at any time.

3.3 Sample Size and Power Determination

The primary outcome is binary: whether total post-enrollment Modified Vesikari Score (MVS) is $\geq$9 during the 2-week follow-up period. The difference between arms will be tested at a 0.05 level. The null and alternative hypotheses are, respectively,

$$H_0 : p_A = p_P \qquad \text{and} \qquad H_1 : p_A \neq p_P,$$

where p_A and p_P are, respectively, the true probabilities of MVS$\geq$9 on the Active drug and on Placebo.

Our pilot data indicate that 25% of patients will have moderate to severe disease (MVS$\geq$9) during the course of their illness. Furthermore, expert surveys indicated that an absolute risk reduction of 10% would constitute a minimal clinically-important difference (MCID). Therefore, our sample size calculation assumed a 25% event rate in the control group for which we desire to detect an absolute beneficial treatment effect of 10% with 90% power (0.1 Type II error rate). Using a two-sided type I error (α) of 0.05 and the hypothesized proportions yields a required total sample size of 670 patients. Our expected power, should we find different event rates in our 2 groups, is displayed in the following table.

Placebo	Active	% Difference	Power
0.30	0.21	9%	0.76
0.30	0.20	10%	0.85
0.25	0.15	10%	0.90
0.25	0.16	9%	0.82
0.25	0.17	8%	0.72
0.20	0.10	10%	0.95
0.20	0.12	8%	0.81
0.20	0.13	7%	0.69

Based on previous work by our group, we assumed 10% loss to follow-up ($670/0.90 = 744$), 5% drop-out, and 3% drop-in (caregivers who buy a probiotic agent to administer to their child) rate ($744/(0.92)^2 = 879$). Adjustment for O'Brien-Fleming monitoring boundaries requires a further 2% increase. Thus, the total number randomized (final target sample size) will be 900.

4 Study Subjects and Analysis Populations

4.1 Analysis Populations

4.1.1 Screening Population

The screening population (SCREEN) includes all patients who are screened for eligibility into the trial, regardless of randomization into the trial or treatment status. This population represents all patients who meet inclusion criteria outlined in the study protocol and who are screened in real-time by study staff at the site. This population will be used for reporting of study flow per CONSORT guidelines.

4.1.2 Intention-to-Treat Population

The Intention-to-Treat (ITT) population includes all subjects who are randomized into the trial, regardless of adherence to the protocol, including, for example, subjects who receive no study drug. The ITT population will be used for the primary efficacy analyses in the study, as well as for main efficacy analyses of secondary and tertiary outcomes. All analyses using the ITT population will be based on each subject's assigned treatment arm, regardless of treatment actually received.

4.1.3 Per-Protocol Efficacy Population

The Per-Protocol (PP) efficacy population includes all subjects in the ITT population who are verified to meet all study inclusion and exclusion criteria, who receive study drug according to their assigned study arm (defined as $\geq$ 7 out of 10 doses). This population will be used to examine whether results seen in the ITT population are maintained in the population adhering to the protocol.

4.1.4 Safety Population

The safety population (SAFETY) includes all subjects who receive any study drug. Reporting of results based on this population will be summarized according to treatment received. This population will be used for analysis of adverse events and (in addition to ITT) to examine safety outcomes.

4.2 Study Subjects

4.2.1 Inclusion and Exclusion Criteria

To be included in the study, patients:

- are between 3 and 48 months of age; AND

- have had 3 or more watery stools within 24 hours prior to screening; AND
- have had vomiting or diarrhea less than 7 days; AND
- have symptoms consistent with acute intestinal infectious process.

The following patients will be excluded from the study:

- patients with an indwelling vascular access line; OR
- patients with structural heart disease excluding non-pathological heart murmurs; OR
- patients receiving immunosuppressive therapy or having history of immunodeficiency; OR
- patients with hematochezia in the preceding 48 hours; OR
- patients with chronic gastrointestinal problems (e.g., short gut syndrome, inflammatory bowel disease); OR
- patients with known pancreatitis; OR
- critically ill patients; OR
- patients having a family member with an indwelling vascular access line, or on immunosuppressive therapy, or with a known immunodeficiency; OR
- patients with bilious emesis; OR
- patients who have used probiotics (supplement) in the preceding 2 weeks; OR
- patients previously enrolled in this trial; OR
- patients who are allergic to lactobacillus or Microcrystalline Cellulose (MCC); OR
- patients who are allergic to erythromycin, clindamycin, AND β-lactam antibiotics (all); OR
- patients who will not be available for daily follow-up while symptomatic; OR
- patients whose parent/guardian do not speak English or Spanish; OR
- patients under 6 months of age AND premature (<37 weeks).

4.3 Enrichment Design

The study design and power analysis described are based on the assumption of homogeneous treatment effect. We may also incorporate an enrichment design to restore the statistical power in case the presence of a subpopulation with a substantially low treatment effect is identified [1]. We are particularly interested in two potential subpopulations: participants with <48 hours of symptoms prior to presentation and those with 48 hours or more of symptoms. Based on pilot data, each subpopulation accounts for approximately 50% of the total population. The decision for enrollment modification will be made at the first interim analysis for efficacy. Specifically, three Mantel-Haenszel z-statistics will be calculated to compare the primary efficacy outcome between Active and Placebo groups for subjects in the total population and the two subpopulations, respectively. Denote these z-statistics as $Z_{0,1}$, $Z_{<48,1}$, and $Z_{>48,1}$. Note that a positive z-statistic sign will be used to indicate superiority of probiotic relative to placebo.

First, $Z_{0,1}$ will be compared to the O'Brien-Fleming boundary to determine whether the trial should be discontinued for efficacy (as described in Section 6.2). If the boundary is not crossed, or if the DSMB will otherwise recommend continuation of the trial, all three z-statistics will be considered. If $Z_{0,1}$ is larger than both $Z_{<48,1}$ and $Z_{>48,1}$, or if both $Z_{<48,1}$ and $Z_{>48,1}$ are >0.3, accrual will continue as before. Otherwise, future patients will be recruited only from the subpopulation with the larger z-statistic. All subjects, regardless of symptom duration will be included in the final analyses. At the second interim analysis and the final analysis (or any additional unplanned interim analysis), the following method will be used to test for efficacy.

Let Z_2 be the Mantel-Haenszel z-statistic that is based only on subjects enrolled since the first interim analysis. Then, the final test statistic to be used is a weighted sum of the statistics from each stage:

$$Z = \left(\frac{n_1}{n_1 + n_2}\right) Z_{0,1} + \left(\frac{n_2}{n_1 + n_2}\right) Z_2,$$

where n_1 and n_2 are, respectively, the number of subects enrolled prior to and following the first interim analysis. Note that, if no enrichment is performed (i.e., accrual from the full population), Z is asymptotically equivalent to the usual z-statistic ignoring the stages. This final Z value will be compared to the quantiles of the standard normal distribution corresponding to the interim or final boundaries described in Section 6.2. A significant result will indicate efficacy in the population that was enrolled following the first interim analysis.

Simulations were performed to evaluate the performance of this design. Additional assumptions/considerations:

- The (untreated) outcome rate in the subpopulation with greater than 2 days of symptoms is 20%, while it is 30% in the other subpopulation.

- Each subpopulation accounts for 50% of the overall population.
- The normal approximation to the binomial distribution was used, rather than a chi-square or Mantel-Haenszel test.
- The interim analyses occur at 35% and 70% of target patient enrollment.

Several different treatment effect scenarios were simulated. The results show that the enrichment design will increase power when the treatment effect differs by subpopulation, but will have little impact on power if the treatment effect is the same in both subpopulations. One slight disadvantage is that, depending on outcome rates, the enrichment design will only accrue a subpopulation after the first interim analysis with probability 0.05–0.35 when, in fact, there is a homogeneous treatment effect.

5 General Analysis Issues

5.1 Analysis Software

Analysis will be performed using SAS® Software version 9.3 or later whenever possible. Other software packages, including R and StatXact®, may be used for particular specialized procedures.

5.2 Methods for Withdrawals, Missing Data, and Outliers

Per the intention-to-treat principle, subjects who withdraw from the study or are lost to follow-up will have all available data used in the analysis. In the event that a substantial number of subjects are withdrawn or lost to follow-up, baseline characteristics and available information on hospital course will be reviewed and compared to subjects not withdrawn or lost, to assess empirically if these subjects differ from those remaining in the study for the scheduled treatment and follow-up time.

Data from the follow-up surveys will be collected in such a way that data for a particular day should be complete. Information may be missing, however, if an entire day is not completed. For cases where the information needed to derive the primary outcome is incomplete, multiple imputation methods will be used. This is described in more detail in Section 7.2.

Outliers will be reviewed for validity. Outliers that are valid, for example, high number of vomiting episodes, will be included in all primary reports from this trial.

5.3 Multicenter Studies

The randomization sequences will be stratified by clinical center, to assure approximate balance of sites between study arms at all times. The primary analysis, and other analyses

will be stratified by clinical center in order to account for possible baseline differences between centers.

5.4 Multiple Comparisons

The secondary outcomes will be subject to adjustment of significance level for multiple comparisons. Diarrhea duration, vomiting duration, return visits, missed daycare, missed work, and household transmission rate will be subject to Holm's stepdown procedure. Specifically, the smallest of the six p-values will be compared to a significance level of 0.05/6. If significance is reached, the next-smallest p-value will be compared to 0.05/5, and so on. The final p-value of the six will be compared to 0.05, assuming that all others are significant. Confirmatory and tertiary outcome results will not be adjusted for multiple comparisons, as these are more exploratory in nature.

5.5 Planned Subgroups, Interactions, and Covariates

There are three subgroup factors prespecified for formal analysis in this trial:

1. Age (under 1 year versus 1 and older)
2. Antibiotic useage during the 14 days prior to ED visit,
3. Infectious agent: virus, bacteria, parasite, or other/unidentified.

We will also consider the factor used to randomize, duration of symptoms (<48 hours vs. 48 hours or more), in subgroup analyses.

5.6 Derived and Computed Variables

All derived and computed variables will be outlined in the analysis dataset specifications for this study. These datasets are independently programmed by two statisticians. The SAS COMPARE procedure will be used to verify that the dual programmed analysis datasets are identical for each variable and each observation.

5.7 Independent Review

All statistical analyses for primary reporting of trial results will be independently verified through dual programming. Two statisticians will each program all datasets and analyses for the DSMB reports and the primary manuscript(s) and the results will be compared. This process will begin at the analysis design stage and will continue through writing of abstracts and manuscripts.

6 Overview of Planned Analyses

6.1 Schedule of Interim Analyses

This study has two planned *formal* interim analyses. There will be additional meetings for review of the protocol, safety of the first 80 patients, and other meetings, as necessary. Formal interim analyses will examine treatment efficacy as well as patient safety. These analyses will be reviewed by the DSMB. The DSMB will, at their discretion, be able to request analyses additional to those described in this SAP.

The total target enrollment for this study is 900 subjects. We estimate that this will take 4 years. The first analysis (safety) will take place after enrollment of 80 subjects (40 of these $<$1 year old and 40 $\geq$1). The first formal efficacy interim analysis will take place after the second year of enrollment, after approximately 350 subjects have been enrolled. The second formal efficacy interim analysis will be performed after approximately 620 subjects have been enrolled. The DSMB is to review interim data and make recommendations regarding continuation or modification of the study.

6.2 Stopping Rules for Interim Analyses

Two-sided O'Brien-Fleming boundaries, implemented using the alpha-spending function approach will be used. Specifically, if the analyses are performed exactly when planned, the significance levels that will be used at interim analyses are 0.00065 and 0.0136. If the trial reaches the anticipated total sample size, a significance level of 0.0457 will be used for the final test. These boundaries assure that the total Type I error rate will be less than or equal to 0.05.

6.3 Blinding in the Interim Analyses

Data center biostatisticians involved in this study will be partially blinded, aware of results by treatment arm, but not of arm identities. Other data center personnel will be blinded to treatment assignments, but data center study team for this trial will have access to individual safety and efficacy data. Personnel at the clinical centers will be blinded to aggregate safety and efficacy data until the time of final analysis or until the decision is made to unblind all investigators to study results.

All by-treatment interim analyses will refer to arms as "A" and "B" throughout the report presented to the DSMB. The DSMB will have the option of being unblinded to treatment arm identity at any time.

6.4 Schedule of Final Analyses

All final, planned analyses identified in the protocol and in this SAP will be performed only after all randomized patients have completed the protocol and the results of all significant queries have been resolved. Any *post hoc*, exploratory analyses completed to support planned study analyses, which were not identified in this SAP, will be documented and reported as such in all study publications.

7 Planned Analyses with Procedures for Completion

7.1 Analysis of Demographic and Other Pre-Treatment Characteristics

Publication of the primary results will include reporting of key baseline characteristics overall, and by assigned treatment arm. These will include, but are not limited to

- gender
- race
- age
- baseline MVS
- duration of symptoms prior to enrollment (<48 hours vs. 48 hours or more)
- breast-feeding status
- antibiotic useage during the 14 days prior to ED visit
- infective agent at baseline (e.g., norovirus, rotavirus)
- hydration assessment (Gorelick dehydration score)
- need for hospitalization from initial ED visit

7.2 Analysis of Primary Outcome

The primary outcome for the study is a post-enrollment (2-week) modified Vesikari Score (MVS), defined as MVS ≥ 9. The scientific hypotheses regarding the primary outcome are described in Section 3.3.

The post-enrollment score is calculated based on the elements of the score collected Day 1 through Day 14, or when symptoms resolve. Resolution of symptoms is defined as the first

Points	**0**	**1**	**2**	**3**
Diarrhea duration	0	1–96 hrs	97–120 hrs	$\geq$ 121 hrs
Max # of diarrheal stools/24 hrs	0	1–3	4–5	$\geq$ 6
Vomiting duration	0	1–24 hrs	25–48 hrs	$\geq$ 49 hrs
Max # of vomiting episodes/24 hrs	0	1	2–4	$\geq$ 5
Max recorded fever	$\leq$ 37°C	37.1–38.4°C	38.5–38.9°C	$\geq$ 39°C
Unscheduled healthcare visit	0	—	Primary Care	Emergency Department
Treatment	None	Rehydration	Hospital Admission	—

Table 1: Modified Vesikari Score

daily survey with absence of both diarrhea and vomiting. Each of the seven components will be assigned a score for the entire study period (from randomization to day 14). Scoring of each component is described in Table 1.

The components will be calculated by the following rules:

1. **Maximum number of episodes of vomiting in a 24-hour period.** The number of episodes of vomiting is not collected precisely in 24-hour blocks. However, this component will be defined as the maximum number of vomiting episodes reported on a single daily follow-up survey.

2. **Maximum number of episodes of diarrhea in a 24-hour period.** This will use the same procedure as the previous component.

3. **Maximum temperature.** This will also use the same procedure as the two above.

4. **Diarrhea duration.** This is calculated as the time between randomization and the last diarrhea episode. When diarrhea is reported to have stopped on one survey, and started again on a later survey, but prior to all symptoms (i.e., vomiting in this case) stopping, the durations of diarrhea episodes are summed. The first diarrhea episode would be calculated from randomization date/time until the first diarrhea stop date/time. Subsequent episodes would be calculated from the beginning (noon) of the survey day on which diarrhea reportedly returned until the next diarrhea stop date/time. In cases where diarrhea stop date/times are not in sync with the expected survey dates, we assume the date/times to be correct as long as a daily duration is no more than 84 hours (i.e., more than 3.5 days) late. Otherwise, we assume the dates are erroneous, and use the expected dates instead of the reported dates. For example, if the stop date on survey 5 should be Jan 5, but is reportedly Feb 5, we would use Jan 5. If the reported date was Jan 8, we would assume that some surveys were skipped, and

use Jan 8. If any duration is less than 0, we use 0 hours. This will happen if diarrhea stops at any time prior to randomization.

5. **Vomiting duration.** This component will be calculated using exactly the same procedure described for diarrhea when vomiting is present at randomization. If vomiting is not present at randomization and never occurs, vomiting duration will be 0. If vomiting starts after randomization, the start time will be the start time (noon) of the first daily survey where vomiting is present.

6. **Unscheduled healthcare visit.** Presence of an unscheduled healthcare visit will be collected on the survey and will be classified as "none", "primary care", or "emergency department".

7. **Treatment.** Treatment will be classified as "none", "rehydration", or "hospital admission". If the patient is admitted directly following their enrollment ED visit and that admission stay is longer than 48 hours, it will be counted as treatment in the post enrollment MVS. If the patient's enrollment admission stay was less than 48 hours, it will not be counted as "treatment". The same logic will be used for "rehydration" during the enrollment ED visit (and admission stay, if admitted).

This outcome will be tabulated by treatment for each stratum (clinical center and duration of symptoms). A Mantel-Haenszel statistic, assessing the effect of treatment on the primary outcome controlling for strata, will be calculated to test the primary hypothesis. Summary statistics for the effect sizes will be given. For dichotomous outomes, Mantel-Haenszel risk ratios will be reported to the DSMB at interim analyses. Choice of final summary statistic reported (e.g., risk difference, risk ratio, or odds ratio) will be based, in part, by which statistic is least variable across subgroups (i.e., shows the smallest degree of interaction across subgroups).

It is expected that, for both the final analyses as well as interim analyses, the asymptotic version of the Mantel-Haenszel test will be appropriate. This analysis will be performed using SAS PROC FREQ. In the case of low outcome counts, which may occur within strata at early interim analyses, the Mantel-Fleiss criterion will be assessed. If the criterion has a value less than 5, the exact version of the Mantel-Haenszel test will be used and the two-sided p-value will be defined as the sum of all probabilities of test statistics with point probabilities less than or equal to that of the observed value.

In the event any patients were randomized within an incorrect stratum (i.e., wrong duration of symptoms) due to misspecification at time of randomization, the actual rather than assigned category will be used in the above analysis.

7.2.1 Multiple Imputation

When the primary outcome is unable to be derived due to missing data, multiple imputation will be used. This method will be applied to the components of the overall MVS: Diarrhea duration, Maximum number of diarrheal stools/24 hours, Vomiting duration, Maximum number of vomiting episodes/24 hours, Maximum fever, Unscheduled ED visit, Unscheduled Primary Care Visit, Rehydration, and Hospital Admission. In the case where no MVS outcome data are available, the subject will not be included in imputations and the outcomes will remain missing.

Sequential Regression Multivariate Imputation Models We will use logistic regression methods to model unscheduled ED visit, Unscheduled Primary Care Visit, Rehydration, and Hospital Admission. We will use Poisson or linear regression methods to model Diarrhea duration, Maximum number of diarrheal stools/24 hours, Vomiting duration, Maximum number of vomiting episodes/24 hours, and Maximum fever. For all models, we will use forward stepwise variable selection to select predictors.

Variables to include in imputation models *Stratum*: Stratify models using treatment assigned
Minimum and maximum possible values will be computed and used as bounds for imputing MVS components: Diarrhea duration, Maximum number of diarrheal stools/24 hours, Vomiting duration, Maximum number of vomiting episodes/24 hours, Maximum fever
General model variables: age, gender, breast-feeding status, hospital/site
Baseline model variables: duration of symptoms prior to enrollment, diarrhea duration, maximum number of diarrheal stools/24 hours, vomiting duration, maximum number of vomiting episodes/24 hours, maximum fever, dehydration status, antibiotics use, Number in household, number infected in household
Post-randomization model variables: Time to symptom resolution, ED disposition, primary care visits, Emergency department visits, hospital admissions, daycare days missed, work days missed, antibiotic use, invasive LGG disease, bloating, gas, intestinal rumbling, blood in stool, abdominal cramps, nausea, loss of appetite, heartburn, constipation, skin rash, muscle aches, sore throat, cough, headache, chills, weakness, runny nose, number infected in household
Minimum and maximum possible values will also be used for imputing the following: Daycare Days Missed, Work Days Missed, number infected in household

Combining results from imputed datasets for nonparametric tests For the main analysis of the primary outcome (Mantel-Haenszel test), the chi-square test statistic (S) will be calculated for each imputed dataset. We will also calculate the MH odds ratio (MHOR), defined so that MHOR<1 suggests that Arm A has a lower rate of the outcome. If MHOR<1,

then let $z = -\sqrt{S}$. Otherwise, $z = \sqrt{S}$. The standard error of z is 1. If the calculated zs are the same among all of the imputations, then the final p-value is that common p-value, and the z-statistic is the common z. If the calculated zs differ, then the z-statistic will be estimated and the corresponding p-value (testing $z = 0$) will be calculated using the imputation-specific zs and standard errors using SAS PROC MIANALYZE.

For the analysis of continuous outcomes using the Van Elteren test, a similar approach will be used. Instead of odds ratios, the mean in each group will be used to determine the direction of the difference. For example, if the mean of Arm A is less than that of B, then $z = -\sqrt{S}$, where S is the Van Elteren chi-square statistic.

Number of imputations We will impute and analyze 10 datasets. We will increase that number if the efficiency of the imputed datasets is less than 0.99.

7.2.2 Additional Analyses of Primary Outcome

A secondary analysis of the primary outcome will test for treatment effect on the primary outcome using a logistic regression model with baseline MVS as a covariate. We will also analyze the MVS outcome as a continuous variable. For this analysis, treatment groups will be compared using a Van Elteren test, stratified by center and duration of symptoms.

We will perform further analyses of the MVS score outcomes by assessing the treatment effect after adjustment for covariates. The covariates that will be included have been described previously. A logistic regression model will be used for the indicator outcome of moderate-severe disease (MVS $\geq$9). Linear models will be used for the continuous MVS score. These models will be evaluated and adjustments will be made if any assumptions appear to be seriously violated. For example, a distribution other than the normal distribution may be used for the MVS score.

7.2.3 Sensitivity Analysis of Primary Outcome

The daily surveys are intended to be completed on or very shortly after the corresponding study day. In some cases, a family may be contacted long after the events in question occurred. In theory, this information should have been recorded real-time in a diary. Also, there is little reason to believe the delayed information would differ in quality by treatment arm. However, we will perform a sensitivity analysis to ascertain whether the primary results hold when information collected after long delay is excluded. For this analysis, we will exclude any patient with a daily survey collected more than 14 days after the day corresponding to the survey and we will repeat the primary analysis.

7.3 Analysis of Secondary Outcomes

7.3.1 Duration of Symptoms, Missed Daycare and Missed Work

Duration of diarrhea, duration of vomiting (components of the MVS), days of missed daycare, and hours of missed work will be analyzed in a similar manner. Groups will be compared using the Van Elteren test, stratified by center and duration of symptoms (pre-enrollment). Analysis of duration of vomiting, missed daycare, and days of missed work will be restricted to the populations in which the measures are applicable. Additional analyses involving these outcomes will include linear regression models that adjust for possible effects of baseline characteristics.

7.3.2 Return Visits

Return visits will be assessed as to whether or not they are related to vomiting, diarrhea, dehydration, fever, or fluid refusal. If any such visit occurs within 2 weeks of the index visit, it will be counted for this outcome. The outcome will be compared between treatment groups using the Mantel-Haenszel test, stratified by clinical center and duration of symptoms.

7.4 Safety Outcomes

7.4.1 Side-Effects

The occurence of any side-effect (listed previously) will be analyzed as a dichotomous variable for two time windows: the initial disease window (through day 14, or until resolution of symptoms) and through day 35 (which includes the initial window). The first of these two windows will not count diarrhea, vomiting, fever, or diaper rash as these will likely be experienced by a vast majority of subjects and are part (with the exception of diaper rash) of the primary and secondary outcomes. The latter window will only count diarrhea, vomiting, fever, or diaper rash that occur after the initial disease window.

The outcome will be compared between treatment groups using a Mantel-Haenszel test, stratified by center and duration of symptoms prior to randomization.

7.4.2 Adverse Events

Adverse events (AEs) will be recorded from the time of randomization through 35 days. Symptoms/side-effects described previously will generally not be counted as AEs, as they will be reported separately. Serious adverse events (SAEs), unexpected medically attended events, and new chronic illnesses will be reported from randomization through one year after last study dose. Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), version 14.0.

All Adverse Events Summaries of incidence rates (frequencies and percentages), intensity, and relationship to study of individual AEs by System Organ Class and Preferred Term (MedDRA) will be prepared. All AEs beginning after randomization but before discharge will be included. Basic summaries by assigned groups will be prepared. The DSMB may request to see more detailed tables. The occurrence of any adverse event will be considered as a dichotomous outcome and will be compared between groups using a Mantel-Haenszel test, stratified by clinical center and duration of symptoms.

Serious Adverse Events/Deaths SAEs/Deaths will be reported separately in a similar fashion to the more general AE reports. In addition, narratives will be available for each event.

7.5 Analysis of Tertiary Outcomes

Tertiary outcomes will be analyzed in a manner similar to the primary and secondary outcomes. Dichotomous outcomes will be compared between groups using the Mantel-Haenszel test, while continuous outcomes will be compared using the Van Elteren test. Both types of tests will be stratified by the randomization stratification factors: clinical center and duration of symptoms.

7.6 Analysis of Subgroups

There are 3 subgroup factors prespecified for formal analysis in this trial:

1. Age (under 1 year versus 1 and older)
2. Antibiotic useage during the 14 days prior to ED visit,
3. Infectious agent: virus, bacteria, parasite, or other/unidentified.

Rates of the primary study outcome will be reported by treatment arm for all prespecified subgroups. Secondary outcomes will also be reported by arm for all subgroups.

A "subgroup" effect will be declared to be significant only if the interaction between assigned treatment and the subgroup factor is significant in the appropriate statistical model testing for each particular interaction, at a significance level of $0.05/3 = 0.017$. These results must still be viewed with caution, given the number of outcomes. For the primary outcome, and other binary outcomes, logistic regression models will be used with a main effect for treatment, a main effect for the subgroup variable of interest, and an interaction between the subgroup variable of interest and the treatment. Interactions for continuous outcomes will be evaluated in a similar manner, using linear regression models. Interactions for count outcomes (missed days of daycare and work) will be evaluated using negative binomial regression models.

References

[1] M Rosenblum and MJ Van Der Laan. Optimizing randomized trial designs to distinguish which subpopulations benefit from treatment. *Biometrika*, 98:845–860, 2011.

[2] The American Statistical Association, Committee on Professional Ethics. Ethical guidelines for statistical practice, August 1999.

Lactobacillus Rhamnosus GG vs. Placebo for Pediatric Acute Gastroenteritis

Summary of SAP changes (from version 1.1- January 2015- to V1.2 April 2017)

1. Section 7.2: The definitions of duration of diarrhea and vomiting were changed to incorporate additional details about handling potentially erroneous dates and times. (Pages 13 and 14)
2. Section 7.2.1: Detail was added on the approach for combining results from nonparametric tests across imputed datasets. (Page 15)
3. Section 7.2: A sensitivity analysis was added. This will look at the primary results after excluding subjects with daily surveys that were completed more than 14 days after the corresponding survey day. (page 16)
4. Section 7.4: Diaper rash was added as one of the symptoms not counted in the initial “side effects” window due to an expected high prevalence at baseline and early follow-up. (Page17)
5. Section 7.6: Detail was given on the analysis of subgroup effects related to count outcomes (negative binomial regression). (page 18)