Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis: Randomized Controlled Trial

This supplement contains the following items:
2. Original statistical analysis plan (August 2013), final statistical analysis plan (November 2017), summary of changes.
Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis: Randomized Controlled Trial

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1.0 THE NEED FOR A TRIAL

1.1 WHAT IS THE PROBLEM TO BE ADDRESSED?

The burden of acute gastroenteritis (AGE) on children and their families continues to be enormous. It accounts for 1.7 million pediatric emergency department (ED) visits annually in the United States and nearly 240,000 in Canada. Children often suffer from prolonged and severe illness; amongst hospitalized Canadian children, 19% have clinical sepsis, 7% seizures and 4% require intensive care unit admission. In a study that we conducted at 11 Canadian EDs, 51% of children experienced moderate to severe disease. Parents rate such episodes as being equivalent to a 10 day admission (moderate) and persistent moderate hearing loss (severe). The burden is augmented by the 50% household transmission rate and 42% prolonged work absenteeism rate. Apart from supportive care, health-care providers have little to offer to relieve suffering.

Probiotics, which are defined as viable microbial preparations that have a beneficial effect on the health of the host, represent a rapidly expanding field. While they are available as over-the-counter products, according to the National Institutes of Health, the Food and Drug Administration has not yet approved a single agent for any health claims. Further, a 2012 meta-analysis concluded that there is limited data to support their indications and no published pediatric gastroenteritis trials reported on side effects. Thus, understanding the benefits and side effects of probiotics is crucial before widespread use can be endorsed. Although probiotic clinical trials have been performed, only one (still unpublished) has been ED based. Most studies to date have been significantly flawed and guidelines do NOT endorse their use stating that well-controlled human trials are needed. Consequently, we and others have found that they are rarely used in clinical practice. Reasons cited include (1) questionable clinical meaning to the outcomes evaluated thus far; (2) absence of studies in the appropriate patient population, and (3) a lack of confidence in the quality of probiotic agents studied.

Our proposed definitive trial is necessary because it addresses the weaknesses and deficiencies in prior studies. We (1) focus on the burden of disease and outcomes of relevance to the infected child and his/her caregiver, (2) study outpatient children (>95% of those infected), (3) employ rigorous methodology and a sample size significantly larger than any prior study, (4) evaluate the side effect profile and conduct subgroup analyses by etiologic agent, and (5) will be free of bias (i.e. industry funding). These elements have not been previously addressed by any pediatric probiotic clinical trial. We will additionally investigate several novel domains: (1) the economics of widespread probiotic use and (2) the in vivo impact on immunoglobulin secretion.

This study will address (1) the needs of the medical community, which is aware of the widening gap between the number of important pediatric and adult trials and (2) the interest of caregivers in “probiotics” - 71% are aware of the term; 31% believe they may be beneficial in children with diarrhea, and > 90% would administer a probiotic if it could make their child better. Furthermore, our pilot study has provided promising preliminary data and has proven the feasibility of our methods. Thus we are poised to conduct a randomized controlled trial (RCT) that will definitively determine if meaningful benefits are derived from probiotic use and will provide critical information regarding their mechanism of action. This information will impact on practice, the burden of disease, and ensure that children receive the best care possible. The results of our proposed RCT will enable guidelines to either clearly endorse or recommend against the routine use of a probiotic agent in children with AGE.

1.2 WHAT ARE THE PRINCIPAL RESEARCH QUESTIONS TO BE ADDRESSED?

Hypotheses: In children aged 3-48 months presenting to an ED with less than 72 hours of AGE like symptoms, compared with placebo, the administration of a probiotic agent:

1. Will result in a significantly lower proportion of children developing moderate to severe disease over the subsequent 2 weeks.
2. Will not be associated with a significantly greater occurrence of minor side effects.
3. Will be associated with a greater increase in secretory IgA (sIgA).

**Specific Aim 1 (Clinical Efficacy):**

**Primary Question:** For previously healthy children, ages 3-48 months, who present to an ED with less than 72 hours of AGE like symptoms, is the proportion who develop moderate to severe disease [Modified Vesikari Score (MVS) ≥ 9] following ED evaluation, significantly different in those who receive a probiotic agent (Lacidofil) compared to those who receive placebo?

**Secondary Questions:** In this group of patients, amongst those receiving active treatment versus placebo:

1. Is there a difference in the (a) duration of diarrhea or (b) duration of vomiting?

2. Is there a difference in the proportion who require an unscheduled health care provider visit?

**Specific Aim 2 (Side Effect Profile):**

**Question:** In this group of patients, is the proportion that experiences a side effect (e.g. bloating, fever, abdominal distention, rash) significantly different in those who receive Lacidofil compared to placebo?

**Specific Aim 3 (Mechanism of Action):**

**Question:** In this group of patients, are fecal sIgA levels 5 days and 4 weeks after the initiation of treatment higher in those who receive Lacidofil compared to those who receive placebo?

### 1.3. WHY IS A TRIAL NEEDED NOW? Definitive data is lacking to guide clinical decision making and most guidelines do not endorse routine probiotic use.\(^{14,25}\) Hence, probiotics are rarely prescribed by North American physicians.\(^{4,19,26}\) However, there are current trends that obligate an urgent assessment. First, since probiotics are sold as food supplements, manufacturers can encourage their use while their relevance has yet to be established.\(^{27}\) Manufacturers have embarked on aggressive campaigns making health claims that may not be supported by rigorous research.\(^{28-31}\) At stake is the world-wide probiotic market which is growing at 13% annually and is valued at $33 billion/year.\(^{32}\) Second, North American and European government agencies remain concerned about their value and safety.\(^{33-35}\) Third, some institutions are now recommending the routine use of probiotics.\(^{36}\) Fourth, parents of affected children are often providing probiotics.\(^{17}\) We are therefore concerned that probiotic consumption is increasing in the absence of solid evidence. This underscores the necessity to conduct this definitive trial without delay. Prior research on the topic suffers from the following important shortcomings:

**1-Outcome measures used to date have limited clinical meaning:** Studies have focused on individual symptoms (e.g. stool duration), without consideration of the full picture of the illness\(^{37}\) (e.g. fever, vomiting, ED visits, hospitalization). A 2010 Cochrane Review concluded that the instruments employed to date are heterogeneous, lack evidence of validity and focus on outcomes that are not important to participants.\(^{38}\) Thus, the significance of conclusions reached are questioned.\(^{39,40}\) We will employ a validated burden of disease score and will focus on outcomes of relevance to children and their caregivers to enable an evidence-based conclusion to be drawn.\(^{12}\)

**2-Populations studied to date do not apply to the majority of children:** Though 95% or more of children are treated as outpatients,\(^{41}\) only a handful of small studies have focused on outpatients.\(^{39}\) Inpatient research cannot be extrapolated to outpatients, as hospitalized children are more likely to benefit from probiotics.\(^{12,42,43}\)

**3-Quality of studies to date is inadequate:** Most are small, single-centre\(^{44}\) and have been conducted by pharmaceutical companies.\(^{45}\) Many negative probiotic studies remain unpublished.\(^{46}\) Design issues are a concern: in a 2010 Cochrane Review, only 16% of studies adequately reported the 4 key methodological assessment parameters (i.e. allocation sequence generation, concealment, blinding, and loss to follow-up).\(^{12}\) Of 175 outstanding dietary research articles selected over the past 7 years by the National
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Institutes of Health, only 2 addressed probiotics and none AGE.\textsuperscript{47} Hence, high quality studies funded by non-vested parties that assess outcomes of interest to children and parents are needed.\textsuperscript{45,48}

4-\textbf{Inadequate data available from research in the relevant patient population:} No studies to date have evaluated the impact of probiotics on children with gastroenteritis treated in primary care. Only a single ED study has been performed: 129 children received a probiotic or placebo agent and the authors found statistically insignificant trends towards a reduction in stool frequency (30\% fewer diarrheal stools) and duration (median 14 hours fewer of diarrhea) amongst those administered a probiotic agent.\textsuperscript{49} The groups did not differ in terms of return to normal activities, return for medical care or the need for hospitalization. In light of these potentially important trends, the conclusions of systematic reviews, and the burden of disease – there are 1.7 million ED visits in the United States and 240,000 ED visits annually in Canada for pediatric gastroenteritis – conclusive data regarding the routine outpatient use of probiotics in North American children with AGE are needed.\textsuperscript{1}

5-\textbf{Knowledge about the in-vivo Mechanism of Action in AGE is lacking:} Our understanding of the mechanism of action of probiotics is limited.\textsuperscript{50-51} Possible methods of action are (1) \textit{Microbiologic} – by improving intestinal mucosal permeability,\textsuperscript{52} modifying the microbiota, inhibiting adherence of pathogenic bacteria, and competing for nutrients;\textsuperscript{53} (2) \textit{Immunologic} – by upregulating gene expression,\textsuperscript{54} inhibiting the activation of pro-inflammatory pathways,\textsuperscript{55} increasing the concentrations of anti-inflammatory cytokines,\textsuperscript{56} and promoting local antigen-specific immunoglobulin A (IgA) responses.\textsuperscript{57} \textbf{Studies incorporating both clinical outcomes and the measurement of biomarkers potentially related to the clinical effects are desperately needed.}\textsuperscript{12,58}

6-\textbf{Lack of Probiotic Quality Control:} As reported in an RCT comparing 5 probiotic products,\textsuperscript{59} not all are equally effective. Strain, viability, and dose are important factors.\textsuperscript{60} In North America, most have never been clinically evaluated,\textsuperscript{61} some claim to contain organisms that do not exist,\textsuperscript{62} others do not match their labeled microbiologic specifications. Our work with Lacidophil has demonstrated that it reduces epithelial injury,\textsuperscript{63,64} prevents bacterial binding, invasion and translocation,\textsuperscript{64,65} reduces gastric inflammation,\textsuperscript{66} attenuates colonic disease and dysfunction,\textsuperscript{64,67,68} improves intestinal barrier function,\textsuperscript{69} normalizes corticosterone release,\textsuperscript{68} and plays an immunomodulatory role.\textsuperscript{64} As a mandatory, yet rarely performed research requirement,\textsuperscript{12,70} \textbf{we have obtained independent analyses to confirm the viable colony forming unit (CFU) count and microbe identity (Appendix 1-Lacidophil).} We have obtained Health Canada approval for our pilot which has guided this proposal’s design. \textbf{Hence our study will provide evidence about a high quality product available in Canada.}\textsuperscript{71}

1.4 \textbf{RELEVANT SYSTEMATIC REVIEWS AND NEED FOR THIS TRIAL IN LIGHT OF THESE REVIEWS.} Meta-analyses\textsuperscript{12,42,45,72,73} are encouraging however, they (1) question the clinical relevance of the outcomes evaluated,\textsuperscript{12,39,45} (2) conclude that publication bias is a concern, and (3) advocate for a large RCT,\textsuperscript{26} funded by an unbiased agency, in an ambulatory pediatric population.\textsuperscript{45} A 2010 Cochrane Review reported reductions in the mean duration of diarrhea (25 hours), diarrhea lasting >4 days (risk ratio 0.41), and stool frequency on day 2 (mean difference 0.8).\textsuperscript{12} Given the limited clinical relevance of these findings, and the significant between-study heterogeneity, the authors of this and other reviews have called for studies that (1) evaluate specific regimens in large numbers of participants, (2) identify infectious causes,\textsuperscript{39} (3) present data separately for important subgroups, (4) include identification of the probiotic being tested, (5) confirm viability and quantity, (6) identify mechanisms underlying the beneficial effects, (7) conduct cost-effectiveness analyses,\textsuperscript{39,74} and (8) are definitive multicentre RCTs.\textsuperscript{12,45,75} Our proposed study, which builds on our promising pilot work, addresses all the limitations raised by the previous reviews and will provide the missing pieces of information.
1.5 HOW WILL THE RESULTS OF THIS TRIAL BE USED? The generalizability of the proposed trial will be excellent. If probiotics are effective, we will develop a knowledge translation (KT) plan to ensure integration into care occurs. We will encourage incorporation into clinical pathways and seek endorsement by knowledge user groups (e.g. Canadian Pediatric Society, Canadian Association of Emergency Physicians). Successful dissemination strategies similar to those previously employed will be adopted. This study, which has been endorsed by Pediatric Emergency Research Canada (PERC), a 2011 winner of the CIHR-CMAJ Top Achievements in Health Research Awards, will be conducted at 5 member sites. The network has recently been awarded funding by the Networks of Centres of Excellence Knowledge Mobilization program to build a 36 site network termed Translating Emergency Knowledge for Kids. The network’s purpose is to optimize the transfer of knowledge into non-academic institutions. As part of this process Dr. Freedman recently received (as Co-PI) a CIHR Knowledge Synthesis Grant (Spring 2012) focused on gastroenteritis which ultimately will lead to a guideline building consensus conference which will incorporate findings from TREKK’s knowledge user work. Subsequent KT efforts will employ the TREKK network to conduct a cluster RCT. David Johnson, a co-investigator, and University of Calgary Site Lead for Knowledge Translation Canada, will play a lead role in disseminating the findings. Ron Goeree will work closely with Canada’s Health Technology Assessment Agency to determine the most efficient and effective ways to implement the knowledge gained. Lastly, this network will facilitate the integration of probiotic use into the national pathways program being developed by PERC investigators.

1.6 PLEASE DESCRIBE ANY RISKS TO THE SAFETY OF THE PARTICIPANTS INVOLVED IN THE TRIALS. Well over 200 billion doses of probiotics have been consumed and no serious side effects have been reported in well people. Five pediatric cases of lactobacillus bacteremia have been reported in which the strain was indistinguishable from the strain administered. The cases include short gut syndrome (3), complex congenital heart disease (1), and cerebral palsy and sepsis (1). There have been no reports of adverse overdose events. There is no evidence that probiotic use will worsen diarrhea, result in complications from the disease process, or introduce new toxicity. In our pilot, adverse events were only reported in the placebo group. Information on Lacidofil - testing, safety data, and research by Dr. Sherman’s lab are available in Appendix 1 - Lacidofil.

2.0 THE PROPOSED TRIAL

2.1 WHAT IS THE PROPOSED TRIAL DESIGN? Randomized, placebo-controlled, double-blind, multicentre (5), Canadian, ED trial. All children aged 3 months to less than 48 months of age who present to a participating ED will be assessed for eligibility. A total of 886 children will be randomized to receive 5 days of a probiotic agent (Lacidofil – 8 x 10^9 CFU/day) or placebo. The study will be conducted employing methodology suggested by the 2010 CONSORT statement.

2.2 WHAT ARE THE PLANNED TRIAL INTERVENTIONS?

**ED Intervention:** The 1st dose will be administered in the ED. The sachet’s contents will be sprinkled into 30 mL of a liquid (ideally ORS) which may be cool (0°C-25°C) but without ice crystal formation. Caregivers will receive instructions on study drug administration, completion of study forms, what and how much fluid to drink, criteria for seeing a health care practitioner or returning to the ED (Appendix 2), and standardized AGE discharge instructions from each hospital.

**Home Intervention:** All patients will take 1 sachet, based on randomization, every 12 hours for 5 days (total of 9 home doses). They will administer the medication at meal time, mixed with 30 mL of an unfrozen beverage with no ice crystal formations (above 0°C) and ingested immediately to optimize viability. Carbonated and highly acidic beverages should be avoided. We will stress the importance of
administering all doses dispensed and the need to communicate with the study team on a daily basis until symptoms resolve. One extra dose/day will be provided (i.e. kits will contain 5 extra doses – total of 15 sachets to account for vomiting or wastage). The dose may be repeated once should the child vomit within 15 minutes of medication administration. Vomiting after medication administration rarely occurs > 1 time. Oral fluid therapy will be encouraged according to established guidelines. Children who are hospitalized will continue as per study protocol as we have successfully done previously.

Hospitalization at a non-study hospital site is very uncommon – 1/800 (0.1%) children in the PERC multicentre bronchiolitis RCT were admitted at an alternative site. Should this occur, caregivers will have a letter describing the study, the care-plan, and the contact information of the Site Investigator.

**Rationale for Treatment Dose**: Although multi-strain products, such as Lacidofil, appear to show greater efficacy than single strains, the optimal CFU/kg dose is unknown. Lacidofil data indicates that a dose of 3-6 x 10⁹ CFU/day is effective. Our pilot trial, which employed low (4 x 10⁹ CFU/day) and high (8 x 10⁹ CFU/day) dose arms, found no side effects with either dose. However, a positive association is postulated to exist between the probiotic dose and clinical benefits with most positive studies employing doses ≥ 6 x 10⁹ CFU/day. Thus, we will employ a dose of 8 x 10⁹ CFU/day. This should enable us to definitively answer our research question and hence influence future usage. The duration of therapy has been selected based on the best available evidence, the recommendations of experts in the field, previous studies, and the typical duration of most episodes of AGE.

**Stool Sample Testing**: In keeping with usual common clinical practice, stool samples from all enrolled children will be sent for bacterial culture. Bulk specimens will be obtained whenever feasible. As was done in our pilot study, for children who do not provide a stool specimen prior to discharge, rectal swabs will be performed. One swab will be sent for bacterial culture and the other will be frozen at -80°C for future viral testing which will be performed in batches. For patients enrolled at The Hospital for Sick Children (HSC) and Alberta Children’s Hospital (ACH) who consent to additional bulk stool sample collection will provide a bulk sample as soon as possible following enrolment, and on Days #5 and #28 to allow for IgA testing. If a sample is unable to be provided on Days 5 and 28, the first sample provided after Day 5 and Day 28 respectively will be accepted. If bulk samples are provided, rectal swabs will not need to be performed. Caregivers of participants at these institutions will be provided with standard stool collection containers in which to place stool specimens. They will contact the site Clinical Research Assistant/Nurse once a sample is obtained. The Clinical Research Assistant or Nurse will arrange for pick up employing a biomedical courier service and processing (Appendix 3-sIgA Procedures). Caregivers may also call the specified courier service for sample pick-up however they must notify the research team that a sample has been provided. Both sites will prepare, store, and ship the bulk samples for analysis. Bulk stool samples will be labeled with the date and time of collection as well as the subject ID. Samples will be stored at -80°C and sent to HSC in bulk shipments once per year over a three year period. IgA analysis will be performed by Dr. Sherman’s laboratory which is located at HSC and is certified to handle human samples.

2.3 WHAT ARE THE PROPOSED PRACTICAL ARRANGEMENTS FOR ALLOCATING PARTICIPANTS TO TRIAL GROUPS? **Sequence Generation**: The Clinical Research Informatics Core (CRIC), based at the University of Alberta, will provide data management services for this study. www.randomize.net, an internet based randomization service, will produce a randomization list stratified by study site, using random-number generating software. The lists will be sent to the central pharmacy (ACH) who will prepare consecutively numbered study kits according to the randomization schedule. These will be couriered to the clinical sites, using proper shipment containers and temperature monitors, where they will be stored in the Research Support Pharmacies. **Allocation Concealment**: www.randomize.net uses industry standard security to send data over the internet. Randomization will
be blocked using random blocks of 4 and 6 with a 1:1 allocation ratio. Stratifying by clinical site and blocked randomization will ensure that variations (e.g. site specific practice patterns, gastrointestinal pathogens) are comparably distributed across treatment arms. Only the research pharmacy at the coordinating centre and www.randomize.net will retain the randomization code. **Implementation:** Potentially eligible patients (i.e. all children with diarrhea who meet age criteria) will be identified by the triage nurses and will be screened by the Clinical Research Assistant or Nurse for eligibility. A log of all screened patients will be maintained. If eligible, the details of the study will be discussed with the caregivers of all eligible children by the Clinical Research Assistant or Nurse who will seek consent. If consent is obtained, enrolled children will consecutively be assigned a patient ID number by the clinical site. The Clinical Research Assistant or Nurse will collect baseline demographic clinical variables and will complete the data collection forms (Appendix 4-Study Subject Timeline). Elements of clinical dehydration (Gorelick Score)\(^6\) and baseline disease severity scores (Modified Vesikari Score)\(^7\) will be assigned to enable baseline comparisons between treatment arms. The Clinical Research Assistant or Nurse will then log into randomize.net which will randomize the patient (i.e. it will provide a kit number that corresponds to a study drug kit at the clinical site which will be given to the patient). Following randomization the first dose will be administered (Section 2.2).

### 2.4 WHAT ARE THE METHODS FOR PROTECTING AGAINST SOURCES OF BIAS?

Bias will be minimized by strictly adhering to the 2010 CONSORT Statement recommendations including the use of "third-party" assignment (Section 2.3).\(^8\) Moreover, because the active ingredient constitutes < 10% of the sachet, the probiotic and placebo powders will be identical in appearance, taste, texture and smell. Thus, participants, families, healthcare providers, data collectors (Research Assistants/Nurses), outcome adjudicators (Research Assistants/Nurses), and data analysts will be blinded, thereby preventing bias in outcome assessment. An intention-to-treat analysis will be performed to minimize bias associated with poor compliance and non-random loss of participants.\(^9\) Co-interventions (e.g. antiemetic, intravenous rehydration, antibiotic administration) and other sources of confounding will be recorded. Reporting bias will be avoided by registering the trial at clinicaltrials.gov. Additionally our use of a published score as an outcome measure will protect against the introduction of bias in the assessment of treatment effects.\(^10\)

### 2.5 WHAT ARE THE PLANNED INCLUSION/EXCLUSION CRITERIA?

All patients with gastroenteritis presenting to the ED of 5 participating hospitals will be eligible. The diagnosis of gastroenteritis is at the discretion of the emergency department supervising physician and may or may not include vomiting. Alternative terminologies that reflect as similar diagnosis are acceptable provided they meet all other eligibility criteria. Examples include: viral illness, diarrhea, vomiting, upper respiratory infection, post-infectious gastroenteritis, antibiotic associated diarrhea, toddlers diarrhea, viral infection, enteritis, viremia, fever, bronchiolitis.

**Inclusion criteria (Patients must meet all of the following criteria to be eligible)**

1. **Presence of diarrhea:** defined as ≥ 3 watery stools in a 24-hour period.\(^9\)
2. **Duration of vomiting or diarrhea < 72 hours:** Early administration = greater efficacy.\(^27,100,101\)
3. **Age 3 to < 48 months:** AGE severity and frequency are greatest amongst young children.\(^102\)

**Exclusion criteria (Patients who meet any one of the following criteria will not be eligible)**

1. **Presence of an indwelling vascular access line or structural heart disease** (bacteremia risk).\(^103\)
2. **Taking immunosuppressive therapy, or known history of immunodeficiency** (bacteremia risk).\(^104\)
3. **Hematochezia in the preceding 72 hours, underlying significant chronic gastrointestinal problem or inflammatory bowel disease:** Not including constipation, gastroesophageal reflux or chronic pain.
4. **Family member with an indwelling vascular access line, on immunosuppressive therapy, or with a known immunodeficiency:** Does not include use of short course oral (<7 days) or inhaled steroids.

5. **Bilious vomitus:** May indicate a diagnosis other than AGE is possible.

6. **Probiotic use (supplement) in the preceding 2 weeks:** However, consumption of foods containing probiotics will not result in exclusion as they are ubiquitous.

7. **Previously enrolled in this trial** (to ensure that the observations on trial patients are independent).

8. **Daily telephone follow-up will not be possible while symptomatic** (travel plans or language barrier).

9. **Allergy to soy:** Lacidofil, as well as the placebo product have come in contact with soy during the manufacturing process.

10. **Pre-existing, or known, pancreatic dysfunction or insufficiency**

11. **Oral or Gastrointestinal surgery within the preceding 7 days:** theoretical wound infection risk.

### Concomitant Medications

The concomitant administration of antibiotics will be permitted and will be at the discretion of the child’s treating physician. Children taking antibiotics will not be excluded as probiotics remain effective when given concomitantly with antibiotics and their survival is not significantly altered. Similar criteria will be applied to the administration of antipyretics, anti-emetics, and any other medications. As per Standard of Care at the participating sites, Oral Rehydration Solution (ORS) will be provided during the emergency department visit to enable the performance of oral rehydration therapy. In keeping with institutional Standard of Care, patient/parent discharge instructions that will be provided, as specified in protocol section 2.2, will encourage the ongoing use of appropriate ORS following discharge.

#### 2.6 WHAT IS THE PROPOSED DURATION OF TREATMENT PERIOD?

Five days.

#### 2.7 WHAT IS THE PROPOSED FREQUENCY AND DURATION OF FOLLOW-UP?

Daily telephone or e-mail survey follow-up will occur, 7 days/week, until both the diarrhea and vomiting have resolved. We will also conduct follow-up on days #5 and #14 even if symptoms have resolved.

#### 2.8 WHAT ARE THE PROPOSED PRIMARY AND SECONDARY OUTCOME MEASURES?

**Primary Outcome (Clinical):** The primary outcome is the development of moderate-severe disease in the 2 weeks after the index ED visit as measured by the MVS (Appendix 5-MVS).7 The original 20 point Vesikari Score has been employed as a dichotomous variable in many clinical studies despite limited evidence supporting its use. However, it has been shown to correlate with other meaningful measures such as caregiver anxiety, helplessness, and stress. Recently, increasing severity scores were associated with higher parental worry, greater changes in the child’s behavior, and trends towards greater impact on the parents’ daily activities and higher parental distress. So, why did we develop a Modified Score? Percent dehydration, an element of the original score, is challenging to determine. While using baseline and rehydrated weights is the gold standard, this is often of limited value due to difficulties in ensuring follow-up, determining when rehydration has occurred, and the variation related to timing of voiding, stooling, eating, and drinking. Moreover, clinical estimates of dehydration are extremely inaccurate. Thus, this element is omitted or incorrectly assigned in most studies. The modified score which we have created includes an important and easy to obtain outcome that reflects global disease severity—need for unscheduled future health care visits within 2 weeks of the index visit. This is supported by evidence that the utilization of professional medical care correlates with disease severity. Unscheduled future health care visits is a powerful marker that has the capacity to alter clinical practice and influence decision makers. Similar modifications have been performed previously when percent dehydration has been unavailable and we have previously shown that because ED care does not alter the disease process in AGE, ED revisits are very common (publication...
attached). The MVS is presented below, with the score structure (0, 1, 2, 3 points) unaltered from the original score.

<table>
<thead>
<tr>
<th>Points</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>≥ 121 hours</th>
<th>≥ 6</th>
<th>≥ 49 hours</th>
<th>≥ 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea Duration (d)</td>
<td>0</td>
<td>1-96 hours</td>
<td>97-120 hours</td>
<td>≥ 121 hours</td>
<td>≥ 6</td>
<td>≥ 49 hours</td>
<td>≥ 5</td>
</tr>
<tr>
<td>Max # of diarrheal stools/24 hr period</td>
<td>0</td>
<td>1-3</td>
<td>4-5</td>
<td>≥ 121 hours</td>
<td>≥ 6</td>
<td>≥ 49 hours</td>
<td>≥ 5</td>
</tr>
<tr>
<td>Vomiting Duration (d)</td>
<td>0</td>
<td>1-24 hours</td>
<td>25-48 hours</td>
<td>≥ 49 hours</td>
<td>≥ 5</td>
<td>≥ 49 hours</td>
<td>≥ 5</td>
</tr>
<tr>
<td>Max # of vomiting episodes/24 hr period</td>
<td>0</td>
<td>1</td>
<td>2-4</td>
<td>≥ 49 hours</td>
<td>≥ 5</td>
<td>≥ 49 hours</td>
<td>≥ 5</td>
</tr>
<tr>
<td>Max Recorded Fever</td>
<td>&lt; 37.0°C R</td>
<td>37.1-38.4°C R</td>
<td>38.5-38.9°C R</td>
<td>≥ 39.0°C R</td>
<td>≥ 5</td>
<td>≥ 49 hours</td>
<td>≥ 5</td>
</tr>
<tr>
<td>Future Health Care Visit</td>
<td>0%</td>
<td>-</td>
<td>Primary Care</td>
<td>Emergency Dept.</td>
<td>Hospitalization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>None</td>
<td>Rehydration</td>
<td>Primary Care</td>
<td>Emergency Dept.</td>
<td>Hospitalization</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Characteristics of the MVS: We prospectively evaluated the MVS in an 11 centre (455 children) ED study in children meeting eligibility criteria as planned for the current proposal (>3 stools in a 24 hour period and <72 hours of symptoms) which found that it effectively measures global disease severity. Factor analysis revealed that item correlations were acceptable and supported the appropriateness of retaining all factors. Multi-collinearity was not a problem and the correlations between the MVS and other measures of clinical significance were in the expected direction. Disease severity was associated with prolonged daycare (P = 0.01) and work (P = 0.002) absenteeism. The MVS had a normal distribution with minimal kurtosis (-0.14; SE: 0.24) and skewing (0.39; SE: 0.12). There was good variation across severity ranges (49% mild; 21% moderate; 30% severe). Variation between institutions was insignificant (P = 0.11) and complete follow-up was achieved in 91% of participants.

How will it be Calculated?: Following enrollment (Time 0), follow-up will occur daily until both the diarrhea and vomiting have resolved (Section 2.7). Once follow-up is complete (Day #14) each variable is assigned a score for the entire study period (Time 0 to Day #14); each patient gets a single total score for the study. Variables are scored based on the worst 24 hour period (e.g. maximal number of episodes of vomiting in a 24 hour period) or on the total duration of symptoms (e.g. number of days of vomiting) or are based on the occurrence of an outcome (e.g. hospitalization).

What if at baseline the pre-enrollment MVS is ≥ 9?: Regardless of the score assigned at Time 0 (i.e. pre-enrollment score), EVERYONE reverts to a score of 0 at enrollment (i.e. the study evaluates the impact on the disease process going forward). The pre-enrollment score, which is based on symptoms in the 72 hours prior to presentation, will serve as a covariate in a secondary analysis of the primary outcome and will be employed for sub-analysis purposes. An example is provided (Appendix 5-MVS). The primary outcome (the presence of moderate-severe disease, as defined by a MVS of ≥ 9 during the 2 week follow-up period) will ONLY include symptoms and outcomes that occur following the ED visit (i.e. after randomization) and will not be directly impacted by the pre-enrollment score.

Why a cut-point of 9?: With the original score, severe disease was defined as ≥ 11,107,108,113,114,121-123 moderate as ≥ 9.124 In our derivation study,7 construct validity was proven 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 [e.g. daycare (P=0.01) and work absenteeism (P=0.002)].

Secondary Outcomes (Clinical): 1. The duration of diarrhea: Time from treatment initiation until the appearance of the last watery stool125-127 as reported during daily phone conversations.

2. The duration of vomiting: Limited data indicate that probiotic administration may reduce vomiting.100,128 Recovery will be evaluated in children who vomit ≥ 3 times over the 24 hours prior to the ED visit and defined as “time from treatment initiation until last vomiting episode.” We have previously reported that vomiting frequency predicts outcomes in AGE.129
3. **Return visits for unscheduled care to a health care provider related to vomiting, diarrhea, dehydration, fever, or fluid refusal, within two weeks:** Not included will be scheduled visits (e.g., re-assessment, vaccinations). This outcome is important as > 50% of children have a follow-up office visit,41 8-18% require an ED visit,130 and 5-8% are hospitalized.41

**Additional Outcomes:** Work and daycare absenteeism.

**Side Effect Profile:** To determine if short course probiotic administration to young children with AGE is associated with an increase in minor side effects. As stated by the NIH, probiotic safety needs to be studied scientifically.131 Groups will be compared regarding the development of any side effects with particular attention paid to bloating, abdominal distention, duration of fever, and buttock rash. The importance of evaluating side effects has been highlighted by a recent adult pancreatitis study which found an unexpected increase in mortality in probiotic treated patients.105

**Mechanism of Action:** To determine if probiotic administration increases fecal secretory IgA levels in children with AGE (Appendix 3). The first stool sample produced following enrollment will be collected along with samples on days 5 and 28. sIgA is a key element in the gastrointestinal immune defense as it agglutinates microorganisms and prevents pathogen adherence to mucosal surfaces.132-135 Evaluating sIgA in children with AGE has been identified as a needed element to advance this field of research.136,137 Animal studies have reported a substantial increase in anaerobic bacteria in the absence of normal sIgA and that normalization of sIgA production results returns intestinal microbiota to its regular composition.138 Probiotics are believed to enhance host immunity by regulating inflammatory cytokines139 and by increasing sIgA production.140,141 In human studies, probiotic administration appears to increase fecal sIgA concentration in healthy adults,142 children,143 infants,144,145 and pre-term infants.146 However, correlation with clinical outcomes has not yet been evaluated. We will determine if fecal sIgA levels are greater amongst children treated with a probiotic agent compared with placebo. Levels will be correlated with clinical findings.

2.9 HOW WILL THE OUTCOME MEASURES BE MEASURED AT FOLLOW-UP? All caregivers will receive discharge instructions that will include information on tasks required following discharge. Training materials have been developed based on the 3 site probiotic pilot study.

1. **Daily Telephone/Survey Communication:** At the index visit, caregivers will be asked their preferred method of communication – electronic (i.e. email survey) versus telephone. Following discharge, site Clinical Research Assistants or Nurses will contact the family daily until both the diarrhea and vomiting have resolved employing the identified method. A standardized script or survey/data collection form will be employed. If phone is opted for, the caller will enquire about ongoing symptoms, medical evaluations, treatments, child care and work absenteeism, and side effects. Detailed questioning will follow positive responses. The survey will employ advanced logic to enhance ease of use. If the caregiver does not complete the survey within 48 hours, a telephone follow-up will be performed. **Compliance** will be assessed on day #5 and final data points will be collected on day #14. Protocols will be developed to deal with caregiver questions in accordance with institutional requirements. To maximize validity, caregivers will be reminded of the importance and method of administering the probiotic/placebo. Similar schemes have been successfully implemented by the principal investigator,177,120 other PERC multicentre studies,80,89 and was employed in the pilot. Caregiver report (telephone/survey) will serve as the primary source document.

2. **Diary:** As has been done previously,89,147 including in our probiotic pilot trial, parents will be given a diary to use as a worksheet. They will record all information related to the questions asked during the telephone calls. Data recorded will serve as a reminder in case there are days when the Clinical Research
Assistant or Nurse is unable to communicate with the caregiver directly (or electronic surveys are not returned). In the previous probiotic trial which employed this measure; although parents enjoyed having the diary as a worksheet, the return rate was suboptimal hence this will not be required.

3. Chart Review: We will verify data regarding revisits, intravenous hydration, hospitalization, and microbiology testing using each centre’s medical record database.

4. Database Reviews: Provincial databases (e.g. National Ambulatory Care Reporting System; Alberta Ambulatory Care Classification System; Alberta Health Care Insurance Plan) and Canadian Institute for Health Information databases will be employed to verify future health care provider use.

2.10 WILL HEALTH SERVICE RESEARCH ISSUES BE ADDRESSED? As called for by the 2010 Cochrane review, an economic evaluation will be conducted by Dr. Willan and Mr. Goeree alongside the clinical trial (Appendix 6-Economic Analysis Plan). We will monitor work absenteeism, as this is the major item contributing to cost. Moreover, days of diarrhea has been found to correlate with work absenteeism, and a recent pediatric, Canadian ED study found that > 50% of the societal costs occur in the 15 days following the ED visit. Hence, if effective, cost savings are likely from a societal perspective due to the inexpensive nature of probiotics and the economic benefit derived from reduced work absenteeism. Because adding a therapeutic intervention may add to overall health care costs, willingness to pay will be determined. The incremental cost effectiveness will be determined by assessing resources and costs associated with the treatment of AGE for children who receive the current standard of care compared to those who receive a probiotic.

2.11 WHAT IS THE PROPOSED SAMPLE SIZE AND WHAT IS THE JUSTIFICATION FOR THE ASSUMPTIONS UNDERLYING THE POWER CALCULATIONS (APPENDIX 7)?

Clinical Outcome: The sample size is based on the assessment of the between-group difference in proportions of children with a post-randomization score ≥ 9 on the MVS. This is a superiority study in which the adoption of probiotic use can be recommended if the rate of the primary outcome is significantly lower amongst those who receive the probiotic medication. Calculations are based on a two-sided type I error (α) of 0.05 and power (1-β) of 0.90. The null hypothesis is \( H_0: P_C - P_T = 0 \), where \( P_C \) and \( P_T \) are the event rates in the intervention and control groups respectively. The alternative hypothesis is \( H_A: |P_C - P_T| > 0.10 \) (i.e. the event rates will differ by at least 10 percentage points).

Minimal Clinically Important Difference (MCID): Ten content experts from the US and Canada were surveyed regarding the MCID. Absolute risk differences ranging from 7.5-15% were suggested. We chose a conservative estimate of 10% for the primary outcome (number needed to treat of 10).

Outcome in Control Group: Our estimate for the development of moderate to severe AGE in the controls is based on data collected as part of our 2009 evaluation of the MVS in 455 children aged 3 – 48 months, with < 72 hours of symptoms, who presented to one of 11 Canadian EDs (Section 2.8). Using the ED visit as time 0, 25% of eligible children had scores consistent with moderate to severe disease following discharge. This is lower than previous reports of ED and community populations because we did not include symptoms that existed prior to the visit. However, Dr. Schnadower’s group in the United States has just completed data collection on 282 children enrolled at 6 sites in the United States and they found that 24% of children in their sample had scores consistent with moderate to severe disease following discharge (personnel communication September 6, 2012). Since our study population and method of MVS calculation in the derivation and recent validation studies and the current proposal are the same, 25% is a very accurate estimate. Given the above, the required sample size to compare proportions between two different groups is 670.

Sample Size Adjustment Calculation: Based on previous work by our group with similar follow-up designs and extensive reviewer feedback, we have assumed a 10% loss to follow-up.
(670/0.9=744), 5% drop out (744/(0.95)^2=825), and 2.5% drop in (caregivers who decide to buy a probiotic agent at a pharmacy to administer to their child) rate (825/(0.975)^2=868). Adjustment for O’Brien-Fleming monitoring boundaries requires a further 2% increase. Thus, the total number randomized (final sample size) will be 886.

**Side Effect Profile:** To date, clinical trials employing probiotics have not attributed any adverse events to probiotic administration.\(^\text{12}\) We suspect that minor side effects have not been documented; however, clinicians need to have an understanding of the side effect profile in order to enable caregivers to make an informed treatment decision. Given our sample size, a significant difference between groups will be easily detected (i.e. 80% power to detect an increase in reported adverse events from 5% to 10%).

**Mechanism of Action:** A study evaluating the impact of formula supplementation with oligosaccharides found fecal sIgA values of 729 and 377 µg/g in the intervention and control groups respectively.\(^\text{158}\) If we assume a clinically significant difference of 300 µg/g, a standard deviation of 500 µg/g, 80% power and a type I error of 0.05, the required sample size is 45 subjects/group. Thus we will aim to include 100 patients which will be recruited from ACH and HSC.

### 2.12 WHAT IS THE PLANNED RECRUITMENT RATE (APPENDIX 8)?

The proposed sites saw 10,344 children aged 3 – 48 months with AGE in 2011 (a 17% increase since 2009). During our pilot RCT, 2.1% of children with AGE aged 0-4 years were enrolled. Based on the published literature and our data: (i) presenting November 1 – May 31 between 8:00 – 24:00 (55%), (ii) meet definition of diarrhea (50%), (iii) < 72 hours of symptoms (45%), (iv) absence of exclusion criteria (80%), and (v) provide consent (50%), our best estimate is that 4.7% of children with AGE aged 3 - 48 months will be enrolled. The difference between our pilot and the best point estimate is due to the requirement of daycare attendance in our pilot study. Based on our experience with AGE,\(^\text{77,159}\) and multicentre trials,\(^\text{80,160}\) we believe that we should employ our worst case scenario recruitment estimate (3.1%) which will enable us to enroll our full sample size over three AGE seasons. The only prior North American ED study, which employed similar eligibility criteria, recruited 129 subjects at 1 site in just 8 months\(^\text{49}\) therefore we believe our recruitment plan is realistic.

### 2.13 ARE THERE LIKELY TO BE ANY PROBLEMS WITH COMPLIANCE?

While infrequently reported and not considered to be problematic,\(^\text{161}\) non-compliance is unlikely related to probiotic side effects.\(^\text{12}\) Participant withdrawal has primarily been related to the primary illness.\(^\text{12}\) A recent study reported 108% compliance due to medication re-administration in subjects who vomited.\(^\text{162}\) As the intervention is of a short duration, the burden to caregivers is minimal. In our pilot, compliance was 91% as reported by caregivers and verified by return sachet counts. This does not reflect the impact of vomiting following medication administration. A recent ED probiotic study reported that 87% of caregivers found the probiotic and placebo powders to be “very” or “somewhat” easy to administer.\(^\text{49}\) Hence, we do not anticipate compliance problems; nonetheless, we will track compliance by obtaining unused sachet counts (day #5) and requesting their return (day #14).

### 2.14 WHAT IS THE LIKELY RATE OF LOSS TO FOLLOW-UP?

Our previous ED pediatric AGE research achieved telephone follow-up rates of 98-99% on Day #3 and 96-99% on day #7.\(^\text{77,120}\) Similar success has been documented in prior PERC (99%)\(^\text{80,89}\) multicentre studies. We will err on the conservative side and 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 databases (Section 2.9) will supplement the daily telephone calls.

### 2.15 HOW MANY CENTRES WILL BE INVOLVED?

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Five EDs that are members of PERC, a network which has extensive experience conducting large scale clinical studies,\textsuperscript{4,80,89,145,163-165} will participate – Alberta Children’s Hospital (Calgary), Hospital for Sick Children (Toronto), Children’s Hospital of Eastern Ontario (Ottawa), Centre Hospitalier Sainte-Justine (Montreal), and IWK Health Centre (Halifax).

2.16 WHAT IS THE PROPOSED TYPE OF ANALYSES?
All analyses will be undertaken by the intention to treat principle. Adverse events will use the “as treated” principle. Patients who drop out or crossover will be followed and included. All statistical tests of hypotheses will be two-sided. Baseline characteristics will be compared between groups using frequency counts and percentages for discrete variables, and means, medians, standard deviations, and interquartile ranges for continuous variables. Baseline characteristics will be analyzed to determine if there is a need to adjust for differences between groups. Sensitivity analyses will be performed to assess the possibility and consequences of losses to follow-up not occurring at random.

**Clinical-Primary Outcome:** The proportion of children with moderate to severe disease (i.e. MVS $\geq 9$) will be analyzed by comparing proportions utilizing a Mantel-Haenszel test, stratified by clinical centre.

**Significance for the primary outcome measure will be determined using a two-sided 0.05 level.** The \textit{pre-enrollment} MVS will not be included in the primary analysis as we do not anticipate the baseline and post-intervention scores to be correlated. Secondary analyses of the primary outcome will employ logistic regression methods to adjust for covariates that may be imbalanced between groups (e.g. age, \textit{pre-enrollment} MVS, severity of baseline diarrhea and vomiting, hydration assessment, need for hospitalization at index visit). We will also analyze the MVS as a continuous variable through a stratified Wilcoxon rank-sum test.

**Clinical-Secondary & Tertiary Outcomes:** The overall significance level for statistical tests on the \textit{secondary outcomes} will be set at 0.05. Holm’s method will be used to adjust for multiple comparisons. The continuous variables of (1) duration of diarrhea and (2) vomiting will be measured in hours and analyzed with a Van Elteren test, stratified by clinical centre. (3) Unscheduled health care visits will be analyzed using a Mantel-Haenszel test, stratified by clinical centre. The \textit{tertiary outcomes} of (4) number of days the child is absent from daycare and the (5) caregiver is absent from work will be analyzed using an appropriate model with robust estimates for standard errors. Dichotomous outcomes to be evaluated but unlikely to achieve significance include ED 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.

**Side Effect Profile:** The proportions of children experiencing any \textit{side 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 side effects, as an aggregate outcome variable.

**Mechanism-Fecal Secretory IgA:** To test for a difference in \textit{fecal secretory IgA} the Wilcoxon rank-sum test will be performed. As this is a mechanistic outcome and the motivation of its study is distinct from other outcomes, the test will be performed at the 0.05 level. Data will be analyzed to determine if fecal secretory IgA levels 5 days and 4 weeks after initiation of treatment are higher amongst children treated with probiotic than those treated with placebo. Fecal sIgA data will also be analyzed by outcome, comparing levels amongst those with mild disease to those with moderate-severe disease.

2.17 WHAT IS THE PROPOSED FREQUENCY OF ANALYSES?
The Data Safety Monitoring Committee (DSMC) will meet after 200 and 500 patients to review enrollment, study procedures, form completion, data quality, loss to follow-up, drop-in rate, and interim safety and efficacy results. The analyses will test the hypothesis that the probability of developing
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moderate to severe AGE in the probiotic arm is equal to that in the placebo arm. Conservative O’Brien-Fleming monitoring boundaries, implemented using the Lan-DeMets alpha-spending function approach, will be used as guidelines for early stopping for safety or efficacy. Based on trends and adverse events, the DSMC may decide to meet sooner than planned using boundaries adjusted accordingly. Because this trial involves children under the age of 6 months, the DSMC has approved a plan to complete an interim safety analysis on the first 20 subjects enrolled under 6 months of age. All serious adverse events will be reported within 24 hours to the DSMC and based on these reports; the DSMC may decide to conduct a safety analysis before the full 20 subjects have been enrolled in this age group. Otherwise, a blinded analysis will be conducted after the 20 subjects < 6 months of age have been enrolled. This data will be unblinded if the DSMC deems it necessary to conduct an unblinded interim safety analysis. The results of this analysis will be communicated to the NHPD branch of Health Canada at the discretion of the DSMC chair should any concerns be identified.

2.18 ARE THERE ANY PLANNED SUBGROUP ANALYSES? (1) The presence of a MVS ≥ 9 will be analyzed by (i) age < 1 year, (ii) breast-feeding status, (iii) antibiotic usage and (iv) protocol compliance. (2) Duration of vomiting will be analyzed only in those patients who have ≥ 3 episodes of vomiting in the 24 hours prior to enrollment. (3) Daycare and work absenteeism will only be analyzed for children who attend daycare and caregivers who work. A subgroup analysis will be performed for children with (4) rotavirus infection by adding an interaction term between treatment and rotavirus positivity in a logistic regression model. The independent variables in the model will be (i) treatment group, (ii) rotavirus positivity (yes/no) and (iii) the interaction between treatment group and rotavirus positivity. Universal rotavirus vaccination does not exist in Canada with the decision being made individually by each province based on the expense as well as feasibility. At present it is included in the provincial schedules in Quebec and Ontario but not in Nova Scotia or Alberta. The varying use of the vaccine and our goal to identify etiologic agents and to conduct sub-analyses will yield very important information related to probiotic use in the presence/absence of rotavirus vaccination. (5) Fecal sIgA levels will be sub-analyzed based on the mother’s breast-feeding status.

2.19 HAS ANY PILOT STUDY BEEN CARRIED OUT USING THIS DESIGN? The participating research team members and PERC network have extensive experience conducting clinical research. The network has monthly conference calls and the executive meets several times per year. Dr. Freedman, the Vice-Chair of PERC, has successfully completed and published several gastroenteritis clinical trials, with publications in BMJ and NEJM. He additionally led a 50 patient multicentre pilot study employing Lacidofil which provided promising preliminary data, evaluated the feasibility of the current proposal and identified potential problems. The pilot included a placebo group and two dosages: 4 x 10⁹ CFU/day and 8 x 10⁹ CFU/day. It did not detect a trend toward increased side effects in the 8 x 10⁹ CFU/day arm; hence, to ensure our study has the optimal ability to answer the primary question, the 8 x 10⁹ CFU/day dose will be used. Overall, 91% of all doses dispensed were administered. Key information data provided by the pilot were: (1) the safety of high dose Lacidofil, (2) anticipated recruitment and compliance estimates, (3) the revision of data collection forms, (4) the use of rectal swab for specimen collection (aside from sIgA), (5) the optimal rectal swab testing device, (6) day #5 instead of 7 compliance assessment, (7) modified follow-up protocol to minimize loss to follow-up, and (8) proved our ability to obtain Health Canada approval.

3.0 TRIAL MANAGEMENT

3.1 WHAT ARE THE ARRANGEMENTS FOR DAY-TO-DAY MANAGEMENT OF THE TRIAL? (APPENDIX 9) CRIC, based at the University of Alberta, will act as a central repository for all study data. Staffing will include a project manager, a medical informatics specialist and an assistant.
CRIC will be responsible for the provision of data collection technology and clinical data management services. CRIC’s staff has extensive experience and expertise in collecting data using REDCap software and managing study data in accordance with Good Clinical Practice requirements including the use of qualified and trained study personnel, study monitoring, standard operating procedures, validated software, data audit trails, and quality assurance. ACH (the PI’s institution) will serve as the coordinating centre, will be in constant communication with CRIC, and will be responsible for study training, monitoring, and progress. Dr. Willan will supervise all data analyses. Dr. Freedman will take overall responsibility for the study. Site Investigators and Clinical Research Assistants/Nurses will share responsibilities including day to day activities, payroll, study promotion, contacting caregivers, and reviewing charts.

Once funding is obtained, REB and Health Canada approvals will be obtained. All ED physicians and nurses will be educated regarding the study and Clinical Research Assistants/Nurses will be trained. Sites have committed to having Clinical Research Assistants or Clinical Research Nurses present 75 hours/week during peak season and volume periods (7 months/year). Their presence will maximize study enrollment by continuously reminding physicians about the study and enrolling eligible children. Participating institutions all have significant infrastructure in place and will use a variety of methods to optimize coverage while minimizing costs including Clinical Research Assistants or Nurses covering multiple studies and volunteer programs (e.g. www.sickkids.ca/HealthcareProfessionalsandStudents/clinical-research/index.html).

The ACH Research Pharmacy will ship the study drug in batches to the participating institutions. The ACH Research Pharmacy will also maintain a batch of sachets which have not been randomized to be sent to high recruiting sites. Collaborating pharmacies will be blinded to study drug and will be responsible for storage and providing study kits to the site Clinical Research Assistants. Regular e-mail, weekly teleconferencing for the first 6 weeks of the trial, and monthly conference calls will be used to monitor start up and to obtain updates on recruitment and issues arising. Real-time data entry will facilitate an ongoing data cleaning plan. Double data entry will be employed on a random sampling of subjects at various time points throughout the study to ensure the data collected is accurate and is being recorded properly.

3.2 WHAT WILL BE THE ROLE OF EACH PRINCIPAL APPLICANT AND CO-APPLICANT PROPOSED? This study, under the umbrella of PERC brings together North American investigators with transdisciplinary expertise. Dr. Freedman who has expertise in AGE research,1,4,7,19,64,120,129,130,168-171 recently reported77 that ondansetron, an antiemetic agent, is effective in pediatric AGE. It is now routinely used to reduce the need for intravenous hydration and hospitalization.78,130,172-174 Dr. Gorelick, a clinical epidemiologist,175-178 with significant network research experience,179-183 has provided senior guidance and high level input from a large research think-tank in the United States (Pediatric Emergency Care Applied Research Network-PECARN). Dr. Schuh81,160 has successfully completed 15 pediatric ED RCTs and has guided the study since its inception. Dr. Johnson,80,160 who has multicentre RCT experience has served as a resource regarding operational issues and will guide KT184-186 efforts. Dr. Schnadower has led efforts to conduct a similar study in the United States and has served as a liaison with the Pediatric Emergency Medicine Collaborative Research Committee (Dr. Freedman is a steering committee member). Site investigators will supervise the study at their respective institutions. Dr. Sherman has experience with LactoRil67,69,187 and his laboratory will perform the fecal sIgA analyses.94,95 Dr. Willan, a PhD statistician, and Mr. Goeree, a health economist, will perform the statistical and economic evaluations. Dr. Willan is extensively involved in methodologic research in the area of health economics and optimizing decision-making in health care research and policy.188-190 All team members will be aided by CRIC, MICYRN, the ACH Program Manager, the Clinical Research Coordinator, and the site Clinical Research Assistants or Nurses.
3.3 DESCRIBE THE TRIAL STEERING COMMITTEE AND THE DATA SAFETY AND MANAGEMENT COMMITTEE. **Trial Steering Committee:** The advisory panel has included knowledge users, caregivers, pediatricians, emergency medicine physicians, gastroenterologists, and infectious disease physicians. The protocol has been revised based on guidance provided by the PERC and PECARN networks. Non-research team members who have had extensive input include clinicians, statisticians, ethicists, and coordinators with multicentre research expertise. Official committee members have included senior clinical research team members (Drs. Gorelick, Schuh, Johnson), Dr. Sherman, a Canada Research Chair in Gastrointestinal Disease (selection of probiotic agent, dose, duration of therapy, and planned translational studies), Dr. Kuppermann, the past-Chair of PECARN, Dr. Dean, expert in conduct of multicentre network research, and Dr. Plint, the Chair of PERC. This has ensured that the study will answer important questions that can readily be applied by these leading KT research networks. **Data Safety Monitoring Board (section 2.17 also):** There will be an independent monitoring committee consisting of a biostatistician (Nick Barrowman, PhD-Ottawa), and two physicians with RCT expertise (Drs. Mark Roback–Minnesota and Terry Klassen (Chair) - Winnipeg). This committee will be independent of the investigators and will be advised of all adverse events.

3.4 ADVERSE EVENT REPORTING

**Adverse Event (AE):** An adverse event is any unfavorable or unintended clinical or other occurrence during the study period that may or may not be the result of participation in the research study.

**Expected Adverse Drug Reactions/Events**

These include the following as they are part of the natural history of the underlying disease process:

- Hospitalization
- Future health care provider visit, ED return visit
- IV rehydration
- Abdominal pain, distension
- Vomiting, diarrhea, fever, flatulence

Because expected adverse events are part of the natural history of acute gastroenteritis and diarrheal illness in children, they will not need to be reported as Adverse Events. This information will be recorded in normal study data collection processes.

**Serious Adverse Events**

Any Serious Adverse Event (SAE) that occurs after the first sachet administered will be reported to the Research Ethics Board (REB) and the study subject will be followed until the conclusion of the event.

A SAE is defined as:

- Results in death.
- Is life-threatening. This refers to an event in which the patient was at immediate risk of death; it does not refer to an event that might have caused death had it been more severe.
- Results in a persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect
- Is medically significant. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

In addition, any serious adverse reaction to the natural health product will be reported to the Natural Health Product Directorate (NHPD).
**Adverse Event Reports**

For unexpected adverse events, we will inform the REB, in addition to the clinical chief of the ED, and the external sponsor within 7 days of learning of the event.

For unexpected SAEs, we will inform the REB, in addition to the clinical chief of the ED, and the external sponsor within 24 hours of learning of the event (by AE form, telephone or email). The SAE information will be sent even if the information is incomplete. A complete follow-up AE report will be submitted as soon as possible but no later than 7 days after the initial reporting.

**Collaborating Study Sites**

The principal investigator or delegate will also submit to the University of Calgary REB information received from other sites. Conversely, adverse events that occur at The Alberta Children’s Hospital (The University of Calgary) will be communicated by the principal investigator to collaborating sites, as their local requirements dictate.

**Health Canada (Natural Health Product Directorate) Reporting**

Adverse drug reactions (ADR) that are both serious and unexpected are subject to expedited reporting to Health Canada (NHPD) by the sponsor. These include reactions;

- Where it is fatal or life-threatening, immediately where possible and, in any event, within 7 days after becoming aware of the information
- A complete follow up report within 8 days which includes an assessment of the importance and implication of any findings including relevant previous experience with the same or similar drugs
- Where it is neither fatal nor life-threatening within 15 days after becoming aware of the information

Each ADR which is subject to expedited reporting will be reported individually in accordance with the data element(s) specified in Section 78 of the NHP Regulations, ICH Guidance Document E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting.

**Emergency Unblinding**

Un-blinding should only occur in the event that there is clinical concern regarding the possibility of bacteremia/septicemia or when it is felt by the treating physician that unblinding would alter the clinical care being provided. All patients whose therapy is intentionally un-blinded will discontinue the experimental therapy. Un-blinding should only occur when future clinical treatment of the patient will depend on prior treatment administered. Approval from the principal investigator or designate will be obtained prior to un-blinding. If the principal investigator cannot be reached, the un-blinding can be performed and the principal investigator informed within 24 hours via e-mail or telephone call.

Accidental and intentional un-blinding will be documented and reported and the subject will be withdrawn from the study.

**3.5 PREMATURE WITHDRAWL/DISCONTINUATION CRITERIA**

The subjects retain the right to withdraw from the study at any time, although withdrawal from the study is strongly discouraged after the subject has been enrolled.

Every effort will be made to contact all subjects for follow-up as scheduled. Subjects will be withdrawn from the study if:

1. After enrollment they are determined to meet any of the exclusion criteria
2. If the subject is admitted to an intensive care unit
3. If it is deemed by the treating physician that the child’s health may be jeopardized by continued participation in the study
4. The patient’s caregivers wish to withdraw their child for whatever reason
If the patient’s caregiver chooses to withdraw their child from the study, they will be provided with a choice regarding their exit from the study:
1. The caregiver may choose to withdraw the child from the study, as well as all data collected from their child’s participation in the study
2. The caregiver may choose to withdraw their child from the study; however they will allow continued use of study data collected from their child.

3.6 RECORD KEEPING
The data produced from this study will be stored in a secure, locked location. Only members of the research team will have access to the data. Following completion of the research study the data will be stored and kept for a minimum of 25 years. The data will then be destroyed in accordance with institutional policy.


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emID%3D88039%26libID%3D87727%3F&ei=FK0SUJXFN4iiAH65oHYDQ&usg=AFQjCNE8lhSeTiAUcY3km
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Date: August 14, 2013
APPENDIX 1: Lacidofil (L. helveticus Rosell-52 and L. rhamnosus Rosell-11)

The probiotic Lacidofil® is a two-strain product which has been commercially available since 1995. It is composed of *L. rhamnosus* R0011 and *Lactobacillus helveticus* R0052 in a 95:5 ratio. *L. rhamnosus* R0011 was isolated from a dairy starter culture in 1976 and deposited at the Institut Pasteur Collection Nationale de Cultures de Microorganismes (CNCM, Paris, France) as I-1720. The *L. helveticus* R0052 was originally isolated in 1990 from a dairy culture for ‘sweet acidophilus milk’ and deposited in the Institut Pasteur CNCM as I-1722. Historically, it had been identified as a *Lactobacillus acidophilus* on the basis of its biochemical and metabolic activities, however, subsequent molecular investigation reclassified the identity of this strain as *L. helveticus*. (1) All studies prior to April 2006 refer to R0052 as *L. acidophilus*. They are both Gram (+) anaerobic bacilli found in pairs or short chains.

**Composition**

<table>
<thead>
<tr>
<th>Active Ingredients (freeze-dried)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lactobacillus rhamnosus</em> R0011</td>
<td>3.8 x 10⁹ CFU/sachet</td>
</tr>
<tr>
<td><em>Lactobacillus helveticus</em> R0052</td>
<td>0.2 x 10⁹ CFU/sachet</td>
</tr>
</tbody>
</table>

It is available in sachets with the total weight of all ingredients = 1 gm. Placebo sachets, which are also supplied by the manufacturer, contain only the non-medicinal excipients and is identical in appearance, smell, and weight to the active sachet (verified in pilot study).

The quantities supplied in each sachet are guaranteed for 18 months after manufacture when stored at ambient temperature. Stability testing shows complete stability when stored under refrigerated conditions with 100% survival of the initial bacterial concentration. At ambient temperature the average monthly loss is 4%. To ensure that each sachet provides the desired dosage (4 x 10⁹ CFU) when stored at room temperature for 18 months, sachets are produced with overages.

**Genome Sequencing**

The strains in Lacidofil were sequenced at Genome Québec (Montréal, Canada) using pyrosequencing (454 Life Sciences, Branford, CT, USA) and the genomes have been uploaded to GenBank. *L. rhamnosus* R0011 Bioproject Genome ID: 51799 (accession: PRJNA51799). *L. helveticus* R0052 accession: AEIR01000000. No antibiotic resistance genes or other genes of concern were observed in the genome of either strain.

**Independent Testing**

Testing of sachets prepared for our pilot study, which were intended to contain 4 x 10⁹ CFU/sachet, was conducted by Dr. Denis Groleau at the Microbial and Enzymatic Technology Process Centre of the National Research Council – Biotechnology Research Institute. Sachets were provided to him for testing by the Research Support Pharmacy (Darcy Nicksy) at The Hospital for Sick Children. He found that the sachets were in the required range (maximum overage = 300%) (2) as specified by the Natural Health Product Directorate of Canada and exceeded the anticipated shelf-life at room temperature (see Lactobacilli Enumeration Letter – page 26). He additionally conducted a molecular biologic analysis using a technique termed RAPD (Random Amplification of Polymorphic DNA – page 27) to confirm the identity of the
organism. Two primers for each species were employed: Primer OPA-18 and Primer M14. The control strains were provided by Institut Rosell-Lallemand: Lactobacillus rhamnosus R0011 and Lactobacillus helveticus R0052. The following image (page 30) is a display of the results: R1, R2 and R3 were DNA originating from 3 colonies picked at random on a selective medium for L. rhamnosus; H1, H2 and H3 were DNA from 3 colonies picked at random on a selective medium for L. helveticus. The electrophoresis gels were prepared, separating the pieces of DNA according to their length. The image (prepared by Dr. Groleau) demonstrates that the bacterial strains in Lacidofil match those in the controls.

Excipients
Maltodextrin (carrier) 217 mg/sachet
Magnesium stearate (lubricant) 7.5 mg/sachet
Ascorbic acid (anti-oxidant) 1 mg/sachet
These excipients have complete chemical insensitivity to the lyophilized bacteria, are suitable for production with the technology of corporate use and ease dispersion.

Pharmacodynamics & Pharmacokinetics
R0052 and R0011 produce lactic acid, a pathogen inhibitory substance, and the addition of either organism to Citrobacter rodentium demonstrates time-dependent growth inhibition. Culture supernatants also inhibit C. rodentium growth implying that inhibitory secretory factors are present. Mouse pretreatment with Lacidofil also reduces C. rodentium colonization. Lacidofil additionally reduces bacterial colonization and gastric inflammation in H. pylori infected mice. Strains R0052 and R0011 also inhibit H. pylori growth and E. coli adhesion in a dose-dependent manner. These strains also resist gastric acid, bile and intestinal secretions and are capable of adhering to human intestinal epithelial cells. There is no evidence that these microbes cross the intestinal barrier. The bacteria preserve the epithelial barrier by maintaining intercellular tight junctions and reducing the translocation of other bacteria.

Stability
Lacidofil has been found to be very stable in beverages aside from soft drinks (low pH) and hot drinks (> 70° C). Testing in apple-based fruit juices has demonstrated that consumers can expect good viability of L. rhamnosus R0011 over a few weeks of storage in a refrigerator, even if the bottles have been opened and cells are exposed to oxygen. Stability in oral rehydration solution is optimal because the pH and osmotic pressure are ideal for microbes (similar to phosphate buffered saline solution used in the laboratory), and this procedures has been done successfully in other studies. Stability testing has been performed by the manufacturer and is as follows:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Initial Concentration/250 mL</th>
<th>Time 0 Concentration/250 mL</th>
<th>Time 0 % Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pedialyte-Unflavored</td>
<td>4 x 10^9</td>
<td>3.34 x 10^9</td>
<td>84%</td>
</tr>
<tr>
<td>Pedialyte-Grape</td>
<td>4 x 10^9</td>
<td>3.44 x 10^9</td>
<td>86%</td>
</tr>
<tr>
<td>Apple juice</td>
<td>4 x 10^9</td>
<td>2.97 x 10^9</td>
<td>74%</td>
</tr>
</tbody>
</table>
Montréal, 25 August 2009

Dr. Stephen Freedman, MDCM, FRCPC, FAAP, M.Sc.
Associate Scientist, Research Institute
Assistant Professor of Paediatrics
The Hospital for Sick Children,
Toronto, Canada

Re: Lactobacilli Enumeration in Sachet

Dear Dr. Freedman,

I am pleased to put in an official letter the results of the lactobacilli enumeration counts I have performed starting from one Lacidofil® sachet in early July 2009.

The work was started in the afternoon of July 10th, 2009. The content (1.0016g) was placed into 99mL of sterile phosphate buffer and the mixture shaken at 266 rpm for 10 minutes in an incubator. The sample was then serially diluted (1mL into 9mL of sterile phosphate buffer) up to dilution 10^-8. The dilutions 10^-6 to 10^-8 were thereafter used for counting. Counting was done as follows: 0.1mL aliquots (in triplicate) was added to each of 3 Petri dishes to which ca. 20mL of reconstituted MRS agar at 45°C was added; mixing was done by manual swirling after which the plates, once the agar had hardened, were incubated at 37°C for ca. 72 hours under anaerobic conditions (GasPak system, BBL).

The counting of colonies was done by Mr. Wayne Levadoux from my group (I was on vacation). Using the 10^-7 and 10^-8 dilution plates (total of 6 plates), the following result was obtained: 11.34 x 10^6 CFU (colony forming units) per sachet (per gram).

I hope that you will find these results satisfactory.

Sincerely yours,

Denis Groleau, PhD (Microbiology)
Group Leader
Microbial and Enzymatic Technology
Bioprocess Centre
NRC-BRI

6100, avenue Royalmount
Montréal (Québec) Canada
H4P 2R2
Montréal, 25 February 2010

Dr. Stephen Freedman, MDCM, FRCPC, FAAP, M.Sc.
Associate Scientist, Research Institute
Assistant Professor of Paediatrics
The Hospital for Sick Children,
Toronto, ON, Canada

Re: Identification of the bacterial population present in lactobacilli sachets using a molecular biology approach (RAPD)

Dear Dr. Freedman:

At your request, I am pleased to submit a short description of the work we did here very recently in order to identify, using a molecular biology approach named RAPD, the lactobacilli population present in one of the lactobacilli sachets used in your study.

The work was divided into two steps:

**Step 1:** Estimation of the total population of the lactobacilli (per gram of dry material) together with an estimate of the population of each one of the two lactobacilli species comprising the population.

**Step 2:** Molecular biology-based (RAPD) identification of the two lactobacilli species
RESULTS

For Step 1

The content of one sachet (1 gram of dry material) was used, as done before, for estimating the total viable lactobacilli population using the MRS medium. The total viable population was found to be between 5.767 x 10(9) and 6.0 x 10(9).

The *Lactobacillus rhamnosus* R0011 population was found to be between 6.533 x 10(9) and 8.0 x 10(9) using MRS medium containing vancomycin.

The *Lactobacillus helveticus* R0052 population was found to be 2.33 x 10(8), or, roughly 3.5% of the total viable lactobacilli population, using MRS medium containing clindomycin.

For Step 2

For each one of the two lactobacilli populations, *L. rhamnosus* and *L. helveticus*, three (3) colonies were selected at random from the appropriate plates, their DNA extracted and processed according to protocols provided by Dr. T. Tompkins from Institut Rosell-Lallemand (Montréal, Qc). The two control strains were treated in an identical manner.

RAPD analysis was done according to a protocol also supplied by Dr. Tompkins. Two primers were used on each sample: Primer OPA-18 and Primeer M14. Please note that, in order to obtain optimal results, Dr. Tomkin's protocol was modified very slightly: The annealing temperature was 45°C instead of 42°C.

Earlier this week, I sent you the electronic pictures of the two electrophoresis gels obtained at the end. The pictures show conclusively that all colonies examined showed a RAPD profile identical to that of the control strain.

Conclusion

The sachet contained the "announced" two lactobacilli species.
I hope that you will be very satisfied with these results.

It has been a real pleasure to interact with you in the last few months.

Best Regards,

Denis Groleau
Group Leader
Microbial and Enzymatic Technology Group
Bioprocess Centre
NRC-BRI
R0052; *L. helveticus*
R0011; *L. rhamnosus*

* Annealing temperature for PCR was modified from 42 to 45°C.
Physiological Characteristics

Resistance to Gastric Acidity: The rate of survival of *L. helveticus* R0052 and *L. rhamnosus* R0011 was measured at 3 pH values (4.0, 3.0, and 2.0) after 30 minutes at 37°C. The strain demonstrated resistance to moderate acid conditions with 87% survival at pH 4.0, and 76% survival at 3.0.\(^5\) In physiologic terms, this is exceptional as it is estimated that every 10 minutes the stomach eliminates 50% of its fluid, thus after 30 minutes in the stomach, it is estimated that 87% of the probiotic bacteria ingested will have been evacuated towards the intestine. *L. rhamnosus* R0011 also has excellent resistance to gastric acid, with > 80% survival in a solution with a pH of 3.0.\(^5\) Nonetheless, we will encourage the administration of Lactobifl/ placebo to occur within 30 minutes of mealtime to avoid ingestion when the stomach pH may potentially be <3.0.

Resistance to Biliary Salt: Experiments were conducted in the following fashion: 2 tubes were inoculated with the same concentration of *L. helveticus* R0052 or *L. rhamnosus* R0011 and incubated at 37°C, one contained bile at 0.3% and a control. The turbidity of the resultant solution quantified by the measure of the wavelength at 600 nm, was used to reflect growth. The results showed that the bile exercises an inhibiting effect on the growth of both *L. helveticus* R0052 and *L. rhamnosus* R0011, but both strains are capable of surviving in the presence of the high concentration of bile.\(^5\)

Adherence to Epithelial Cells: *L. helveticus* R0052 and *L. rhamnosus* R0011 show very strong adhesion to the surface of epithelial cells.\(^5\)

Action on the Intestinal Epithelial Lining and Microflora: *L. rhamnosus* R0011 has been demonstrated to stimulate the proliferation of intestinal epithelial cells.\(^5\) This stimulation might confer protection against potentially pathogenic agents and aid in replacing damaged epithelial cells thereby reinforcing the physical barrier which separates the intestinal pathogens from the general blood circulation.

Inhibition of Pathogen Growth: In vitro research has found that both *L. helveticus* R0052 and *L. rhamnosus* R0011 prevent injury of the epithelial cell barrier induced by attaching-effacing bacterial enteropathogens.(12) There is also in vitro evidence that a non-viable constituent derived from *L. helveticus* R0052 is effective in interrupting the infectious process of intestinal pathogens.(13)

Stimulation of the Immune System: *L. helveticus* R0052 and *L. rhamnosus* R0011 have the capacity to activate in vitro the multiplication of immune cells.\(^5\) On contact with these probiotic agents, immune cells isolated from a mouse’s spleen show a dose-dependent proliferation response. This response appears to be greatest with *L. rhamnosus* R0011. On the other hand, *L. helveticus* R0052 induces mitogenic and polyclonal activation of B lymphocytes in vitro, stimulating the release of antibodies against pathogens. *L. helveticus* R0052 additionally stimulates the production of cytokines (Tumour Necrosis Factor alpha and interleukin-6) by macrophages.

Toxicology
Animal and human safety studies were performed in 1992-93 by Drs. Smirnov and Khchenko in the Ukraine. The results were reported directly to Institut Rosell Inc.\(^5\) They sacrificed 25 rats following 14 days of Lacidofil administration.\(^5\) Compared with 15 controls, no differences were detected. In the intestines, the Lacidofil treated rats had greater microbial counts but significantly less hemolytic and non-lactose utilizing bacteria indicating a reduction in potentially pathogenic bacteria. A 28-day toxicity study in 10 rats who received 10 times the maximal human recommended dose found no changes indicative of toxicity. When a dose 3500 times the recommended daily human dose was given for 70 days, there were no deaths, adverse reactions, or gross anatomical changes.\(^14\)

Lacidofil has been sold as a dietary supplement or a pharmaceutical product as a powder in capsules or in sachet format in a number of countries since 1995. During the period of March 2003 to March 2008 exposure to Lacidofil was estimated to include 12,606,701 patients.\(^15\) A total of 6 non-serious spontaneous case reports related to Lacidofil were collected by the Post-Marketing Surveillance system which included a search in “REACTIONS,” “MEDLINE,” “EMBASE” and “INIST” using the search criteria “lactobacillus.” They were:

<table>
<thead>
<tr>
<th>Country</th>
<th>Source</th>
<th>Gender</th>
<th>Age</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>Naturopath</td>
<td>F</td>
<td>56</td>
<td>Pain, swelling, redness, itching, tenderness of outer labia</td>
</tr>
<tr>
<td>USA</td>
<td>Naturopath</td>
<td>F</td>
<td>55</td>
<td>Pain, swelling, redness, itching, tenderness of outer labia</td>
</tr>
<tr>
<td>USA</td>
<td>Naturopath</td>
<td>M</td>
<td>65</td>
<td>Rash in groin area</td>
</tr>
<tr>
<td>Poland</td>
<td>Competent authority</td>
<td>M</td>
<td>Child</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>Poland</td>
<td>Consumer</td>
<td>Child</td>
<td></td>
<td>Diarrhea</td>
</tr>
<tr>
<td>Poland</td>
<td>Health professional</td>
<td>Child</td>
<td></td>
<td>Macular rash</td>
</tr>
</tbody>
</table>

**Lacidofil – Research by Dr. Sherman’s Lab**

**Lacidofil inhibits enterohaemorrhagic Escherichia coli O157:H7**: Lacidofil administration reduces epithelial injury following exposure to Escherichia coli O157:H7 and E. coli O127:H6.\(^16\) When human epithelial cells were treated with protein extract alone, infected with E. coli O157:H7, or pretreated with surface layer protein extract from Lacidofil prior to infection,(13) pre-treatment with protein extracts decreased pathogen adherence and attaching-effacing lesions in addition to preserving the barrier function of monolayers. Thus, a non-viable constituent derived from a Lacidofil may prove effective in interrupting the infectious process of an intestinal pathogen. Additionally, pretreatment with the probiotic *L. rhamnosus* reduced morphological changes and diminished the number of attaching and effacing lesions induced in response to EHEC O157:H7 infection.(12) There was a corresponding attenuation of the EHEC-induced drop in electrical resistance and the increase in barrier permeability assays. In addition, *L. rhamnosus* protected epithelial monolayers against EHEC-induced redistribution of tight junction proteins. Collectively, these findings provide in vitro evidence that treatment with *L. rhamnosus* could be an effective treatment for preventing injury by enteropathogens.

**Ability of Lacidofil to prevent Campylobacter jejuni disease**: Human colon and embryonic intestine epithelial cells were pretreated with Lacidofil and then infected with two C. jejuni
pathogens. (17) *L. helveticus* strain R0052 reduced *C. jejuni* invasion by 35-41%. It also adhered to both epithelial cell types, which suggest that competitive exclusion could contribute to protection by probiotics.

**Ability of Lacidofil to ameliorate the effects of Inflammatory Cytokines:** Following *L. rhamnosus* inoculation, interferon-gamma priming, and 24 hour tumour necrosis factor-alpha stimulation, epithelial cells maintained transepithelial electrical resistance and zona occludens-1 distribution. (18) *L. rhamnosus* diminished the nuclear translocation of p65. These findings indicate that *L. rhamnosus* ameliorates the effects of pro-inflammatory cytokines on epithelial barrier integrity and inflammation mediated, at least in part, through inhibition of tumour necrosis factor signaling.

**Animal Studies**

**Ability of Lacidofil to colonize and reduce gastric inflammation in *H. pylori*-infected mice:** Mouse pretreatment with Lacidofil suppresses colonization by *H. pylori*. (4) Thirty mice were divided into four groups: Group A was fed sterile water, group B received probiotics in sterile drinking water, group C was challenged orogastrically with *H. pylori*, and group D was pretreated with probiotics in drinking water prior to and following challenge with *H. pylori*. Probiotics in drinking water did not affect the overall well-being of mice (Group B). Pretreatment with probiotics reduced the number of mice with *H. pylori* growth from stomach homogenates and the percentage of mice with moderate-severe *H. pylori*-induced inflammation in the gastric antrum was reduced (71% to 29%).

**Lacidofil reduces the effects of *Citrobacter rodentium* infection in mice:** Mouse pretreatment with Lacidofil attenuates *C. rodentium*-induced colonic disease. (5) Mice were administered sterile drinking water, Lacidofil in sterile drinking water, maltodextrin in sterile drinking water, orogastric *C. rodentium*, or maltodextrin in sterile drinking water for 1 week before *C. rodentium* infection, or they were pretreated with Lacidofil for 1 week before challenge with *C. rodentium*. Mice that received Lacidofil remained healthy. Dr. Sherman subsequently found that *C. rodentium* infection in newborn mice causes death and that probiotics promote survival. (19) Infection with *C. rodentium* was induced at postnatal day 14 however a subset of mice were pretreated orally with Lacidofil starting at 7 days. Survival was maintained by daily treatment with Lactobacilli. Weight loss, colonic epithelial cell hyperplasia, mucosal barrier dysfunction, and elevated serum corticosterone levels in *C. rodentium*-infected mice were ameliorated by Lacidofil.

**Lacidofil prevents bacterial translocation and improves intestinal barrier function**: (7) Rats were subjected to either water avoidance stress or sham stress for one hour per day for 10 consecutive days. Additional animals received seven days of Lacidofil in the drinking water prior to stress and remained on these probiotics for the duration of the study. 70% of rats subjected to water avoidance stress had bacterial translocation to mesenteric lymph nodes while there was no bacterial translocation in controls. Water avoidance stress induced increased ileal short circuit current was reduced with Lacidofil whereas there was no impact on altered conductance. Pretreatment with Lacidofil completely abrogated water avoidance stress induced bacterial adhesion and prevented translocation of bacteria to mesenteric lymph nodes. Thus, Lacidofil prevents stress induced intestinal abnormalities.
**Lacidofil normalizes corticosterone release and improves colonic dysfunction**<sup>(20)</sup>: Maternal separation pups were separated from the dam for 3 hours per day from days 4 to 19; non-separated pups served as controls. Twice per day during the separation period, Lacidofil was administered to maternal separation and non-separated pups; vehicle-treated pups received saline. There was increased adhesion and penetration of total bacteria in maternal separation pups, but a significant reduction in Lactobacillus species. Probiotic administration ameliorated the maternal separation-induced gut functional abnormalities and bacterial adhesion and penetration at both day 20 and 60, and reduced the elevated corticosterone levels at day 20. Thus altered enteric flora is responsible for colonic pathophysiology. Lacidofil improves gut dysfunction in part by normalization of the hypothalamic-pituitary-axis activity.

**Human Clinical Trials**

In a randomized trial in Prague of 113 outpatients aged 1-6 years with diarrhea, 1 capsule of Lacidofil (2 x 10<sup>9</sup> CFU) administered daily resulted in normalization of stool consistency more rapidly than placebo (4.0 vs 5.5 days).<sup>(21)</sup> There was no change in stool frequency. Rotavirus accounted for 49% of the cases. In children treated with Lacidofil there was a significant reduction in abdominal gas causing severe distention, P=0.03. In a non-randomized study of 75 children in 2 hospitals in Prague,<sup>(22)</sup> 33 children were administered 1-3 capsules (2 – 6 x 10<sup>9</sup> CFU) of Lacidofil per day for 10-14 days and 42 received clay and smectite. Lacidofil resulted in more rapid resolution of diarrhea (3.1 vs 6.8 days).

In a recent open label clinical study, the ability of *L. rhamnosus* R0011 and *L. acidophilus* R0052 to survive during the passage through the human digestive tract was evaluated.<sup>(23)</sup> Fourteen healthy patients were administered Lacidofil in addition to bacterial spores of *B. stearothermophilus* as a transit marker. Quantification of both strains and the bacterial groups in the feces were performed in a pre-treatment exclusion period, the consumption period and during a 12 day wash-out period. The mean population of *L. rhamnosus* R0011 found in the feces after the consumption period was high. The dominant microbiota of the stool (*Clostridium coccoides, Bifidobacterium* sp., *Bacteroides* sp., and *Clostridium leptum* groups) was not significantly altered by the consumption of Lacidofil. In a prospective study, 57 children aged 1 – 16 years were randomly assigned to receive Lacidofil while they took a course of antibiotics.<sup>(24)</sup> Diarrhea occurred in 7% of children taking Lacidofil vs. 37% of children in the control group. Clostridial toxins were detected in the stool of 7% in the Lacidofil group vs. 43% of the control group.

A controlled study of 248 children with AGE demonstrated that Lacidofil supplementation (1 capsule, 3 times daily for 14 days) increased phagocytosis, as well as levels of IgA (0.59 ± 0.12 g/l before treatment, 0.68 ± 0.08 g/l after treatment) and IgG (6.34 ± 0.31 g/l prior to treatment, 7.62 ± 0.43 g/l post treatment).<sup>(25)</sup> Conversely, a controlled study showed no significant increase in saliva or stool IgA concentrations after a lower dose of Lacidofil supplementation (1 capsule, once daily for 10 days) in infants with AGE.<sup>(21)</sup>
A summary of human studies with gastrointestinal disorders is provided (26):

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study type</th>
<th>Study size</th>
<th>Dosing regime</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic-associated diarrhoea</td>
<td></td>
<td>36 children with mucoviscidosis (18 conventional therapy with Lacidofil + 18 conventional therapy alone)</td>
<td>age &lt;12 months: 1 capsule qd, 1-3 yrs: 1 capsule bid, 3-12 yrs: 1 capsule tid, &gt;12 yrs: 2 capsules tid</td>
<td>decreased AAD development in children with Lacidofil; improved intestinal microbial balance</td>
</tr>
<tr>
<td>Arayev and Kononenko, 2009</td>
<td>randomised</td>
<td>34 children aged 10 months-3 yrs with respiratory pathology (16 antibacterial therapy with Lacidofil + 16 antibacterial therapy alone)</td>
<td>age &lt;1 yr: 1 capsule qd, 1-3 yrs: 1 capsule bid for 2-4 weeks</td>
<td>decreased incidence and duration of AAD; more normalized intestinal microbiome following therapy</td>
</tr>
<tr>
<td>Marushko et al., 2007</td>
<td>randomised</td>
<td>244 children aged 0-17 yrs with acute respiratory, urinary, or digestive exacerbations (117 antibiotic therapy with Lacidofil + 127 antibiotic therapy alone)</td>
<td>age &lt;1 yr: 1 capsule qd, 1-3 yrs: 1 capsule bid, 3-12 yrs: 1 capsule bid or tid, &gt;12 yrs: 1-2 capsules tid for 14-21 days</td>
<td>lower incidence/decreased duration of AAD with Lacidofil; decreased <em>Clostridium difficile/toxin carriage with Lacidofil</em></td>
</tr>
<tr>
<td>Maydannik et al., 2010</td>
<td>randomised</td>
<td>59 children aged 3-18 yrs with pulmonary TB (23 anti-TB chemotherapy with Lacidofil + 36 anti-TB chemotherapy alone)</td>
<td>3 capsules/day for 1 month, 6 capsules/day for 1 month</td>
<td>reduced C. difficile toxins in stool with 6 capsules/day; alleviated gastrointestinal symptoms; normalized stool; disappearance of glossitis</td>
</tr>
<tr>
<td>Patsera et al., 2010</td>
<td>randomised</td>
<td>214 adults with respiratory tract infections on antibiotics (103 Lacidofil + 111 placebo)</td>
<td>1 capsule bid for 14 days vs. placebo (1 capsule bid for 14 days)</td>
<td>improved bowel frequency and consistency with Lacidofil; no difference in AAD occurrence (possibly too small dose of Lacidofil given)</td>
</tr>
<tr>
<td>Song et al., 2010</td>
<td>randomised</td>
<td>35 adults with <em>H. pylori</em> associated duodenal ulcer; (20 RAB/AMO/CLA with Lacidofil + 15 RAB/AMO/CLA alone)</td>
<td>2 capsules tid for 20 days</td>
<td>increased eradication of <em>H. pylori</em> with Lacidofil; faster alleviation of dyspeptic symptoms; improved duodenal mucous coat; improved restoration of intestinal microbiota</td>
</tr>
<tr>
<td>Halicobacter pylori eradication</td>
<td></td>
<td>45 children with <em>H. pylori</em> (25 anti-<em>Helicobacter</em>/therapy with Lacidofil + 20 anti-<em>Helicobacter</em> therapy alone)</td>
<td>1 capsule bid for 20 days</td>
<td>decreased incidence of AAD with Lacidofil</td>
</tr>
<tr>
<td>Babik, 2007</td>
<td>randomised</td>
<td>49 adults with <em>H. pylori</em> (25 triple therapy with Lacidofil + 24 triple therapy alone)</td>
<td>2 capsules bid for 10 days</td>
<td>increased eradication of <em>H. pylori</em>; increased healing of ulcers</td>
</tr>
<tr>
<td>Gnaytenko et al., 2009</td>
<td>non-randomised</td>
<td>841 adults with <em>H. pylori</em> (192 PPI/CLA/AMO + 241 PPI/TET/TNZ/ bismuth salts + 53 according to antibiogram + 102 according to antibiogram + 53 PPI/CLA/AMO with Lacidofil)</td>
<td>2 capsules bid for 20 days</td>
<td>increased eradication of <em>H. pylori</em> with Lacidofil</td>
</tr>
<tr>
<td>Vdovychenko et al., 2008</td>
<td>controlled</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ziemniak, 2006</td>
<td>open label</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paediatric gastrointestinal diseases</td>
<td></td>
<td>248 paediatric patients with acute intestinal infection (225 conventional treatment + 23 conventional treatment with Lacidofil)</td>
<td>1 capsule tid for 14 days</td>
<td>restored intestinal microbiome with probiotic; improved immune status (increased phagocytosis); increased IgA/IgG with probiotic</td>
</tr>
<tr>
<td>Skorodumova et al., 2007</td>
<td>controlled</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Health Canada and Food and Drug Administration (FDA) Issues: An IND application is under review by the FDA (reference #: BB-IND 14190) and we are compiling the necessary data to respond to the FDA’s requests. A Clinical Trial Application has already been approved by Health Canada (CTA #136815) for the pilot study on which the current protocol is based.

Pediatric Dosing: The optimal pediatric dose is unknown but the minimum effective dose for therapeutic purposes has been reported to be a daily intake of $10^6 - 10^9$ CFU. While a meta-analysis concluded that a positive linear association exists between the log of the probiotic dose and the reduction in diarrhea duration, no optimal CFU/kg dose has ever been determined. Comparative studies to determine the minimum effective and optimal doses, frequency of administration, delivery vehicles, and duration of treatment are needed. Our three arm (placebo, low dose, high dose) probiotic clinical trial has found no trend towards increased side effects with the higher ($8 \times 10^9$ CFU/day) dose. Thus, to ensure that a sufficient dose is administered, the high dose ($8 \times 10^9$ CFU/day) will be employed.
APPENDIX 2: Discharge Instructions

**Medication**
Please administer the medication we have provided you with TWICE DAILY for the next 5 DAYS. The medication should be given, mixed with 1 ounce (30 mL) of an unfrozen beverage (containing no ice crystals), at mealtime in the morning and again in the evening. It also recommended that you do not mix the medication with carbonated or highly acidic beverages. Each medication should be separated by 12 hours (minimum of 8 hours) and taken within 30 minutes of food/drink. It is very important that ALL 10 MEDICATION DOSES are administered.

If your child vomits the medication within 15 minutes of administration, please repeat the dose. If he/she vomits a second time during the same dose, do not repeat again; wait until the next dose is scheduled.

**Diary**
Please record any information related to visits to health care providers, ongoing vomiting and diarrhea, daycare and work attendance, monetary expenses, and time spent related to the child’s illness. This information will be helpful when completing the follow up phone calls or email surveys.

**Follow Up Phone Calls/Email Surveys**
Every day until your child’s symptoms resolve, you will receive a phone call or email survey asking questions related to your child’s health and progress. You will also receive a survey/phone call on Day 5 and Day 14 of the study, regardless of your child’s current symptoms.

**Stool Sample Collection (if you have agreed to provide additional samples)**
Please use the gloves, collection container and stick/basinet provided to collect the first available stool sample from your child (and again on days 5 and 28 - we will call/email reminders). Remember to collect the sample in the appropriately labeled container (Day 0, Day 5, or Day 28). Once collected in the provided container, place the container in the bag provided and store in your refrigerator until the sample is picked up. Please call the following telephone number for sample pick up as soon as possible: (XXX) XXX-XXXX.

**Indications to see a health care provider**
 a. Blood in vomit
 b. Bilious (green) vomit
 c. Constant vomiting or diarrhea
 d. Blood in stools
 e. Signs of dehydration
 f. Dry tongue or mouth (dry lips do not indicate dehydration)
 g. Sunken eyes
 h. Decreased urine frequency and volume
 i. Decreased tears
 j. Rapid, deep breathing
 k. Very sleepy, hard to arouse, little interaction with surroundings
 l. A very bad headache
m. A very sore neck  
n. Worsening abdominal pain  
For additional information, please review the handouts you have been provided on “diarrhea”, “dehydration”, or “gastroenteritis”.
**APPENDIX 3: Fecal Secretory IgA Procedures**

**Fecal Secretory IgA:** Processing will be performed as previously described. The frozen fecal samples will be defrosted on ice and 10% weight to fecal volume homogenates will be made by weighing 1 gm of feces, adding 9 mL of phosphate buffered saline with a pH of 7.4 and homogenizing this suspension for 10 minutes in a stomacher (IUL Instruments). One mL of the homogenized suspension will be directly fixed in 3 mL of freshly prepared 4% weight to volume paraformaldehyde in phosphate buffered saline and incubated overnight at 40°C. Fixed samples will then be separated into aliquots and stored at -80°C until they are used for the analysis of secretory IgA.

The analysis of secretory IgA will be performed similarly to what has been previously described. NUNC 96-well Immuno-maxisorp plates will be coated overnight at 4°C with mouse α human secretory component (Sigma, clone GA-1), 1:10,000 in phosphate buffered saline. After thoroughly washing with wash buffer (0.005% Tween-20 in phosphate buffered saline), the plates will be incubated for one hour at room temperature with phosphate buffered saline containing 1% of bovine serum albumin to block nonspecific protein binding sites. Supernatants of the fecal homogenates, defrosted on ice, vortexed, and centrifuged at 16,000 x g for 5 minutes at 4°C will be diluted in phosphate buffered saline/bovine serum albumin between 2,500 and 125,000 times. Purified human IgA isolated from Colostrum (Sigma I-1010) will be used as the standard calibration curve and serial diluted in phosphate buffered saline/bovine serum albumin starting from 80 μg/L. After blocking, the plates will be washed and the samples and standards incubated for two hours at room temperature. Plates will then be washed and biotin conjugated mouse α human IgA1/IgA2 monoclonal antibody (Pharmingen, clone G20-359) will be added to the plates at a concentration of 1 mg/L phosphate buffered saline/bovine serum albumin. After one hour of incubation at room temperature, the plates will be washed again and finally incubated with Streptavidin conjugated horseradish peroxidase (CLB, M2051), 1:10,000 diluted in phosphate buffered saline/bovine serum albumin for 30 minutes at room temperature. Plates will then be washed and incubated with 1-step Ultra TMB substrate solution (Pierce, 34028), 2 times diluted with 0.1 mol/L sodium acetate, pH 5.5 (Merck, 1.06268). The enzymatic color development will then be stopped by adding stop solution (1.8 mol/L H2SO4), and then the absorbance will be measured at 450 nm in a plate reader, secretory IgA concentrations of the fecal samples will be calculated from a calibration curve. Samples will be assayed on two consecutive days in duplicate in 2 different dilutions.
### APPENDIX 4: Study Subject Timeline

| Day #0       | - Present to ED  
|             | - Triaged → potentially eligible children identified  
|             | - Clinical Research Nurse Coordinator applies inclusion/exclusion criteria  
|             | - If eligible, consent will be requested  
|             | - Consent obtained  
|             | - Data collection form completed  
|             | - Randomization performed (www.randomize.net)  
|             | - Medication administered in ED  
|             | - Study drug kit provided to family  
|             | - Diary and return envelope provided  
|             | - Discharge teaching and instructions provided  
|             | - Stool sample obtained  

| Day #1       | - Administer study medication twice daily  
|             | - Contacted by study coordinator (method to be selected by family – phone, e-mail, survey) for data collection  

| Day #2       | - Administer study medication twice daily  
|             | - Contacted by study coordinator for data collection  

| Day #3       | - Administer study medication twice daily  
|             | - Contacted by study coordinator for data collection  

| Day #4       | - Administer study medication twice daily  
|             | - Contacted by study coordinator for data collection  

| Day #5       | - Administer last dose of study medication  
|             | - Contacted by study coordinator for data collection & compliance assessment  
|             | - Calls continue while symptomatic  

| Day #14      | - Contacted by study coordinator regarding need to return unused medication and to complete blinding assessment  

| Day #21      | - Chart reviewed to confirm caregiver report and diary information  

APPENDIX 5: Modified Vesikari Score

The manuscript describing the development, evaluation and validation of the Modified Vesikari Score (MVS) which is being employed as the primary outcome measure of this study (MVS) has been published in Pediatrics (see Publications Section of the grant). (32) Below is a summary of the rationale for the development of a Modified Vesikari Score.

The primary outcome measure, the Modified Vesikari Score is a composite measure which includes several aspects which represent the severity of disease. The original score was first described by Drs. Ruuska and Vesikari in 1990. (33) Since then the score has been extensively employed in clinical research, particularly those evaluating the effectiveness of rotavirus vaccines. (33-44) The score incorporates features of gastroenteritis that are of importance to a variety of stakeholders including parents, children and physicians. It includes the duration of diarrhea and vomiting, the frequency of diarrhea and vomiting, the maximal height of fever, the presence of dehydration and the medical interventions required. The maximal score is 20 points. Most studies have defined severe disease as a score ≥ 11. (35, 38-40, 43, 45-47) Perhaps the best known use of the Vesikari score was in the rotavirus vaccine safety and efficacy evaluation study, published in the New England Journal of Medicine, whose primary outcome, severe rotavirus gastroenteritis, was defined as a score of ≥ 11. (41) In keeping with previous research, we will define moderate gastroenteritis as a score of ≥ 9 points. (48)

**Original Vesikari Score**

<table>
<thead>
<tr>
<th>Points</th>
<th>0</th>
<th>1-4 days</th>
<th>5 days</th>
<th>≥ 6 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea Duration (days)</td>
<td>0</td>
<td>1-3</td>
<td>4-5</td>
<td>≥ 6</td>
</tr>
<tr>
<td>Maximum # of diarrheal stools/24 hr period</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>≥ 3</td>
</tr>
<tr>
<td>Vomiting Duration (days)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>≥ 5</td>
</tr>
<tr>
<td>Maximum # of vomiting episodes/24 hr period</td>
<td>0</td>
<td>1</td>
<td>2-4</td>
<td>≥ 5</td>
</tr>
<tr>
<td>Maximal Recorded Fever*</td>
<td>&lt; 37.0°C Rectal</td>
<td>37.1-38.4 °C Rectal</td>
<td>38.5-38.9°C Rectal</td>
<td>≥ 39.0°C Rectal</td>
</tr>
<tr>
<td>Percent Dehydration</td>
<td>0%</td>
<td>-</td>
<td>1-5%</td>
<td>≥ 6%</td>
</tr>
<tr>
<td>Treatment</td>
<td>None</td>
<td>Rehydration</td>
<td>Hospitalization</td>
<td>-</td>
</tr>
</tbody>
</table>

*Temperatures will be adjusted for location of measurement: 1.1°C will be added to axillary temperatures and 0.6°C will be added to oral temperatures. (49)

Unfortunately, one element of the score, percent dehydration is very difficult to assess accurately, particularly in the ED. It is well known that the clinical assessment of dehydration is inaccurate; (50) thus the difference between the baseline and fully rehydrated weights is considered the gold standard. (51) However, even this is of limited value in the outpatient setting due to difficulties in determining when full rehydration has occurred, the variations that occur due to timing of voiding and stooling, and the difficulties in obtaining repeat weights. Thus, we
have elected to capture another very important outcome measure – the need for future health care use - which can easily be done in the outpatient setting. **We have modified the Vesikari score by replacing percent dehydration by the need for an unscheduled future health care visit within 2 weeks.** This modification is supported by evidence that (1) the demand for professional medical care correlates with clinical severity - 41% with severe versus only 5% of mild cases require hospitalization.(52) (2) Modifications of the original Vesikari score have been routinely performed when the outcome of dehydration has been unavailable.(52, 53) (3) In the original dataset, the outcome of ≥ 6% dehydration occurred in 6% of the sample. This is similar to gastroenteritis ED revisit data in children 0-4 years of age provided to us by the Pediatric Emergency Care Applied Research Network (PECARN; courtesy of Dr. Marc Gorelick). Of a total of 12,694 visits to 4 PECARN sites, 682 revisits occurred within 2 weeks (5.4%). Based on the above, we have replaced the outcome of percent dehydration with need for future health care provider visits. The original score structure for this variable (0, 2, 3 points) has been maintained.

**0 Points:** No future health care provider visit related to vomiting, diarrhea, fever or fluid refusal  
**2 Points:** Community based health care provider visit related to vomiting, diarrhea, fever or fluid refusal  
**3 Points:** ED health care provider visit related to vomiting, diarrhea, fever or fluid refusal

The calculation of the MVS will be based on data collected via daily telephone or email/survey follow-up during the 2 weeks following the index visit with **all data points employing the ED visit as Time 0** (i.e. the score prior to administration of the first dose – the pre-enrollment score - will not be included in the score calculated for assessment of the study’s primary outcome). Interventions that occur at the index visit (i.e. following randomization) will only be counted if the child remains in the hospital for greater than 2 evenings (i.e. past midnight on 2 consecutive days). Should the latter occur, the child will be deemed to have required both hospitalization and an ED revisit as that is evidence that the child’s disease severity requires hospitalization. This is based on the expectation of an early onset of benefit as a meta-analysis has reported a day #2 reduction of 1.6 stools/day (95% CI: 0.7, 2.6). Thus, the primary outcome will be the presence of moderate-severe disease, as defined by a maximum MVS of ≥ 9 during the 2 week follow-up period.

Our estimate for the development of moderate to severe gastroenteritis in controls is based our 2009 evaluation of the MVS in 455 children aged 3 – 48 months who presented to one of 11 Canadian EDs with < 72 hours of gastroenteritis symptoms.(32) We calculated the MVS employing symptoms that occurred following the ED visit (i.e. using the ED visit as time zero and not including anything that occurred up to and including the ED visit – these symptoms will be employed to calculate a pre-enrollment score) and found that **25% of children had scores consistent with moderate to severe disease following discharge.** This point estimate is lower than previously reported in ED(40, 47) and community populations, (39, 46, 48) as we did not include the symptoms that brought them to the ED (pre-enrollment symptoms). Since the estimate was derived in the same population as the proposed clinical trial and was calculated in a manner identical to that proposed, 25% is likely a very accurate point estimate for the outcome in our control population.

The following is a description of the distribution of the MVS in our prospective cohort.
Examples Describing Calculation of the Modified Vesikari Score
(Including handling of baseline score – i.e. the pre-enrollment score)

Example #1
A 2 year old child who presents with the following symptom complex:

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea for 36 hours</td>
<td>1</td>
</tr>
<tr>
<td>Maximal stools in a 24 hour period = 8</td>
<td>3</td>
</tr>
<tr>
<td>Vomiting for 36 hours</td>
<td>2</td>
</tr>
<tr>
<td>Maximal vomiting episodes in a 24 hour period = 7</td>
<td>2</td>
</tr>
<tr>
<td>Maximal recorded fever = 39.5 axillary</td>
<td>3</td>
</tr>
<tr>
<td>Health care provider visit = emergency department</td>
<td>3</td>
</tr>
<tr>
<td>Treatment = rehydration</td>
<td>1</td>
</tr>
</tbody>
</table>

TOTAL SCORE at ENROLLMENT (i.e. pre-enrollment score) = 15

Following enrollment the caregiver is contacted as per the protocol and we record the following information:

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea for 72 more hours</td>
<td>1</td>
</tr>
<tr>
<td>Maximal stools in a 24 hour period = 2</td>
<td>1</td>
</tr>
<tr>
<td>Vomiting = none</td>
<td>0</td>
</tr>
<tr>
<td>Maximal vomiting episodes in a 24 hour period = 0</td>
<td>0</td>
</tr>
<tr>
<td>Maximal recorded fever = 37.5 axillary (= 38.6 rectal)</td>
<td>2</td>
</tr>
<tr>
<td>Health care provider visit = none</td>
<td>0</td>
</tr>
<tr>
<td>Treatment = none</td>
<td>0</td>
</tr>
</tbody>
</table>
TOTAL MODIFIED VESIKARI SCORE (MVS) for STUDY PERIOD (PRIMARY OUTCOME) = 4

For subject #1, the score of 4 implies that the primary outcome did NOT occur (i.e. did not develop moderate to severe disease following enrollment as the MVS is < 9) despite having a baseline score of ≥ 9. The baseline score of 15 however will be employed as a covariate to enable us to adjust the primary analysis should that be necessary. Moreover a subgroup analysis will be conducted comparing outcomes amongst those with moderate to severe disease at enrollment to those with mild disease at enrollment.

**Example #2**

A 6 month old child who presents with the following symptom complex:

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea for 26 hours</td>
<td>1</td>
</tr>
<tr>
<td>Maximal stools in a 24 hour period = 3</td>
<td>1</td>
</tr>
<tr>
<td>Vomiting = none</td>
<td>0</td>
</tr>
<tr>
<td>Maximal vomiting episodes in a 24 hour period = 0</td>
<td>0</td>
</tr>
<tr>
<td>Maximal recorded fever = 37.5 rectal</td>
<td>1</td>
</tr>
<tr>
<td>Health care provider visit = emergency department</td>
<td>3</td>
</tr>
<tr>
<td>Treatment = rehydration</td>
<td>1</td>
</tr>
</tbody>
</table>

TOTAL SCORE at ENROLLMENT (*i.e. pre-enrollment score*) = 7

Following enrollment the caregiver is contacted as per the protocol and we record the following information:

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea for 7 more days</td>
<td>3</td>
</tr>
<tr>
<td>Maximal stools in a 24 hour period = 12</td>
<td>3</td>
</tr>
<tr>
<td>Vomiting for 36 hours</td>
<td>2</td>
</tr>
<tr>
<td>Maximal vomiting episodes in a 24 hour period = 10</td>
<td>3</td>
</tr>
<tr>
<td>Maximal recorded fever = 38.5 axillary (= 39.6 rectal)</td>
<td>3</td>
</tr>
<tr>
<td>Health care provider visit = emergency department</td>
<td>3</td>
</tr>
<tr>
<td>Treatment = hospitalization</td>
<td>2</td>
</tr>
</tbody>
</table>

TOTAL MODIFIED VESIKARI SCORE (MVS) for STUDY PERIOD (PRIMARY OUTCOME) = 19

For subject #2, the score of 19 implies that the primary outcome DID occur (i.e. the child developed moderate to severe disease following enrollment as the MVS is ≥ 9) despite having a baseline score of < 9. The baseline score of 7 however will be employed a covariate to enable us to adjust the primary analysis should that be necessary. Moreover a subgroup analysis will be conducted comparing outcomes amongst those with moderate to severe disease at enrollment to those with mild disease at enrollment.
APPENDIX 6: Economic Analysis Plan

Prior Experience - Economic Analyses
The research team includes experts in the conduct of economic analyses. Dr. Willan(54) and Mr. Goeree, MA, have successfully collaborated on many studies including economic evaluations in the context of clinical trials(55-62) and multinational studies.(63-65) Moreover, Dr. Willan is an expert on the conduct of cost-effectiveness analyses of RCTs with binary measures of effectiveness employing an interacting covariate,(66) on methods to account for between-country differences in the analysis of cost-effectiveness data, on the value of information, and cost-effectiveness thresholds.(67, 68) The extensive involvement of these co-investigators will ensure that the cost analysis is successfully completed employing innovative methodology.

Preliminary Data - AGE
Dr. Freedman, the Co-PI recently published an economic analysis evaluating the financial impact of ED ondansetron administration to children with AGE.(69) This analysis, which was conducted from both the Canadian and American perspectives, demonstrated that there can be significant clinical and economic benefits when an effective intervention is administered to children with AGE. In fact, we concluded that the administration of ondansetron to eligible children would prevent approximately 4,065 intravenous insertions and 1,003 hospitalizations annually in Canada alone. Moreover, at the current average wholesale price, and employing cost data, its routine administration to eligible children would annually save society US$1.7 million and healthcare payers US$1.2 million. Thus, there is evidence that the implementation of a simple intervention in children with AGE can result in significant economic benefits.

METHODS

Cost-Effectiveness
This specific aim is designed to prospectively collect and analyze data on resource utilization. All children enrolled will participate in this analysis. An economic analysis will be conducted to evaluate the overall cost-effectiveness of routinely administering Lacidofil to eligible children. This analysis will be performed in keeping with current recommendations on the conduct of cost-effectiveness analyses conducted as part of a clinical trial.(70, 71) In this analysis, all costs will be expressed in monetary terms. In our model, the strategies compared will be (1) administering Lacidofil in addition to routine care versus (2) routine care alone. The measure of effectiveness will be avoidance of the primary outcome (i.e. avoiding an episode of moderate-severe disease). Discounting will not be considered given the 14-day time horizon of the trial.

Measurement of Resource Utilization
The study viewpoints will be those of society and the healthcare payer. We will collect patient-specific data on healthcare resource utilization during the child’s condition, namely, the administration of interventions and treatments. The minimum amount of information that is required to address our proposed analysis will be collected. Data forms will integrate the economic questions to minimize repetition, simplify the questionnaires, and maximize follow-up while minimizing missing data. These forms were included in our pilot study. Price weight estimates will be based on national standards and will be performed by staff under the supervision of Mr. Goeree at the Program for Assessment of Technology in Health (PATH) Research Institute at McMaster University. All cost data will be reported adjusted for use of

Version 2.0
Date: August 14, 2013
2013 as the base year. Conversions will be conducted using indices commonly employed to adjust for inflation,(72) and the Statistics Canada Health and Personal Care Consumer Price Index.(73) Direct costs will include medical expenses: hospitalization, ED visits, physician office visits, medications, intravenous solutions, radiographs, and laboratory tests in addition to private out-of-pocket costs. Also included will be professional services, supplies, and equipment associated with the use of these resources. Nonmedical costs will include caregiver-foregone earnings, travel time, parking costs, extra diapers, special foods, ORS, and childcare. Data related to nonmedical costs will be supplied by the parent. When the caregiver misses workdays, foregone earnings will be calculated. The cost of work lost can be calculated directly when hourly wage information is available. If individual hourly wages are unavailable, the annual income and the number of hours worked weekly will be used to determine an average hourly wage. If income information is unavailable, then the value of lost wages will be estimated by applying a postal code adjusted mean wage rate to missed work time, obtained from Statistics Canada. The point estimate charge for Lactobaby administration will be the average wholesale price (AWP). The AWP has been selected as it is almost always greater than the wholesale acquisition cost and lists prices that are much higher than public or private payers are likely to pay, reflecting the opportunity cost.(74)

**Cost Analysis**

An average cost per patient will be calculated for overall, direct and indirect costs per treatment group. The analysis will first determine if one treatment dominates another (i.e. lower costs and better clinical outcomes). If a trade-off exists between costs and outcomes we will estimate the incremental cost-effectiveness ratio (ICER) together with the 90% confidence interval. The ICER is the additional cost at which probiotics provides one additional unit of health benefit (i.e. moderate or severe disease episode avoided), relative to routine care.(75) The incremental net benefit (INB)(76) and the corresponding 90% confidence intervals will be plotted as functions of the threshold value for avoiding a moderate or severe disease episode. Expressing INB in units of cost will allow us to examine cost minimization, incremental cost-effectiveness and INB in a single graph, complete with the corresponding statistical inferences.(77) Because the tests of hypothesis for cost-effectiveness are one-sided (i.e. INB ≤ 0 vs. INB > 0), if the lower limit of the 90% confidence interval is > 0, then you can reject the null hypothesis and limit the type I error probability to 5%. A cost-effectiveness acceptability curve (CEAC) will be calculated. The CEAC, which is the probability that the INB is positive, as a function of the threshold value, provides an excellent means of quantifying the cost-effectiveness of a new healthcare intervention and its uncertainty. A gamma model for cost will be used to account for right skewing.(66) Key cost drivers will be identified.

**Value of Information Analysis**

Value of Information (VOI) methods are a systematic approach to inform optimal research design and prioritization. In health technology assessment, decision makers face situations where, having reviewed the best available evidence of the costs and effectiveness of a new healthcare intervention (i.e. probiotics) relative to existing practice, the new intervention has positive but uncertain net benefit at a decision maker’s threshold value for health effects. Assuming no further evidence from other jurisdictions is expected, decision makers then have 3 options: (1) adopt the new intervention without further research; (2) adopt the new intervention and undertake another trial; or (3) delay the decision and undertake a trial.(78) We will use VOI
at the completion of our trial to determine if further research is potentially worthwhile. (79) This will be achieved by determining if the information from our RCT is sufficient for decision making. (80) A sensitivity analysis will be conducted with respect to the threshold willingness-to-pay and the time horizon.

**Willingness to Pay**

This method of quantifying the valuation of clinical outcomes to the patient/caregiver provides a measure of how much an individual values the particular clinical benefit of treatment. This methodology is gaining popularity. (81, 82) Moreover, because the analysis of INB requires the specification of the willingness-to-pay (WTP) for a unit of effectiveness, (83) we will conduct a willingness to pay evaluation in the ED of The Alberta Children’s Hospital. Formal study design and pilot testing will be performed as has been done previously by Ron Goeree and staff at the PATH Research Institute at McMaster University. (84-89) Caregivers surveyed will be those who bring their child to the ED for symptoms unrelated to AGE. Given their broad demographics this will allow for an accurate representation of society’s willingness to pay for the use of probiotics given a variety of postulated clinical benefits. Surveys will be performed by select groups of trained research volunteers who sole roles are to support research in their respective EDs. This crucial evaluation will allow policy makers to evaluate whether they should provide a supply of probiotic to eligible children upon discharge from the ED. Otherwise caregivers will have to pay out of pocket for the cost of the probiotic agent. At present, insurance companies do not reimburse such expenses. Thus, it is important to know if the valuation of benefits of probiotics outweighs the costs.
APPENDIX 7: Sample Size/Power Analysis

Sample Size
The following formula\(^{(90)}\) for was used to calculate the required sample size \((2N)\) to compare proportions between two different groups where \(N\) is the size of each treatment group:

\[
P_C = \text{Probability in the control group} = 0.25 \quad P = (P_C + P_I)/2
\]
\[
P_I = \text{Probability in the treatment group} = 0.15 \quad Q = 1-P
\]
\[
\Delta = P_C - P_I
\]

\[
N = \frac{2(Z_{a/2}\sqrt{2PQ + Z_{b}\sqrt{P_CQ_C + P_IQ_I}})^2}{\Delta^2} + \frac{2}{\Delta}
\]

\(2N = 670\) participants
\(N=335\)

Power Analysis
Based on a sample size of 670, we obtain the following results for the primary outcome:

<table>
<thead>
<tr>
<th>(P_C)</th>
<th>(P_I)</th>
<th>(\Delta)</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.30</td>
<td>0.21</td>
<td>9%</td>
<td>0.76</td>
</tr>
<tr>
<td>0.30</td>
<td>0.20</td>
<td>10%</td>
<td>0.85</td>
</tr>
<tr>
<td>0.25</td>
<td>0.15</td>
<td>10%</td>
<td>0.90</td>
</tr>
<tr>
<td>0.25</td>
<td>0.16</td>
<td>9%</td>
<td>0.82</td>
</tr>
<tr>
<td>0.25</td>
<td>0.17</td>
<td>8%</td>
<td>0.72</td>
</tr>
<tr>
<td>0.20</td>
<td>0.10</td>
<td>10%</td>
<td>0.95</td>
</tr>
<tr>
<td>0.20</td>
<td>0.12</td>
<td>8%</td>
<td>0.81</td>
</tr>
<tr>
<td>0.20</td>
<td>0.13</td>
<td>7%</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Sample Size Adjustment
Since daily follow-up will be required for 2 weeks, based on previous data\(^{(91)}\) and to err on the conservative side, our calculations assumed:

A- 9% loss to follow-up,
\[
= 670/(1-0.10) = 744
\]

B- 5% drop out and 2.5% drop in rate = 7.5% non-compliance
\[
= 744/(1-0.075)^2 = 868
\]

C- O’Brien-Fleming
\[
= 868 * 1.02 = 886
\]
**APPENDIX 8: Recruitment**

**Site Requirements:** Five eligible sites have been identified within the PERC network. Sites have been selected based on volume and success with participation in prior PERC clinical trials. Specifically, we required a sufficient number of annual visits by children aged 0 - 4 years with gastroenteritis, adequate geographic diversity to ensure generalizability, and a Site Investigator willing and capable of acting as a “champion” for the study at their respective institution.

Number of ED visits for gastroenteritis in children 3 month and 48 months of age at the 5 participating sites:

- **2009:** 8,855
- **2010:** 9,596
- **2011:** 10,399

We have set the minimum accrual estimate at 90% of the data points presented above (9,359). This is likely a low estimate as 2009 to 2011 data have in fact identified a 17% increase.

We have included the IWK Health Centre (Halifax) for several reasons: (1) They enable us to have geographic diversity across Canada (only Maritime site); (2) they have a track record of excellent recruitment, retention and high quality data performance, (3) financial support will be in large proportional to recruitment. Thus, our study Steering Committee has agreed that the selection of IWK Health Centre would represent an excellent study site.

**Percent Presenting November 1 – May 31 (2009 – 2011 data)**

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<thead>
<tr>
<th>Location</th>
<th>Percent</th>
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<tbody>
<tr>
<td>Alberta Children’s Hospital</td>
<td>74%</td>
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<tr>
<td>The Hospital for Sick Children</td>
<td>76%</td>
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<tr>
<td>The Children’s Hospital of Eastern Ontario</td>
<td>78%</td>
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<tr>
<td>Sainte-Justine</td>
<td>67%</td>
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<tr>
<td>IWK Health Centre</td>
<td>75%</td>
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<tr>
<td><strong>TOTAL</strong></td>
<td><strong>74%</strong></td>
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**Percent Meeting Definition of Diarrhea**

Data from The Children’s Hospital and Regional Medical Center ED in Seattle, Washington, reported that of children who presented with diarrhea (N=1,626), the mean number of episodes in the 24 hours prior to presentation was 8 (median = 6).³² Thus, we anticipate that the majority (95%) of children with diarrhea will meet our definition of 3 stools in the preceding 24 hours. However, it is unknown what proportion of children do not have any diarrhea at the time of presentation (i.e. have isolated vomiting). In a 5 year chart review of children diagnosed with gastroenteritis at The Hospital for Sick Children we found that 51% had ≥ 3 diarrheal episodes in a 24 hour period. This is in large part due to the diagnosis of gastroenteritis often being assigned to children who have isolated vomiting. Thus we will use a point estimate of 50%.

**Percent with < 72 hours of Symptoms at Presentation**

Data from Children’s Hospital and Regional Medical Center ED in Seattle, Washington, reported that of children who presented with diarrhea, the median duration at the time of presentation was 3 days.³² Thus, to err on the conservative side, we will assume that 55% of children will have
had diarrhea for > 72 hours at the time of ED assessment. In our 5 year chart review we found that 58% had symptoms for <72 hours. For accrual analysis purposes, we will assume that only 45% of children evaluated will have had symptoms for < 72 hours.

Presence of Hematochezia
Data recently published by the PI (S.F.) from The Hospital for Sick Children(93) reported that over 5 years, only 4% of children had hematochezia, hence a conservative estimate of 5% will be employed.

Other Eligibility Requirements (telephone access, indwelling vascular access line, congenital heart disease, immunosuppressive therapy, immunodeficiency, inflammatory bowel disease, exclusively breastfed, bilious or bloody vomitus, probiotic use, language barrier, soy allergy, pre-existing pancreatic dysfunction, oral or gastrointestinal surgery)
These features are uncommon as individual entities, however, when combined they may have an effect on recruitment, particularly as enrollment will be occurring at tertiary care centers. This may represent up to 20% of children with gastroenteritis.

Percent Presenting Between 8:00 – 24:00
In a database of 3508 AGE visits at The Hospital for Sick Children, 82% of children were triaged between 8:00 – 24:00. As presentation patterns and coverage may vary between institutions and we must account for sick days, our point estimate will be 75%.

Consent
Data from a recently published PERC multi-centre RCT (CanBEST) reported an overall consent rate of 52% (N=1540).(94) The consent rate in our pilot study was 56%. Thus we will estimate a 50% consent rate.

**Summary:** Taking all the above factors into account, our best point estimate is that we will be able to enroll approximately 494 children/year. However, slight alterations in our best estimates result in a reduction to approximately 294 children/year. Our collective research expertise is that recruitment never follows the best point estimates and hence we anticipate that we will require three gastroenteritis recruiting seasons (winter/spring) to achieve our necessary sample size (886).

<table>
<thead>
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<th>Total # visits</th>
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<tr>
<td></td>
<td>9,359</td>
<td>10,399</td>
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<tr>
<td>% November 1 – May 31</td>
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<td>74</td>
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<tr>
<td>% Meeting Definition of Diarrhea</td>
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<tr>
<td>% &lt; 72 hours of Symptoms</td>
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<tr>
<td>% Absence of Hematochezia</td>
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<td>95</td>
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<tr>
<td>% Absence of other exclusion criteria</td>
<td>75</td>
<td>80</td>
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<tr>
<td>% Presenting Between 8-24</td>
<td>70</td>
<td>75</td>
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<tr>
<td>% Consenting</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td><strong>TOTAL ANNUAL ACRUAL/Year</strong></td>
<td><strong>294</strong></td>
<td><strong>494</strong></td>
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Estimates from the Probiotic Pilot RCT: The probiotic pilot RCT that we conducting has screening log data available for analysis. The overall number of children enrolled 50/2235 (2.2% of all children screened) is slightly lower that the estimates above (minimum = 3.1%; best point = 4.7%). This difference is likely due to the requirement of daycare attendance in children included in the pilot. Daycare attendance was the only identifiable exclusion criteria in 187 children screened. Given the 56% consent rate in our pilot (50/90), if these additional 187 patients were eligible, as would be the case with the proposed RCT, then the overall number of enrolled children would be 155, yielding an eligibility rate of 6.9%.
APPENDIX 9: Data Management, Training, and Site Monitoring

Data Management

Data management will be performed by the Women & Children’s Health Research Institute's Clinical Research Informatics Core (CRIC), located at the University of Alberta in Edmonton, Alberta. CRIC uses a state-of-the-art computer and network infrastructure housed within a dedicated server room with fully redundant power and cooling systems. Access to the server room is restricted to authorized staff. Servers are backed up daily and tapes are stored in a fireproof safe in the Faculty of Medicine and Dentistry at the University of Alberta. Communication over public networks is encrypted using 128 bit SSL encryption and direct access to the servers is only available to users located within the CRIC office or via the Faculty’s virtual private network (VPN).

In accordance with the Tri-Council Policy Statement, direct subject identifiers will not be stored in the study database. A REDCap data collection application will be employed. It is protected by appropriate security measures which include password protection, enforced password complexity, enforced password changes, inactivity timeout and user lockout following failed login attempts. Inactive user accounts are automatically suspended by the system. Defined user roles ensure that data access is appropriate to the user’s job function. A detailed audit trail monitoring system will be instituted to document the person, time, and nature of data access, with flags for aberrant use and "abort" algorithms to end questionable or inappropriate access.

A complete data management plan will be designed in conjunction with the Clinical Research Informatics Core (CRIC) to ensure that the information collected will enable us to conduct all required analyses. This management plan will be incorporated into the Manual of Operations. Case report forms (CRFs) will be developed for each data recording step (e.g., ED visit data, telephone follow-up data, stool specimen collection data). Paper CRFs will be completed by study personnel or families as appropriate. Data from the paper CRFs will be entered into a Web-accessible database (REDCap(95)) by study personnel at each site. The data entry interface will include range and logic checks to enable the conduct of exploratory data analysis as data entry is ongoing (and will be repeated as the first stage of the analysis) to minimize data entry errors.(96) Following data entry a data quality audit will be performed to ensure that data is complete and accurate. Data will be entered in a timely fashion, with all CRFs entered within 7 days of completion. Automatic alerts will be triggered by incomplete, out-of-range, or logically inconsistent data. Paper CRFs will be stored in a secure fashion at participating sites. They will be available for review by site monitors (see below).

A complete quality assurance and site monitoring plan will be designed in conjunction with CRIC when the Manual of Operations is developed.

Training

A training module will be developed for all study personnel, including Site Investigators, Site Physicians, and Clinical Research Assistants/Nurses. The specific training will be tailored to the role of the individual in the study. All Clinical Research Assistants/Nurses will be familiar with Good Clinical Practice guidelines prior to collecting any study related data, and all study
personnel must meet human subjects and research ethics training requirements at their home institution. Prior to the start of the study, there will be an in-person training session for all Site Investigators, Clinical Research Nurses, and Research Assistants. Site Investigators and Coordinators will then be responsible for ensuring the training of other personnel involved in study procedures at their sites. Written documentation of such training will be required. Refresher training sessions will be conducted a month prior to each enrollment season. Monthly conference calls during the enrollment seasons will be conducted for site investigators, coordinators, and research assistance, during which study procedures will be reviewed and issues discussed. Standardization of data collection procedures will be developed and reinforced during personnel training sessions.

Site Monitoring Plan
Site monitoring visits will be coordinated by the Clinical Research Project Manager, to ensure that all regulatory requirements are being met and to monitor the quality of the data collected. Each site will have a remote monitoring visit within 2 months of the start of patient enrollment during which, patient forms and original source documents will also be inspected. In addition, each site will have an on-site visit at the end of the first enrollment season. Essential document binders and CRFs will be reviewed. 100% of informed consent documents, 100% of inclusion/exclusion criteria, and 100% of the serious adverse events will be reviewed. In addition, the first 10 subjects at a site will have 100% of the CRFs reviewed and a minimum of 10% of the subjects will have 100% of the CRFs reviewed thereafter. Additionally, site monitors will assess and verify adequate patient accrual and review regulatory documents.

Remote site monitoring will also be performed by the Clinical Research Project Manager to verify selected data elements. This will require the clinical site staff to locate the relevant source document, make a copy, de-identify the material, and send the copy to the ACH and CRIC where the information will be compared with data stored in the REDCap system. Remote monitoring reports will be distributed to the Study Investigators and Coordinators. The documents will be retained in accordance with federal requirements.
APPENDIX 10: Probiotic and Administration of Antibiotics

June 9, 2006

Re: Probiotics and timing of antibiotics

We performed a study where we gave hospitalized patients on antibiotics, capsules of
Lacidofil provided by Rosell Institut Lallemand containing Lactobacillus acidophilus and
Lactobacillus rhamnosus. The patients had all been prescribed antibiotics both oral and
intravenous formulations particularly those to which the lactobacilli was sensitive. The
study was performed to determine whether the organisms could survive in this setting.
The probiotic capsules or sachets were given to the patients with their meals to try to
protect them from destruction in the stomach by the otherwise acidic conditions of
the stomach. The antibiotics were given on different schedules and there was no attempt to
time the probiotic doses related to the receipt of antibiotics.

We discovered that we could recover viable Lactobacilli in the stool in the patients and
that the recovery appeared to depend mainly on the initial dose of probiotic given and did
not appear to be correlated with the timing of the antibiotic doses.

Should you require any further information do not hesitate to contact me.

Yours truly,

Sandra Dial, MD, MSc
Assistant Professor, McGill University
Department of Medicine.

3755, ch. de la Côte-Sainte-Catherine, Montréal, Québec H3T 1E2
TP (514) 340-8222  F (514) 340-7510
www.jgh.ca
APPENDIX REFERENCES


# Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis: Randomized Controlled Trial

**Sponsor:** The University of Calgary

**Principal Investigator:** Dr. Stephen Freedman

The Alberta Children’s Hospital

**Co-Investigators:**

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<tr>
<th>Dr. David Johnson</th>
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<th>Dr. Katrina Hurley</th>
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<th>Dr. Andrew Willan</th>
<th>Dr. Marc Gorelick</th>
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<td>Medical College of Wisconsin</td>
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<th>Dr. David Schnedower</th>
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<th>Dr. Yaron Finkelstein</th>
<th>Dr. Linda Chui</th>
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<th>Dr. Bonita Lee</th>
<th>Dr. Xiao-Li Pang</th>
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<th>Dr. Marie Louie</th>
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<th>Dr. Naveen Poonai</th>
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**Funding Agency:** Canadian Institutes for Health Research (CIHR)

**Other Support:** Lallemand Health Solutions

Dr. Thomas Tompkins
Protocol Version: 7.0
Protocol Date: November 1, 2017
1.0 THE NEED FOR A TRIAL

1.1 WHAT IS THE PROBLEM TO BE Addressed?

The burden of acute gastroenteritis (AGE) on children and their families continues to be enormous. It accounts for 1.7 million pediatric emergency department (ED) visits annually in the United States and nearly 240,000 in Canada.\(^1\) Children often suffer from prolonged\(^2\) and severe illness; amongst hospitalized Canadian children, 19% have clinical sepsis, 7% seizures and 4% require intensive care unit admission.\(^3\) In a study that we conducted at 11 Canadian EDs, 51% of children experienced moderate to severe disease.\(^4\) Parents rate such episodes as being equivalent to a 10 day admission (moderate) and persistent moderate hearing loss (severe).\(^5\) The burden is augmented by the 50% household transmission rate\(^6,9\) and 42% prolonged work absenteeism rate.\(^7\) Apart from supportive care, health-care providers have little to offer to relieve suffering.\(^8\)

Probiotics, which are defined as viable microbial preparations that have a beneficial effect on the health of the host,\(^9\) represent a rapidly expanding field. While they are available as over-the-counter products, according to the National Institutes of Health, the Food and Drug Administration has not yet approved a single agent for any health claims.\(^10\) Further, a 2012 meta-analysis concluded that there is limited data to support their indications and no published pediatric gastroenteritis trials reported on side effects.\(^11\) Thus, understanding the benefits and side effects of probiotics is crucial before widespread use can be endorsed. Although probiotic clinical trials have been performed,\(^12\) only one (still unpublished) has been ED based.\(^13\) Most studies to date have been significantly flawed and guidelines do NOT endorse their use stating that well-controlled human trials are needed.\(^14\) Consequently, we and others have found that they are rarely used in clinical practice.\(^4,15-19\) Reasons cited include (1) questionable clinical meaning to the outcomes evaluated thus far; (2) absence of studies in the appropriate patient population, and (3) a lack of confidence in the quality of probiotic agents studied.\(^19\)

Our proposed definitive trial is necessary because it addresses the weaknesses and deficiencies in prior studies. We (1) focus on the burden of disease and outcomes of relevance to the infected child and his/her caregiver, (2) study outpatient children (>95% of those infected), (3) employ rigorous methodology and a sample size significantly larger than any prior study,\(^12\) (4) will evaluate the side effect profile and conduct subgroup analyses by etiologic agent, and (5) will be free of bias (i.e. industry funding).\(^20,21\) These elements have not been previously addressed by any pediatric probiotic clinical trial. We will additionally investigate several novel domains: (1) the economics of widespread probiotic use and (2) the in vivo impact on immunoglobulin secretion.

This study will address (1) the needs of the medical community, which is aware of the widening gap between the number of important pediatric and adult trials\(^22,23\) and (2) the interest of caregivers in “probiotics” - 71% are aware of the term; 31% believe they may be beneficial in children with diarrhea, and > 90% would administer a probiotic if it could make their child better.\(^24\) Furthermore, our pilot study has provided promising preliminary data and has proven the feasibility of our methods. Thus we are poised to conduct a randomized controlled trial (RCT) that will definitively determine if meaningful benefits are derived from probiotic use and will provide critical information regarding their mechanism of action. This information will impact on practice, the burden of disease, and ensure that children receive the best care possible. The results of our proposed RCT will enable guidelines to either clearly endorse or recommend against the routine use of a probiotic agent in children with AGE.

We also hypothesize that the therapeutic benefits of probiotics in children with AGE vary by infecting pathogen (Appendix 1 Pathogen-Specific Effectiveness). We have assembled a team to bridge the gap between the clinical RCT team, molecular diagnostics, and immunologic to quantify the pathogen-specific effects of probiotics. The latter is likely because there are distinct mechanisms (e.g. invasive, inflammatory, non-inflammatory) by which pathogens cause clinical symptoms.\(^25\) Similarly, probiotic effects are exerted through multiple modes-of-action (e.g. direct antimicrobial activity, competitive exclusion, immune response stimulation, inhibition of virulence gene or protein
The simultaneous evaluation of pathogen-specific effects on clinical, microbiological and immunological levels has not previously been performed.

The knowledge gained through this multi-faceted approach will inform understanding of the probiotic-host-pathogen interactions that are responsible for improved clinical outcomes in children with AGE. Our study population, outpatient children, is both the main group of patients who suffer from AGE as well as the main consumer of probiotics. Thus, our findings will be relevant and ready for translation into clinical care while simultaneously opening up avenues for future research.

1.2 WHAT ARE THE PRINCIPAL RESEARCH QUESTIONS TO BE ADDRESSED?

Hypotheses: In children aged 3-48 months presenting to an ED with less than 72 hours of AGE like symptoms, compared with placebo, the administration of a probiotic agent:
1. Will result in a significantly lower proportion of children developing moderate to severe disease over the subsequent 2 weeks.
2. Will not be associated with a significantly greater occurrence of minor side effects.
3. Will be associated with a greater increase in secretory IgA (sIgA).
4. Will have varying effects based on the etiologic pathogen, given the diverse underlying pathophysiologic processes induced by the causative agents and the multiple mechanisms of action of probiotics.

Clinical Efficacy:
Primary Question: For previously healthy children, ages 3-48 months, who present to an ED with less than 72 hours of AGE like symptoms, is the proportion who develop moderate to severe disease [Modified Vesikari Score (MVS) ≥ 9] following ED evaluation, significantly different in those who receive a probiotic agent (Lacidofil) compared to those who receive placebo?
Secondary Questions: In this group of patients, amongst those receiving active treatment versus placebo:
1. Is there a difference in the (a) duration of diarrhea or (b) duration of vomiting?
2. Is there a difference in the proportion who require an unscheduled health care provider visit?
3. Is there a difference in the effectiveness of treatment based on the infecting pathogen?

Side Effect Profile:
Question: In this group of patients, is the proportion that experiences a side effect (e.g. bloating, fever, abdominal distention, rash) significantly different in those who receive Lacidofil compared to placebo?

Mechanism of Action:
Question: In this group of patients, are fecal sIgA levels 5 days and 4 weeks after the initiation of treatment higher in those who receive Lacidofil compared to those who receive placebo?

Microbiologic – Stool Pathogen-Specific Load:
Question: In this group of patients, is there a difference in the pathogen specific reduction in stool pathogen load in those who receive Lacidofil compared to those who receive placebo?

1.3. WHY IS A TRIAL NEEDED NOW? Definitive data is lacking to guide clinical decision making and most guidelines do not endorse routine probiotic use. Hence, probiotics are rarely prescribed by North American physicians. However, there are current trends that obligate an urgent assessment. First, since probiotics are sold as food supplements, manufacturers can encourage their use while their relevance has yet to be established. Manufacturers have embarked on aggressive campaigns making health claims that may not be supported by rigorous research. At stake is the world-wide probiotic market which is growing at 13% annually and is valued at $33 billion/year.
Second, North American and European government agencies remain concerned about their value and safety.35-37 Third, some institutions are now recommending the routine use of probiotics.38 Fourth, parents of affected children are often providing probiotics.17 We are therefore concerned that probiotic consumption is increasing in the absence of solid evidence. This underscores the necessity to conduct this definitive trial without delay. Prior research on the topic suffers from the following important shortcomings:

1-Outcome measures used to date have limited clinical meaning: Studies have focused on individual symptoms (e.g. stool duration), without consideration of the full picture of the illness39 (e.g. fever, vomiting, ED visits, hospitalization). A 2010 Cochrane Review concluded that the instruments employed to date are heterogeneous, lack evidence of validity and focus on outcomes that are not important to participants.40 Thus, the significance of conclusions reached are questioned.41,42 We will employ a validated burden of disease score and will focus on outcomes of relevance to children and their caregivers to enable an evidence-based conclusion to be drawn.12

2-Populations studied to date do not apply to the majority of children: Though 95% or more of children are treated as outpatients,43 only a handful of small studies have focused on outpatients.41 Inpatient research cannot be extrapolated to outpatients, as hospitalized children are more likely to benefit from probiotics.12,44,45

3-Quality of studies to date is inadequate: Most are small, single-centre46 and have been conducted by pharmaceutical companies.47 Many negative probiotic studies remain unpublished.48 Design issues are a concern: in a 2010 Cochrane Review, only 16% of studies adequately reported the 4 key methodological assessment parameters (i.e. allocation sequence generation, concealment, blinding, and loss to follow-up).12 Of 175 outstanding dietary research articles selected over the past 7 years by the National Institutes of Health, only 2 addressed probiotics and none AGE.49 Hence, high quality studies funded by non-vested parties that assess outcomes of interest to children and parents are needed.47,50

4-Inadequate data available from research in the relevant patient population: No studies to date have evaluated the impact of probiotics on children with gastroenteritis treated in primary care. Only a single ED study has been performed: 129 children received a probiotic or placebo agent and the authors found statistically insignificant trends towards a reduction in stool frequency (30% fewer diarrheal stools) and duration (median 14 hours fewer of diarrhea) amongst those administered a probiotic agent.51 The groups did not differ in terms of return to normal activities, return for medical care or the need for hospitalization. In light of these potentially important trends, the conclusions of systematic reviews, and the burden of disease – there are 1.7 million ED visits in the United States and 240,000 ED visits annually in Canada for pediatric gastroenteritis – conclusive data regarding the routine outpatient use of probiotics in North American children with AGE are needed.1

5-Knowledge about the in-vivo Mechanism of Action in AGE is lacking: Our understanding of the mechanism of action of probiotics is limited.52-55 Possible methods of action are (1) Microbiologic – by improving intestinal mucosal permeability,54 modifying the microbiota, inhibiting adherence of pathogenic bacteria, and competing for nutrients;55 (2) Immunologic – by upregulating gene expression,56 inhibiting the activation of pro-inflammatory pathways,57 increasing the concentrations of anti-inflammatory cytokines,58 and promoting local antigen-specific immunoglobulin A (IgA) responses.59 Studies incorporating both clinical outcomes and the measurement of biomarkers potentially related to the clinical effects are desperately needed.12,60

6-Lack of Probiotic Quality Control: As reported in an RCT comparing 5 probiotic products,61 not all are equally effective. Strain, viability, and dose are important factors.62 In North America, most have
never been clinically evaluated,63 some claim to contain organisms that do not exist,64 others do not match their labeled microbiologic specifications. Our work with Lactobacillus has demonstrated that it reduces epithelial injury,65,66 prevents bacterial binding, invasion and translocation,66,67 reduces gastric inflammation,68 attenuates colonic disease and dysfunction,66,69,70 improves intestinal barrier function,71 normalizes corticosterone release,70 and plays an immunomodulatory role.66 As a mandatory, yet rarely performed research requirement,12,72 we have obtained independent analyses to confirm the viable colony forming unit (CFU) count and microbe identity (Appendix 2-Lactobacillus). We have obtained Health Canada approval for our pilot which has guided this proposal’s design. Hence our study will provide evidence about a high quality product available in Canada.73

1.4 RELEVANT SYSTEMATIC REVIEWS AND NEED FOR THIS TRIAL IN LIGHT OF THESE REVIEWS. Meta-analyses12,44,47,74,75 are encouraging however, they (1) question the clinical relevance of the outcomes evaluated,12,41,47 (2) conclude that publication bias is a concern, and (3) advocate for a large RCT,28 funded by an unbiased agency, in an ambulatory pediatric population.47 A 2010 Cochrane Review reported reductions in the mean duration of diarrhea (25 hours), diarrhea lasting >4 days (risk ratio 0.41), and stool frequency on day 2 (mean difference 0.8).12 Given the limited clinical relevance of these findings, and the significant between-study heterogeneity, the authors of this and other reviews have called for studies that (1) evaluate specific regimens in large numbers of participants, (2) identify infectious causes,41 (3) present data separately for important subgroups, (4) include identification of the probiotic being tested, (5) confirm viability and quantity, (6) identify mechanisms underlying the beneficial effects, (7) conduct cost-effectiveness analyses,41,76 and (8) are definitive multicentre RCTs.12,47,77 Our proposed study, which builds on our promising pilot work, addresses all the limitations raised by the previous reviews and will provide the missing pieces of information.

1.5 HOW WILL THE RESULTS OF THIS TRIAL BE USED? The generalizability of the proposed trial will be excellent. If probiotics are effective for specific pathogens, we will develop a knowledge translation (KT) plan to ensure integration into care occurs. We will encourage incorporation into clinical pathways and seek endorsement by knowledge user groups (e.g. Canadian Pediatric Society, Canadian Association of Emergency Physicians).78 Successful dissemination strategies similar to those previously employed will be adopted.79-84 This study, which has been endorsed by Pediatric Emergency Research Canada (PERC), a 2011 winner of the CIHR-CMAJ Top Achievements in Health Research Awards, will be conducted at 6 member sites. The network has recently been awarded funding by the Networks of Centres of Excellence Knowledge Mobilization program to build a 36 site network termed Translating Emergency Knowledge for Kids. The network’s purpose is to optimize the transfer of knowledge into non-academic institutions.

Dr. Finkelstein, editor of “Kid Drug Alert Journal Club”, Journal of Clinical Pharmacology and Population Therapeutics, will disseminate our findings to parents and professionals through this open access venue. Integrated methods will be employed to ensure the lessons learned at ProvLab and the Sherman Lab are rapidly disseminated through publications in peer-reviewed journals enabling others to replicate the process. Epidemiologic findings will be disseminated annually to share new knowledge of circulating pathogens. End-of-grant activities, as described above, will be performed focusing on infectious disease, microbiology, laboratory medicine and public health communities given our strong ties to Alberta Health the Public Health Agency of Canada. From a consumer perspective; our efforts would focus on enhancing the accuracy of labeling of the over-the-counter products, based on our results.

1.6 PLEASE DESCRIBE ANY RISKS TO THE SAFETY OF THE PARTICIPANTS INVOLVED IN THE TRIALS. Well over 200 billion doses of probiotics have been consumed85 and
no serious side effects have been reported in well people.\textsuperscript{12} Five pediatric cases of lactobacillus bacteremia have been reported in which the strain was indistinguishable from the strain administered.\textsuperscript{86-88} The cases include short gut syndrome (3), complex congenital heart disease (1), and cerebral palsy and sepsis (1). There have been no reports of adverse overdose events.\textsuperscript{16} There is no evidence that probiotic use will worsen diarrhea, result in complications from the disease process, or introduce new toxicity. In our pilot, adverse events were only reported in the placebo group. Information on Lacidofil testing, safety data, and research by Dr. Sherman’s lab are available in Appendix 2 - Lacidofil.

2.0 THE PROPOSED TRIAL

2.1 WHAT IS THE PROPOSED TRIAL DESIGN? Randomized, placebo-controlled, double-blind, multicentre (6), Canadian, ED trial. All children aged 3 months to less than 48 months of age who present to a participating ED will be assessed for eligibility. A total of 886 children will be randomized to receive 5 days of a probiotic agent (Lacidofil – \(8 \times 10^9\) CFU/day) or placebo. The study will be conducted employing methodology suggested by the 2010 CONSORT statement.\textsuperscript{89,90}

2.2 WHAT ARE THE PLANNED TRIAL INTERVENTIONS?

\textbf{ED Intervention:} The 1\textsuperscript{st} dose will be administered in the ED. The sachet’s contents will be sprinkled into 30 mL of a liquid (ideally ORS) which may be cool (0\textdegree C-25\textdegree C) but without ice crystal formation. Caregivers will receive instructions on study drug administration, completion of study forms, what and how much fluid to drink, criteria for seeking a health care practitioner or returning to the ED (Appendix 4), and standardized AGE discharge instructions from each hospital.

\textbf{Home Intervention:} All patients will take 1 sachet, based on randomization, every 12 hours for 5 days (total of 9 home doses). They will administer the medication at meal time, mixed with 30 mL of an unfrozen beverage with no ice crystal formations (above 0\textdegree C) and ingested immediately to optimize viability. Carbonated and highly acidic beverages should be avoided. We will stress the importance of administering all doses dispensed and the need to communicate with the study team on a daily basis until symptoms resolve. One extra dose/day will be provided (i.e. kits will contain 5 extra doses – total of 15 sachets to account for vomiting or wastage). The dose may be repeated once should the child vomit within 15 minutes of medication administration. Vomiting after medication administration rarely occurs > 1 time.\textsuperscript{79} Oral fluid therapy will be encouraged according to established guidelines.\textsuperscript{14} Children who are hospitalized will continue as per study protocol as we have successfully done previously.\textsuperscript{91} Hospitalization at a non-study hospital site is very uncommon – 1/800 (0.1\%) children in the PERC multicentre bronchiolitis RCT were admitted at an alternative site.\textsuperscript{91} Should this occur, caregivers will have a letter describing the study, the care-plan, and the contact information of the Site Investigator.

\textbf{Rationale for Treatment Dose:} Although multi-strain products, such as Lacidofil, appear to show greater efficacy than single strains,\textsuperscript{92} the optimal CFU/kg dose is unknown.\textsuperscript{93} Lacidofil data indicates that a dose of 3-6 \(\times 10^9\) CFU/day is effective.\textsuperscript{94} Our pilot trial, which employed low (4 \(\times 10^9\) CFU/day) and high (8 \(\times 10^9\) CFU/day) dose arms, found no side effects with either dose. However, a positive association is postulated to exist between the probiotic dose and clinical benefits\textsuperscript{47} with most positive studies employing doses \(\geq 6 \times 10^9\) CFU/day.\textsuperscript{44} Thus, we will employ a dose of 8 \(\times 10^9\) CFU/day. This should enable us to definitively answer our research question and hence influence future usage. The duration of therapy has been selected based on the best available evidence, the recommendations of experts in the field, previous studies, and the typical duration of most episodes of AGE.\textsuperscript{95}

\textbf{Stool Sample Testing:} In keeping with usual common clinical practice, stool samples from all enrolled children will be sent for bacterial culture. Bulk specimens will be obtained whenever feasible. As was done in our pilot study, for children who do not provide a stool specimen prior to discharge, rectal swabs
(2 swabs) will be performed. One sample will be collected for bacterial culture according to site specific practices. The second sample will be collected using a flocked tipped sterile swab (FLOQSwabs™ Flocked Swabs, Copan) and will be stored and frozen (-80°C) in Universal Transport Media (UTM; Copan). This approach allows us to obtain a specimen for molecular pathogen identification prior to discharge (i.e. prior to probiotic administration altering the accuracy of pathogen identification) on all study participants and will only be tested if an ED bulk stool is not obtained. Viral testing will be performed in batches. We will also attempt to collect a bulk stool sample from all RCT participants in the ED prior to discharge. This specimen is the preferred specimen for pathogen identification testing.

**Bulk Stool from Home (Pathogen Identification):** Patients enrolled at all sites will be asked to provide additional bulk samples at home. Patients may decline, when obtaining informed consent, to collect bulk stool at home. The need to provide bulk stool samples will be stressed as these samples are required to perform pathogen-specific load quantification (i.e. cannot be performed on rectal swabs).

**DAY #0:** We will collect a bulk stool sample from all study participants who do not provide specimens in the ED prior to discharge.

**DAY #5:** We will collect a bulk stool sample from all study participants who provided a Day #0 bulk stool sample.

**DAY #28:** We will collect a Day #28 bulk stool sample from all study participants who consent to provide a Day #0 and #5 bulk stool sample. To collect specimens, caregivers will be provided with instructions (see Appendix 5) along with stool collection containers.

Initial pathogen identification testing will employ the sample (either bulk stool or rectal swab) obtained in the ED to minimize the impact of probiotic administration on test results. The specimen will be tested using the Luminex xTAG GPP. Day #0 bulk stool specimens collected at home will only undergo pathogen identification testing if the ED rectal swab test does not identify a pathogen. This will ensure that negative rectal swab test results do not reflect inadequate sampling (i.e. rectal swab performed but insufficient stool obtained thereby yielding a false negative test). Day #5 and Day #28 specimens will only be tested if the Day #0 specimen identifies a pathogen. The pathogen identification data is required to assign an etiology to all study participants; this information will be employed to determine the pathogen-specific response across all study aims.

**Bulk Stool from Home (secretory IgA):** In addition to pathogen identification and quantification, bulk specimens provided by participants on Days #0, #5, #28 will be sent to the Hospital for Sick Children (HSC) to the lab of Dr. Philip Sherman for sIgA testing (Appendix 6-sIgA Procedures). Samples will be stored at -80°C and will be sent to the Hospital for Sick Children (HSC) in bulk shipments from the labs of Dr. Linda Chui and Dr. Xiao-Li Pang. Fecal sIgA analysis will be performed by Dr. Sherman’s laboratory which is certified to handle human specimens.96,97

If a sample is unable to be provided at Enrolment, on Days 5 and 28, the first sample provided after Enrolment, the Day 5, and Day 28 time points respectively will be accepted.

Patients/caregivers will receive a reminder telephone call or email correspondence, based on preferred method of follow up, one day prior to the scheduled sample return date (i.e. on Day 4 and Day 27).

All specimens will be labeled with the date and time of collection and the subject’s study identification number. Once a sample is obtained, caregivers will contact a contracted biomedical courier service who will transport the specimens to the enrolment site with shipment costs covered by study funds. Upon receipt at the laboratory, each sample will be frozen and split appropriately for future testing. This procedure is based on work by the Centers for Disease Control,143 and our colleagues.144,145
Sites will batch ship all frozen stool samples to the Alberta Provincial Laboratory (ProvLab) and the lab of Dr. Xiao-li Pang in Edmonton, Alberta on a regular basis to enable interim laboratory analyses to verify collection and processing procedures. Regular shipments will minimize shipping costs and is acceptable given the stability of nucleic acid in frozen stool samples. All the analyses will be conducted blinded to patient allocation.

2.3 WHAT ARE THE PROPOSED PRACTICAL ARRANGEMENTS FOR ALLOCATING PARTICIPANTS TO TRIAL GROUPS? **Sequence Generation:** The Women & Children’s Health Research Institute (WCHRI), based at the University of Alberta, will provide data management services for this study. Randomize.net (www.randomize.net), an internet based randomization service, will produce a randomization list stratified by study site, using random-number generating software. The lists will be sent to the central pharmacy (ACH) who will prepare consecutively numbered study kits according to the randomization schedule. These will be couriered to the clinical sites, using proper shipment containers and temperature monitors, where they will be stored in the Research Support Pharmacies. **Allocation Concealment:** Randomize.net uses industry standard security to send data over the internet. Randomization will be blocked using random blocks of 4 and 6 with a 1:1 allocation ratio. Stratifying by clinical site and blocked randomization will ensure that variations (e.g. site specific practice patterns, gastrointestinal pathogens) are comparably distributed across treatment arms. Only the research pharmacy at the coordinating centre and www.randomize.net will retain the randomization code. **Implementation:** Potentially eligible patients (i.e. all children with diarrhea who meet age criteria) will be identified by the triage nurses and will be screened by the Clinical Research Assistant or Nurse for eligibility. A log of all screened patients will be maintained. If eligible, the details of the study will be discussed with the caregivers of all eligible children by the Clinical Research Assistant or Nurse who will seek consent. If consent is obtained, enrolled children will consecutively be assigned a patient ID number by the clinical site. The Clinical Research Assistant or Nurse will collect baseline demographic clinical variables and will complete the data collection forms (Appendix 7-Subject timeline) either on paper or directly into the secure online REDCap database via electronic tablet. Elements of clinical dehydration (Gorelick Score) and baseline disease severity scores (Modified Vesikari Score) will be assigned to enable baseline comparisons between treatment arms. The Clinical Research Assistant or Nurse will then log into randomize.net which will randomize the patient (i.e. it will provide a kit number that corresponds to a study drug kit at the clinical site which will be given to the patient). Following randomization the first dose will be administered (Section 2.2).

2.4 WHAT ARE THE METHODS FOR PROTECTING AGAINST SOURCES OF BIAS? Bias will be minimized by strictly adhering to the 2010 CONSORT Statement recommendations including the use of “third-party” assignment (Section 2.3). Moreover, because the active ingredient constitutes < 10% of the sachet, the probiotic and placebo powders will be identical in appearance, taste, texture and smell. Thus, participants, families, healthcare providers, data collectors (Research Assistants/Nurses), outcome adjudicators (Research Assistants/Nurses), and data analysts will be blinded, thereby preventing bias in outcome assessment. An intention-to-treat analysis will be performed to minimize bias associated with poor compliance and non-random loss of participants. Co-interventions (e.g. antiemetic, intravenous rehydration, antibiotic administration) and other sources of confounding will be recorded. Reporting bias will be avoided by registering the trial at clinicaltrials.gov. Additionally our use of a published score as an outcome measure will protect against the introduction of bias in the assessment of treatment effects.
2.5 WHAT ARE THE PLANNED INCLUSION/EXCLUSION CRITERIA? All patients with gastroenteritis presenting to the ED of 6 participating hospitals will be eligible. The diagnosis of gastroenteritis is at the discretion of the emergency department supervising physician and may or may not include vomiting. Alternative terminologies that reflect as similar diagnosis are acceptable provided they meet all other eligibility criteria. Examples include: viral illness, diarrhea, vomiting, upper respiratory infection, post-infectious gastroenteritis, antibiotic associated diarrhea, toddlers diarrhea, viral infection, enteritis, viremia, fever, and bronchiolitis.

Inclusion criteria (Patients must meet all of the following criteria to be eligible)
1. **Presence of diarrhea**: defined as ≥ 3 watery stools in a 24-hour period.\textsuperscript{101}
2. **Duration of vomiting or diarrhea < 72 hours**: Early administration = greater efficacy.\textsuperscript{29,102,103}
3. **Age 3 to < 48 months**: AGE severity and frequency are greatest amongst young children.\textsuperscript{104}

Exclusion criteria (Patients who meet any one of the following criteria will not be eligible)
1. **Presence of an indwelling vascular access line or structural heart disease** (bacteremia risk).\textsuperscript{105}
2. **Taking immunosuppressive therapy, or known history of immunodeficiency** (bacteremia risk).\textsuperscript{106}
3. **Hematoma in the preceding 72 hours, underlying significant chronic gastrointestinal problem or inflammatory bowel disease**: Not including constipation, gastroesophageal reflux or chronic pain.
4. **Family member with an indwelling vascular access line, on immunosuppressive therapy, or with a known immunodeficiency**: Does not include use of short course oral (<7 days) or inhaled steroids.
5. **Bilious vomiting**: May indicate a diagnosis other than AGE is possible.
6. **Probiotic use (supplement) in the preceding 2 weeks**: However, consumption of foods containing probiotics will not result in exclusion as they are ubiquitous.
7. **Previously enrolled in this trial** (to ensure that the observations on trial patients are independent).
8. **Daily telephone follow-up will not be possible while symptomatic** (travel plans or language barrier).
9. **Allergy to soy**: Lacidofil, as well as the placebo product have come in contact with soy during the manufacturing process.
10. **Pre-existing, or known, pancreatic dysfunction or insufficiency**\textsuperscript{107}
11. **Oral or Gastrointestinal surgery within the preceding 7 days**: theoretical wound infection risk.

Concomitant Medications
The concomitant administration of antibiotics will be permitted and will be at the discretion of the child’s treating physician. Children taking antibiotics will not be excluded as probiotics remain effective when given concomitantly with antibiotics\textsuperscript{108} and their survival is not significantly altered. Similar criteria will be applied to the administration of antipyretics, anti-emetics, and any other medications. As per Standard of Care at the participating sites, Oral Rehydration Solution (ORS) will be provided during the emergency department visit to enable the performance of oral rehydration therapy. In keeping with institutional Standard of Care, patient/parent discharge instructions that will be provided, as specified in protocol section 2.2, will encourage the ongoing use of appropriate ORS following discharge.

2.6 WHAT IS THE PROPOSED DURATION OF TREATMENT PERIOD? Five days.

2.7 WHAT IS THE PROPOSED FREQUENCY AND DURATION OF FOLLOW-UP? Daily telephone or e-mail survey follow-up will occur, 7 days/week, until both the diarrhea and vomiting have resolved. We will also conduct follow-up on days #5 and #14 even if symptoms have resolved.

2.8 WHAT ARE THE PROPOSED PRIMARY AND SECONDARY OUTCOME MEASURES?
**Primary Outcome (Clinical):** The primary outcome is the development of moderate-severe disease in the 2 weeks after the index ED visit as measured by the MVS (Appendix 8-MVS).\textsuperscript{7} The original 20 point Vesikari Score has been employed as a dichotomous variable in many clinical studies\textsuperscript{109-117} despite
limited evidence supporting its use. However, it has been shown to correlate with other meaningful measures such as caregiver anxiety, helplessness, and stress.\textsuperscript{118} Recently, increasing severity scores were associated with higher parental worry, greater changes in the child’s behavior, and trends towards greater impact on the parents’ daily activities and higher parental distress.\textsuperscript{119} \textbf{So, why did we develop a Modified Score?:} Percent dehydration, an element of the original score, is challenging to determine. While using baseline and rehydrated weights is the gold standard,\textsuperscript{98} this is often of limited value due to difficulties in ensuring follow-up, determining when rehydration has occurred, and the variation related to timing of voiding, stooling, eating, and drinking. Moreover, clinical estimates of dehydration are extremely inaccurate.\textsuperscript{120} Thus, this element is omitted or incorrectly assigned in most studies. The modified score which we have created includes an important and easy to obtain outcome that reflects global disease severity-\textit{need for unscheduled future health care visits within 2 weeks of the index visit.}\textsuperscript{7} This is supported by evidence that the utilization of professional medical care correlates with disease severity.\textsuperscript{118} \textit{Unscheduled future health care visits} is a powerful marker that has the capacity to alter clinical practice and influence decision makers. Similar modifications have been performed previously when percent dehydration has been unavailable\textsuperscript{118,121} and we have previously shown that because ED care does not alter the disease process in AGE, ED revisits are very common (publication attached).\textsuperscript{79,122} The MVS\textsuperscript{7} is presented below (Table 1), with the score structure (0, 1, 2, 3 points) unaltered from the original score.

<table>
<thead>
<tr>
<th>Table 1. Modified Vesikari Scale Score</th>
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<tbody>
<tr>
<td>Points</td>
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<tr>
<td>Diarrhea Duration (d)</td>
</tr>
<tr>
<td>Max # of diarrheal stools/24 hr period</td>
</tr>
<tr>
<td>Vomiting Duration (d)</td>
</tr>
<tr>
<td>Max # of vomiting episodes/24 hr period</td>
</tr>
<tr>
<td>Max Recorded Fever</td>
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<tr>
<td>Unscheduled Future Health Care Visit</td>
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<tr>
<td>Treatment Administered</td>
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\textbf{Characteristics of the MVS:} We prospectively evaluated the MVS in an 11 centre (455 children) ED study\textsuperscript{7} in children meeting eligibility criteria as planned for the current proposal (≥3 stools in a 24 hour period and <72 hours of symptoms) which found that it effectively measures global disease severity. Factor analysis revealed that item correlations were acceptable and supported the appropriateness of retaining all factors. Multi-collinearity was not a problem and the correlations between the MVS and other measures of clinical significance were in the expected direction. Disease severity was associated with prolonged daycare (P = 0.01) and work (P = 0.002) absenteeism. The MVS had a normal distribution with minimal kurtosis (-0.14; SE: 0.24) and skewing (0.39; SE: 0.12). There was good variation across severity ranges (49% mild; 21% moderate; 30% severe). Variation between institutions was insignificant (P = 0.11) and complete follow-up was achieved in 91% of participants.

\textbf{How will it be Calculated?:} Following enrollment (Time 0), follow-up will occur daily until both the diarrhea and vomiting have resolved (Section 2.7). Once follow-up is complete (Day #14) each variable is assigned a score for the entire study period (Time 0 to Day #14); each patient gets a single total score for the study. Variables are scored based on the worst 24 hour period (e.g. maximal number of episodes of vomiting in a 24 hour period) or on the total duration of symptoms (e.g. number of days of vomiting) or are based on the occurrence of an outcome (e.g. hospitalization).

\textbf{What if at baseline the pre-enrollment MVS is ≥ 9?:} Regardless of the score assigned at Time 0 (i.e. pre-enrollment score), EVERYONE reverts to a score of 0 at enrollment (i.e. the study evaluates the
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impact on the disease process going forward). The pre-enrollment score, which is based on symptoms in the 72 hours prior to presentation, will serve as a covariate in a secondary analysis of the primary outcome and will be employed for sub-analysis purposes. An example is provided (Appendix 8-MVS). The primary outcome (the presence of moderate-severe disease, as defined by a MVS of > 9 during the 2 week follow-up period) will ONLY include symptoms and outcomes that occur following the ED visit (i.e. after randomization) and will not be directly impacted by the pre-enrollment score. Why a cut-point of 9?: With the original score, severe disease was defined as ≥ 11,109,110,115,116,123-125 moderate as ≥ 9.126 In our derivation study, construct validity was proven 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 [e.g. daycare (P=0.01) and work absenteeism (P=0.002)].

Secondary Outcomes (Clinical):
1. The duration of diarrhea: Time from treatment initiation until the appearance of the last watery stool127-129 as reported during daily phone conversations.
2. The duration of vomiting: Limited data indicate that probiotic administration may reduce vomiting.102,130 Recovery will be evaluated in children who vomit ≥ 3 times over the 24 hours prior to the ED visit and defined as “time from treatment initiation until last vomiting episode.” We have previously reported that vomiting frequency predicts outcomes in AGE.131
3. Return visits for unscheduled care to a health care provider related to vomiting, diarrhea, dehydration, fever, or fluid refusal, within two weeks: Not included will be scheduled visits (e.g. re-assessment, vaccinations). This outcome is important as > 50% of children have a follow-up office visit.43 8-18% require an ED visit,132 and 5-8% are hospitalized.43

Additional Outcomes: Work and daycare absenteeism.

Side Effect Profile: To determine if short course probiotic administration to young children with AGE is associated with an increase in minor side effects. As stated by the NIH, probiotic safety needs to be studied scientifically.133 Groups will be compared regarding the development of any side effects with particular attention paid to bloating, abdominal distention, duration of fever, and buttock rash. The importance of evaluating side effects has been highlighted by a recent adult pancreatitis study which found an unexpected increase in mortality in probiotic treated patients.107

Mechanism of Action: To determine if probiotic administration increases fecal secretory IgA levels in children with AGE (Appendix 6). The first stool sample produced following enrollment will be collected along with samples on days 5 and 28. slgA is a key element in the gastrointestinal immune defense as it agglutinates microorganisms and prevents pathogen adherence to mucosal surfaces.134-137 Evaluating slgA in children with AGE has been identified as a needed element to advance this field of research.138,139 Animal studies have reported a substantial increase in anaerobic bacteria in the absence of normal slgA and that normalization of slgA production results returns intestinal microbiota to its regular composition.140 Probiotics are believed to enhance host immunity by regulating inflammatory cytokines141 and by increasing slgA production.142,143 In human studies, probiotic administration appears to increase fecal slgA concentration in healthy adults,144 children,145 infants,146,147 and pre-term infants.148 However, correlation with clinical outcomes has not yet been evaluated. We will determine if fecal slgA levels are greater amongst children treated with a probiotic agent compared with placebo. Levels will be correlated with clinical findings. However, experiments correlating probiotic administration, clinical outcomes, and fecal slgA levels in the context of enteric infection have not been conducted. Specifically, we will determine, at a pathogen-specific level, if fecal slgA levels are higher in children treated with a probiotic agent compared with placebo, and if higher fecal slgA levels are associated with improved clinical outcomes.
Pathogen Load Quantification: To determine if a 5-day probiotic treatment course administered to children with AGE results in pathogen-specific reductions in stool pathogen load. Our team, which includes experts in molecular diagnostics, virology and bacteriology, has the capacity to quantify the impact of probiotic administration on stool pathogen infectious loads. These measures represent disease severity in individuals with AGE; higher stool loads are associated with more severe symptoms, prolonged shedding, hospitalization, and the presence of virus in the blood (i.e., viremia). In children with AGE, stool viral loads correlate (r = 0.80, P<0.001) with the Vesikari Score. Bacterial loads, analyzed from other biological specimens, also have clinical relevance – for example, sputum Pseudomonas aeruginosa loads correlate with clinical status and those of Neisseria meningitides in serum are associated with death and permanent sequelae. All of this work builds on the model of human immunodeficiency disease, where serum viral load has been a key prognostic marker for decades. Consequently, stool infectious load quantification is increasingly encouraged. Our team, which has led many key advances in molecular virology, has developed a standardized approach (see Section 2.9.2) to quantify stool viral and bacterial loads, enabling us to quantify an objective marker of disease severity.

2.9 HOW WILL THE STUDY AIMS AND OUTCOMES BE ACHIEVED?

2.9.1 Aim #1: Clinical Benefits – Modified Vesikari Scale Score
The Modified Vesikari Scale score will be assigned based on data collected during the follow-up period via electronic survey or phone call. A single score is assigned to each of the 7 elements representing either symptom duration, the maximal frequency of vomiting, maximal frequency of diarrhea, maximal recorded body temperature, and subsequent healthcare use and treatments provided. Each participant will have a single, Modified Vesikari Scale score assigned at the conclusion of the follow-up period which reflects the severity of the child’s disease. The relationship between the assigned score, the identified pathogen, and probiotic exposure (active/placebo) will be quantified.

2.9.2 Aim #2: Microbiologic – Stool Pathogen-Specific Load
All children with a Day #0 viral or bacterial pathogen identified and who provided bulk stool specimens on Days #0, #5, and Day #28 will have samples tested for pathogen-specific load quantification. Results will be reported as NA copies of pathogen/gm and the difference between Days #0 and SUBSEQUENT TEST DAYS will represent the participant’s pathogen-specific load reduction. The relationships between pathogen-specific load reduction, infecting pathogen, and probiotic exposure (active/placebo) will be quantified. In addition, to enhance the clinical interpretation of pathogen load reduction, we will explore the relationship between Modified Vesikari Score and pathogen load reduction separately.

Quantification procedures will be standardized to ensure that the homogeneity and proportion of stool included in each analysis is consistent between samples (intra- and inter-patient) and hence per reporting unit (gm). To achieve this degree of standardization, a 20% (weight/volume) suspension of stool specimen will be prepared with phosphate-buffered saline (PBS) and clarified by centrifugation. Standardization will be facilitated by conducting batch analyses including Days #0 and #5, and Day #28 specimens from each participant in the same run, thereby eliminating inter-run variation.

Quantification of enteric viruses: This will be performed as previously described by our team (Pang, Lee). In brief, samples will be thawed, mixed by vortexing and a 20% stool specimen suspension will be prepared and clarified by centrifugation. Total NA will then be extracted and eluted using the NucliSENS easyMAG® automated system (bioMérieux, Durham). Viral NA prepared from non-study stool samples testing positive for well-characterized enteric viruses (i.e. rotavirus, norovirus GI/GII, and adenovirus 40/41) will be used as positive controls. The primers and probes for the detection of norovirus, rotavirus, and adenovirus will be labeled with Fam detector and Tamara quencher dyes (Applied Biosystems). Individual real-time PCR reactions for each virus will be performed. After
incubation for denaturing, PCR amplification will be performed and profiles will be collected and analyzed using Sequence Detection Software version 1.0. To quantify the 3 viruses, an external standard curve will be established using 10-fold dilutions from 1 copy to 1.0 × 10⁸ copies.

**Quantification of enteric bacteria:** Building on our prior work and collaborating with team members (Pang, Lee), we will employ methodology as described above for the viral targets, to quantify bacterial loads. This will be determined for stool samples positive for each bacteria using singleplex real-time PCR assays for each respective bacteria (*Salmonella, Campylobacter, Shigella, E. coli, Yersinia*). Standard curves correlating CFU and crossing point of the real-time PCR assay for each organism will be created by performing real-time PCR on 10-fold dilutions of standardized bacterial suspensions that will also be plated onto sheep blood agar plate to determine the CFU count.

### 2.9.3 Aim #3: Immune Response – Fecal Secretory Immunoglobin A (sIgA) Quantification

sIgA testing will be performed employing the Eagle Biosciences Secretory IgA ELISA kits (catalog #: SGA35-K01) in accordance with the manufacturers instructions.

### 2.10 HOW WILL THE OUTCOME MEASURES BE MEASURED AT FOLLOW-UP? All caregivers will receive discharge instructions that will include information on tasks required following discharge. Training materials have been developed based on the 3 site probiotic pilot study.

1. **Daily Telephone/Survey Communication:** At the index visit, caregivers will be asked their preferred method of communication – electronic (i.e. email survey) versus telephone. Surveys (telephone and email) will be offered in French and English for sites requiring bilingual data collection. Following discharge, site Clinical Research Assistants or Nurses will contact the family daily until both the diarrhea and vomiting have resolved employing the identified method. A standardized script or survey/data collection form will be employed. If phone is opted for, the caller will enquire about ongoing symptoms, medical evaluations, treatments, child care and work absenteeism, and side effects. Detailed questioning will follow positive responses. The survey will employ advanced logic to enhance ease of use. If the caregiver does not complete the survey within 48 hours, a telephone follow-up will be performed. **Compliance** will be assessed on day #5 and final data points will be collected on day #14. Protocols will be developed to deal with caregiver questions in accordance with institutional requirements. To maximize validity, caregivers will be reminded of the importance and method of administering the probiotic/placebo. Similar schemes have been successfully implemented by the principal investigator,⁷⁹,¹²² other PERC multicentre studies,⁸²,⁹¹ and was employed in the pilot. Caregiver report (telephone/survey) will serve as the primary source document.

2. **Chart Review:** We will verify data regarding revisits, intravenous hydration, hospitalization, and microbiology testing using each centre’s medical record database.

3. **Database Reviews:** Provincial databases (e.g. National Ambulatory Care Reporting System; Alberta Ambulatory Care Classification System; Alberta Health Care Insurance Plan) and Canadian Institute for Health Information databases will be employed to verify future health care provider use.

### 2.11 WILL HEALTH SERVICE RESEARCH ISSUES BE ADDRESSED? As called for by the 2010 Cochrane review,¹² an economic evaluation will be conducted by Dr. Willan and Mr. Goeree¹⁷²-¹⁷⁷ alongside the clinical trial (Appendix 9-Economic Analysis Plan). We will monitor work absenteeism, as this is the major item contributing to cost.¹²² Moreover, days of diarrhea has been found to correlate with work absenteeism,¹⁷⁸ and a recent pediatric, Canadian ED study found that > 50% of the societal costs occur in the 15 days following the ED visit.¹⁷⁹ Hence, if effective, cost savings are likely from a societal perspective due to the inexpensive nature of probiotics and the economic benefit derived from
reduced work absenteeism. Because adding a therapeutic intervention may add to overall health care costs, willingness to pay will be determined. The incremental cost effectiveness will be determined by assessing resources and costs associated with the treatment of AGE for children who receive the current standard of care compared to those who receive a probiotic.

2.12 WHAT IS THE PROPOSED SAMPLE SIZE AND WHAT IS THE JUSTIFICATION FOR THE ASSUMPTIONS UNDERLYING THE POWER CALCULATIONS (APPENDIX 10)?

Clinical Outcome: The sample size is based on the assessment of the between-group difference in proportions of children with a post-randomization score ≥ 9 on the MVS. This is a superiority study in which the adoption of probiotic use can be recommended if the rate of the primary outcome is significantly lower amongst those who receive the probiotic medication. Calculations are based on a two-sided type I error (α) of 0.05 and power (1-β) of 0.90. The null hypothesis is \( H_0: P_C - P_I = 0 \), where \( P_I \) and \( P_C \) are the event rates in the intervention and control groups respectively. The alternative hypothesis is \( H_A: |P_I - P_C| > 0.10 \) (i.e. the event rates will differ by at least 10 percentage points).

Minimal Clinically Important Difference (MCID): Ten content experts from the US and Canada were surveyed regarding the MCID. Absolute risk differences ranging from 7.5-15% were suggested. We chose a conservative estimate of 10% for the primary outcome (number needed to treat of 10).

Outcome in Control Group: Our estimate for the development of moderate to severe AGE in the controls is based on data collected as part of our 2009 evaluation of the MVS in 455 children aged 3 – 48 months, with < 72 hours of symptoms, who presented to one of 11 Canadian EDs (Section 2.8). Using the ED visit as time 0, 25% of eligible children had scores consistent with moderate to severe disease following discharge. This is lower than previous reports of ED \(^{110,125}\) and community populations \(^{109,124,126}\) because we did not include symptoms that existed prior to the visit. However, Dr. Schnadower’s group in the United States has just completed data collection on 282 children enrolled at 6 sites in the United States and they found that 24% of children in their sample had scores consistent with moderate to severe disease following discharge (personnel communication September 6, 2012). Since our study population and method of MVS calculation in the derivation and recent validation studies and the current proposal are the same, 25% is a very accurate estimate. Given the above, the required sample size to compare proportions between two different groups is 670. \(^{180}\)

Sample Size Adjustment Calculation: Based on previous work by our group with similar follow-up designs \(^{79,91,181}\) and extensive reviewer feedback, we have assumed a 10% loss to follow-up (670/0.9 = 744), 5% drop out (744/(0.95) = 825), and 2.5% drop-in (caregivers who decide to buy a probiotic agent at a pharmacy to administer to their child) rate (825/(0.975) = 868). Adjustment for O’Brien-Fleming monitoring boundaries requires a further 2% increase. Thus, the total number randomized (final sample size) will be 886.

Side Effect Profile: To date, clinical trials employing probiotics have not attributed any adverse events to probiotic administration. \(^{12}\) We suspect that minor side effects have not been documented; however, clinicians need to have an understanding of the side effect profile in order to enable caregivers to make an informed treatment decision. Given our sample size, a significant difference between groups will be easily detected (i.e. 80% power to detect an increase in reported adverse events from 5% to 10%).

Mechanism of Action: A study evaluating the impact of formula supplementation with oligosaccharides found IgA values of 729 and 377 μg/g in the intervention and control groups respectively. \(^{182}\) If we assume a clinically significant difference of 300 μg/g, a standard deviation of 500 μg/g, 80% power and a type I error of 0.05, the required sample size is 45 subjects/group. Thus we will aim to include a minimum of 100 patients which will be recruited from all study sites, with the exception of the IWK Health Centre.
Pathogen-Specific-Effectiveness Study (Table 2): Home Stool Collection on Days #0 and #5 will be completed at all 6 study sites. It is anticipated that bulk stool will be collected on 25% of children in the ED and 75% of those requiring home Day #0 collection. Of those providing an ED/home Day #0 specimen, 75% will provide a Day #5 sample. We will collect specimens to enable pathogen identification on all study subjects (n=886). These will be paired with Modified Vesikari Scale score data from the estimated 797 children (90%) who will complete follow-up. Data from these 797 children will support the conduct of Aim #1 analyses. Assuming ~50% viral (n=399), ~40% unidentified (n=318), and ~10% bacterial (n=80), and trusting randomization (~50% probiotics, ~50% placebo) we anticipate a minimum of 40 children per arm in our smallest group. Day #0 and 5 paired samples will be obtained from ~465 children of which ~232 will be positive for a virus and ~46 for a bacteria. Thus, pathogen load reduction calculations will be performed for 278 participants. These accrual estimates are summarized in a diagram in Appendix 12.

<table>
<thead>
<tr>
<th>Table 2. Current and Anticipated Enrollment and Specimens per Study Aim</th>
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<td><strong>Required</strong></td>
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</table>
| Aim #1 | • Modified Vesikari Scale score  
• ED stool sample or rectal swab | 77 (actual) | 120 | 200 | 200 | 200 | 797 |
| Aim #2 | • Days #0 and #5 stool samples  
• Positive pathogen identification | 14 | 43 | 74 | 74 | 73 | 278 |
| Aim #3 | • Days #0 and #5 stool samples | 24 (actual) | 72 | 123 | 123 | 123 | 465 |

2.13 WHAT IS THE PLANNED RECRUITMENT RATE (APPENDIX 11)?
The 5 original proposed study sites saw 10,344 children aged 3 – 48 months with AGE in 2011 (a 17% increase since 2009). During our pilot RCT, 2.1% of children with AGE aged 0-4 years were enrolled. Based on the published literature and our data: (i) presenting November 1 – May 31 between 8:00 – 24:00 (55%), (ii) meet definition of diarrhea (50%), (iii) < 72 hours of symptoms (45%), (iv) absence of exclusion criteria (80%), and (v) provide consent (50%), our best estimate is that 4.7% of children with AGE aged 3 - 48 months will be enrolled. The difference between our pilot and the best point estimate is due to the requirement of daycare attendance in our pilot study. Based on our experience with AGE, and multicentre trials, we believe that we should employ our worst case scenario recruitment estimate (3.1%) which will enable us to enroll our full sample size over three AGE seasons. The only prior North American ED study, which employed similar eligibility criteria, recruited 129 subjects at 1 site in just 8 months therefore we believe our recruitment plan is realistic. The data outlined in Appendix 11 is for the initial 5 sites. A sixth study site has been added to improve enrolment and projected timelines. Data related to gastroenteritis visits is unavailable for the sixth study site.

2.14 ARE THERE LIKELY TO BE ANY PROBLEMS WITH COMPLIANCE?
While infrequently reported and not considered to be problematic, non-compliance is unlikely related to probiotic side effects. Participant withdrawal has primarily been related to the primary illness. A recent study reported 108% compliance due to medication re-administration in subjects who vomited. As the intervention is of a short duration, the burden to caregivers is minimal. In our pilot, compliance was 91% as reported by caregivers and verified by return sachet counts. This does not reflect the impact of vomiting following medication administration. A recent ED probiotic study reported that 87% of caregivers found the probiotic and placebo powders to be “very” or “somewhat” easy to administer. Hence, we do not anticipate compliance problems; nonetheless, we will track compliance by obtaining unused sachet counts (day #5) and requesting their return (day #14).

2.15 WHAT IS THE LIKELY RATE OF LOSS TO FOLLOW-UP?
Our previous ED pediatric AGE research achieved telephone follow-up rates of 98-99% on Day #3 and 96-99% on day #7. Similar success has been documented in prior PERC (99%) multicentre studies. We will err on the conservative side and 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 databases (Section 2.10) will supplement the daily telephone calls.

2.16 HOW MANY CENTRES WILL BE INVOLVED?
Six EDs that are members of PERC, a network which has extensive experience conducting large scale clinical studies, will participate – Alberta Children’s Hospital (Calgary), Hospital for Sick Children (Toronto), Children’s Hospital of Eastern Ontario (Ottawa), Centre Hospitalier Sainte-Justine (Montreal), IWK Health Centre (Halifax), and the London Children’s Hospital (London).

2.17 WHAT IS THE PROPOSED TYPE OF ANALYSES?
All analyses will be undertaken by the intention to treat principle. Adverse events will use the “as treated” principle. Patients who drop out or crossover will be followed and included. All statistical tests of hypotheses will be two-sided. Baseline characteristics will be compared between groups using frequency counts and percentages for discrete variables, and means, medians, standard deviations, and interquartile ranges for continuous variables. Baseline characteristics will be analyzed to determine if there is a need to adjust for differences between groups. Sensitivity analyses will be performed to assess the possibility and consequences of losses to follow-up not occurring at random, as well as to assess the classification of children who have multiple pathogens identified (<5%). Initial classification will be based on Day #0 load (i.e. classified based on higher load); re-classification will evaluate the impact of classification according to the agent with the lower pathogen load.

Clinical-Primary Outcome: The proportion of children with moderate to severe disease (i.e. MVS ≥ 9) will be analyzed by comparing proportions utilizing a Mantel-Haenszel test, stratified by clinical centre. Significance for the primary outcome measure will be determined using a two-sided 0.05 level. The pre-enrollment MVS will not be included in the primary analysis as we do not anticipate the baseline and post-intervention scores to be correlated. Secondary analyses of the primary outcome will employ logistic regression methods to adjust for covariates that may be imbalanced between groups (e.g. age, pre-enrollment MVS, severity of baseline diarrhea and vomiting, hydration assessment, need for hospitalization at index visit). We will also analyze the MVS as a continuous variable through a stratified Wilcoxon rank-sum test. The mean benefit will be explored, separately, in relation to:

1. Pathogen-group: virus vs. bacteria vs. not identified
2. Viral agent: rotavirus vs. norovirus vs. adenovirus
3. Bacterial agent: Campylobacter vs. Salmonella (only ones anticipated to have sufficient numbers)

All analyses will first employ 2-way ANOVA to assess main effects and interactions of treatment assignment and pathogen group. To assess for other covariates and potential confounders, multivariable
regression models including treatment, pathogen and other key covariates (e.g. age, sex, Modified Vesikari Scale score at enrollment, hospitalization, antibiotic use) will be constructed.

**Clinical-Secondary & Tertiary Outcomes:** 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. The continuous variables of (1) duration of diarrhea and (2) vomiting will be measured in hours and analyzed with a Van Elteren test, stratified by clinical centre. (3) Unscheduled health care visits will be analyzed using a Mantel-Haenszel test, stratified by clinical centre. The tertiary outcomes of (4) number of days the child is absent from daycare and the (5) caregiver is absent from work will be analyzed using an appropriate model with robust estimates for standard errors. Dichotomous outcomes to be evaluated but unlikely to achieve significance include ED 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.

**Side Effect Profile:** The proportions of children experiencing any side 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 side effects, as an aggregate outcome variable.

**Mechanism-Fecal Secretory IgA:** To test for a difference in fecal secretory IgA the Wilcoxon rank-sum test will be performed. As this is a mechanistic outcome and the motivation of its study is distinct from other outcomes, the test will be performed at the 0.05 level. Data will be analyzed to determine if fecal secretory IgA levels 5 days and 4 weeks after initiation of treatment are higher amongst children treated with probiotic than those treated with placebo. Fecal sIgA data will also be analyzed by outcome, comparing levels amongst those with mild disease to those with moderate-severe disease.

**Pathogen Load Quantification:** To determine if a 5-day probiotic treatment course administered to children with AGE results in pathogen-specific reductions in stool pathogen load. Benefit is defined as the difference in stool pathogen load between Days #0, #5, and #28. The analysis will employ a 2-way ANOVA followed by multivariable linear regression models adjusted for pathogen, interaction and important covariates (e.g. age, sex, baseline Modified Vesikari Scale score, baseline pathogen load, antibiotic use, increase in fecal sIgA). The analysis will determine if reduction in pathogen-specific load is independently related to treatment and pathogen. Based on the distribution of the reduction in pathogen-specific loads, the mean or median reductions will be explored in relation to pathogen, comparing:

1. **Pathogen-group:** virus vs. bacteria
2. **Viral agent:** rotavirus vs. norovirus vs. adenovirus
3. **Bacterial agent:** *Campylobacter* vs. *Salmonella* (only ones anticipated to have sufficient numbers)

Since there is the potential that clinical response, pathogen load reduction, and fecal sIgA are related outcomes, we will explore the overall simultaneous change in the means of the outcomes due to treatment arm by performing a Hotelling’s t-test on the three response vectors (i.e. differences in Modified Vesikari Scale score, and the Days #0 and 5 and Days #0 and 28 changes in infectious load and fecal sIgA).

**2.18 WHAT IS THE PROPOSED FREQUENCY OF ANALYSES?**

The Data Safety Monitoring Committee (DSMC) will meet after 200 and 500 patients to review enrollment, study procedures, form completion, data quality, 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 AGE in the probiotic arm is equal to that in the placebo arm. Conservative O’Brien-Fleming monitoring boundaries, implemented using the Lan-DeMets alpha-spending function approach,
will be used as guidelines for early stopping for safety or efficacy. Based on trends and adverse events, the DSMC may decide to meet sooner than planned using boundaries adjusted accordingly. Because this trial involves children under the age of 6 months, the DSMC has approved a plan to complete an interim safety analysis on the first 20 subjects enrolled under 6 months of age. All serious adverse events will be reported within 24 hours to the DSMC and based on these reports; the DSMC may decide to conduct a safety analysis before the full 20 subjects have been enrolled in this age group. Otherwise, a blinded analysis will be conducted after the 20 subjects < 6 months of age have been enrolled. This data will be unblinded if the DSMC deems it necessary to conduct an unblinded interim safety analysis. The results of this analysis will be communicated to the NNHPD branch of Health Canada at the discretion of the DSMC chair should any concerns be identified.

2.19 ARE THERE ANY PLANNED SUBGROUP ANALYSES? (1) The presence of a MVS ≥ 9 will be analyzed by (i) age < 1 year, (ii) breast-feeding status, (iii) antibiotic usage and (iv) protocol compliance. (2) Duration of vomiting will be analyzed only in those patients who have ≥ 3 episodes of vomiting in the 24 hours prior to enrollment. (3) Daycare and work absenteeism will only be analyzed for children who attend daycare and caregivers who work. A subgroup analysis will be performed for children with (4) rotavirus infection by adding an interaction term between treatment and rotavirus positivity in a logistic regression model. The independent variables in the model will be (i) treatment group, (ii) rotavirus positivity (yes/no) and (iii) the interaction between treatment group and rotavirus positivity. Universal rotavirus vaccination does not exist in Canada with the decision being made individually by each province based on the expense as well as feasibility. At present it is included in the provincial schedules in Quebec and Ontario but not in Nova Scotia or Alberta. The varying use of the vaccine and our goal to identify etiologic agents and to conduct sub-analyses will yield very important information related to probiotic use in the presence/absence of rotavirus vaccination. (5) Fecal sIgA levels will be sub-analyzed based on the mother’s breast-feeding status.

2.20 DATA SHARING
Participant data will be stored in an online electronic data capture system (REDCap). Collected data will be downloaded at the coordinating centre in Calgary, Alberta Canada. In order to complete the planned subgroup and economic analyses, a de-identified dataset containing only the variables required will be shared with collaborating institutions. The planned economic analyses will be performed by the Program for Assessment of Technology in Health (PATH) Research Institute at McMaster University (Appendix 9-Economic Analysis Plan) located in Hamilton, Ontario Canada. Data will also be shared with the University of Utah Data Coordinating Center (DCC) located in Salt Lake City, Utah USA. The DCC will integrate our study data with those from a companion clinical trial taking place in the United States (co-PIs Dr. Stephen Freedman and Dr. David Schnadower). Integration of data will allow for additional analyses to be performed that would be underpowered for either study to perform them in isolation.

Pathogen Load Quantification Data: Specimens are received de-identified by the processing labs. Results of the pathogen load testing performed by Drs. Xiao-Li Pang, Linda Chui, and Bonita Lee will be compiled and entered into a simple database. The de-identified database will be sent to the coordinating centre in Calgary, Alberta using a secure email service (Alberta Health Services). These results may be shared with the DCC in Salt Lake City, Utah.

Fecal Secretory IgA Data: Fecal sIgA results will be entered in to a simple database. The database will be encrypted and sent to the Principal Investigator at the coordinating centre via institutional email. All participant results will be de-identified. De-identified specimens are received at the lab of Dr. Sherman located at the Hospital for Sick Children. These results may be shared with the DCC in Salt Lake City, Utah.
2.21 HAS ANY PILOT STUDY BEEN CARRIED OUT USING THIS DESIGN?

The participating research team members and PERC network have extensive experience conducting clinical research\textsuperscript{4,82,91,147,191}. The network has monthly conference calls and the executive meets several times per year. Dr. Freedman, the Vice-Chair of PERC, has successfully completed and published several gastroenteritis clinical trials\textsuperscript{66,194} with publications in BMJ\textsuperscript{122} and NEJM.\textsuperscript{79} He additionally led a 50 patient multicentre pilot study employing Lactobacillus which provided promising preliminary data, evaluated the feasibility of the current proposal and identified potential problems. The pilot included a placebo group and two dosages: $4 \times 10^9$ CFU/day and $8 \times 10^9$ CFU/day. It did not detect a trend toward increased side effects in the $8 \times 10^9$ CFU/day arm; hence, to ensure our study has the optimal ability to answer the primary question, the $8 \times 10^9$ CFU/day dose will be used. Overall, 91% of all doses dispensed were administered. Key information data provided by the pilot were: (1) the safety of high dose Lactobacillus, (2) anticipated recruitment and compliance estimates, (3) the revision of data collection forms, (4) the use of rectal swab for specimen collection (aside from sIgA), (5) the optimal rectal swab testing device, (6) day #5 instead of 7 compliance assessment, (7) modified follow-up protocol to minimize loss to follow-up, and (8) proved our ability to obtain Health Canada approval.

3.0 TRIAL MANAGEMENT

3.1 WHAT ARE THE ARRANGEMENTS FOR DAY-TO-DAY MANAGEMENT OF THE TRIAL? (APPENDIX 13) WCHRI, based at the University of Alberta, will act as a central repository for all study data. Staffing will include a project manager, a medical informatics specialist and an assistant. WCHRI will be responsible for the provision of data collection technology and clinical data management services. WCHRI’s staff has extensive experience and expertise in collecting data using REDCap software and managing study data in accordance with Good Clinical Practice requirements including the use of qualified and trained study personnel, study monitoring, standard operating procedures, validated software, data audit trails, and quality assurance. Study participating sites will retain the option of using the developed REDCap database as the primary method of data collection and storage. Due to the extensive validation completed by WCHRI, data can be obtained from the patient and then directly entered into the secure REDCap database via an electronic tablet (e.g. iPad\textsuperscript{6}). Study sites may also collect data on paper case report forms, which would then be transcribed into the REDCap database. For all study data collected, source documentation will be defined in the Manual of Operations. The Alberta Children’s Hospital (the PI’s institution) serving as the coordinating centre, will be in constant communication with WCHRI, and will be responsible for study training, monitoring, and progress. Drs. Willan and Nettel-Aguirre will supervise all data analyses. Dr. Freedman will take overall responsibility for the study. Site Investigators and Clinical Research Assistants/Nurses will share responsibilities including day to day activities, payroll, study promotion, contacting caregivers, and reviewing charts.

Research Ethics Board (REB) and Health Canada approvals will be obtained. All ED physicians and nurses will be educated regarding the study and Clinical Research Assistants/Nurses will be trained. Sites have committed to having Clinical Research Assistants or Clinical Research Nurses present 75 hours/week during peak season and volume periods (7 months/year). Their presence will maximize study enrollment by continuously reminding physicians about the study and enrolling eligible children. Participating institutions all have significant infrastructure in place and will use a variety of methods to optimize coverage while minimizing costs including Clinical Research Assistants or Nurses covering multiple studies and volunteer programs (e.g. www.sickkids.ca/HealthcareProfessionalsandStudents/clinical-research/index.html).

The AHS Research Pharmacy will ship the study drug in batches to the participating institutions. The AHS Research Pharmacy will also maintain a batch of sachets which have not been randomized to be
sent to high recruiting sites. Collaborating pharmacies will be blinded to study drug and will be responsible for storage and providing study kits to the site Clinical Research Assistants/Nurses. Regular e-mail, weekly teleconferencing for the first 6 weeks of the trial, and monthly conference calls will be used to monitor start up and to obtain updates on recruitment and issues arising. Real-time data entry will facilitate an ongoing data cleaning plan. Double data entry will be employed on a random sampling of subjects at various time points throughout the study to ensure the data collected is accurate and is being recorded properly.

Drs. Pang, Louie and Chui will take responsibility for microbiologic testing, specimen storage and data management at ProvLab AB. They have extensive experience managing stool specimens and will correspond with the study team at ACH on a weekly basis.

3.2 WHAT WILL BE THE ROLE OF EACH INVESTIGATOR AND COLLABORATOR? This study, under the umbrella of PERC brings together North American investigators with transdisciplinary expertise. Dr. Freedman who has expertise in AGE research,\(^1,4,7,19,66,122,130,152,194-197\) recently reported\(^79\) that ondansetron, an antiemetic agent, is effective in pediatric AGE. It is now routinely used to reduce the need for intravenous hydration and hospitalization.\(^80,132,198-200\) Dr. Gorelick, a clinical epidemiologist,\(^201-204\) with significant network research experience,\(^205-209\) has provided senior guidance and high level input from a large research think-tank in the United States (Pediatric Emergency Care Applied Research Network-PECARN). Dr. Schuh\(^83,186\) has successfully completed 15 pediatric ED RCTs and has guided the study since its inception. Dr. Johnson,\(^82,186\) who has multicentre RCT experience has served as a resource regarding operational issues and will guide KT\(^210-212\) efforts. Dr. Schnadower has led efforts to conduct a similar study in the United States and has served as a liaison with the Pediatric Emergency Medicine Collaborative Research Committee (Dr. Freedman is a steering committee member). Site investigators will supervise the study at their respective institutions. Dr. Philip Sherman has experience with Lacidofil\(^69,71,213\) and his laboratory will perform the fecal slgA analyses.\(^96,97\) Drs. Willan and Nettel-Aguirre, both PhD statisticians, and Mr. Goeree, a health economist, will perform the statistical and economic evaluations. Dr. Willan is extensively involved in methodologic research in the area of health economics and optimizing decision-making in health care research and policy.\(^214-216\) **Dr. Nettel-Aguirre** (co-applicant) is a biostatistician with extensive experience in analyzing health outcomes and related data from large, complex, linked datasets and in designing healthcare studies.\(^217-221\)

Microbiologic Team: **Dr. Yaron Finkelstein** (co-PA), a board-certified clinical pharmacologist and pediatric emergency medicine physician has conducted multiple RCTs exploring pharmacometrics and safety in infected pediatric\(^222,223\) and general\(^224\) populations in addition to pathogen-specific efficacy studies in infectious gastrointestinal diseases.\(^225,226\) Drs. Freedman and Finkelstein have successfully collaborated on several pediatric AGE and clinical medication studies.\(^224,227-229\) Our team includes **Drs. Xiao-li (Lilly) Pang and Bonita Lee** (co-applicants) who have collaborated extensively\(^167,230\) and have developed numerous assays for virus detection,\(^163,164,169,230,231\) and quantification\(^166,232-234\). They will share joint responsibility for all viral analyses. **Dr. Linda Chui** (co-applicant), who has done extensive work in the development of protocols for the molecular detection of non-traditional enteric bacteria (i.e. non-O157 STEC) employing real-time PCR\(^235-239\) will be responsible for the quantification of stool bacterial load which is a natural extension of her molecular work and expertise in this area.\(^232,237,240,241\) **Dr. Marie Louie** (co-applicant), an infectious disease specialist and medical microbiologist with expertise investigating and managing the public health implications of enteric pathogens,\(^240,242,243\) will lead knowledge translation efforts within the microbiology community.

Working with stool from children with norovirus (n=244) and rotavirus (n=102), our team (Pang, Lee) has developed and validated real-time quantitative PCR assays to measure enteric virus genomic nucleic
acid (NA) in stool (i.e. quantify stool viral load; see Appendix 3). A standard curve has been established employing known genomic copies of DNA fragments, which have then undergone 10-fold dilutions from a single copy to 1 x 10^8 copies. Our team (Chui) has established a bacterial DNA extraction protocol which yields high quality and quantity of DNA. This led to the whole-genome sequencing of 200 bacterial isolates which identified biomarkers for the development of amplification assays, both loop-mediated isothermal amplification (LAMP) and real-time polymerase chain reaction (PCR) assays. Both assays have excellent sensitivity and no evidence of cross reactivity has been observed. These quantitative assays have been correlated with colony forming unit (CFU) counts with crossing point values in the real-time PCR assay.

All team members will be aided by WCHRI, MICYRN, the Clinical Research Coordinator, and the site Clinical Research Assistants or Nurses. Each study site has a dedicated study research coordinator who is responsible for organizing the conduct of the study at their respective institutions. Supporting the pathogen effectiveness work, lab research technologists have extensive experience with specimen processing, handling, storage, and testing.

3.3 DESCRIBE THE TRIAL STEERING COMMITTEE AND THE DATA SAFETY AND MANAGEMENT COMMITTEE. **Trial Steering Committee:** The advisory panel has included knowledge users, caregivers, pediatricians, emergency medicine physicians, gastroenterologists, and infectious disease physicians. The protocol has been revised based on guidance provided by the PERC and PECARN networks. Non-research team members who have had extensive input include clinicians, statisticians, ethicists, and coordinators with multicentre research expertise. Official committee members have included senior clinical research team members (Drs. Gorelick, Schuh, Johnson), Dr. Sherman, a Canada Research Chair in Gastrointestinal Disease (selection of probiotic agent, dose, duration of therapy, and planned translational studies), Dr. Kuppermann, the past-Chair of PECARN, Dr. Dean, expert in conduct of multicentre network research, and Dr. Plint, the Chair of PERC. This has ensured that the study will answer important questions that can readily be applied by these leading KT research networks. **Data Safety Monitoring Board (section 2.18 also):** There will be an independent monitoring committee consisting of a biostatistician (Nick Barrowman, PhD-Ottawa), and two physicians with RCT expertise (Drs. Mark Roback–Minnesota and Terry Klassen (Chair) - Winnipeg). This committee will be independent of the investigators and will be advised of all adverse events.

3.4 ADVERSE EVENT REPORTING

**Adverse Event (AE):** An adverse event is any unfavorable or unintended clinical or other occurrence during the study period that may or may not be the result of participation in the research study.

**Expected Adverse Drug Reactions/Events**
These include the following as they are part of the natural history of the underlying disease process:

- Hospitalization
- Future health care provider visit, ED return visit
- IV rehydration
- Abdominal pain, distension
- Vomiting, diarrhea, fever, flatulence

Because expected adverse events are part of the natural history of acute gastroenteritis and diarrheal illness in children, they will not need to be reported as Adverse Events. This information will be recorded in normal study data collection processes.

**Serious Adverse Events**
Any Serious Adverse Event (SAE) that occurs after the first sachet administered will be reported to the Research Ethics Board (REB) and the study subject will be followed until the conclusion of the event.

A SAE is defined as:
- Results in death.
- Is life-threatening. This refers to an event in which the patient was at immediate risk of death; it does not refer to an event that might have caused death had it been more severe.
- Results in a persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect
- Is medically significant. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

In addition, any serious adverse reaction to the natural health product will be reported to the Natural Health Product Directorate (NHPD).

**Adverse Event Reports**
For unexpected adverse events, we will inform the REB, in addition to the clinical chief of the ED, and the external sponsor within 7 days of learning of the event, if applicable and deemed necessary by the Principal Investigator.

For unexpected SAEs, we will inform the REB, in addition to the clinical chief of the ED, and the external sponsor within 24 hours of learning of the event (by AE form, telephone or email). The SAE information will be sent even if the information is incomplete. A complete follow-up AE report will be submitted as soon as possible but no later than 7 days after the initial reporting.

**Collaborating Study Sites**
The principal investigator or delegate will also submit to the University of Calgary REB information received from other sites. Conversely, serious adverse events that occur at The Alberta Children’s Hospital (The University of Calgary) will be communicated by the principal investigator to collaborating sites, as their local requirements dictate. To ensure that data remains confidential and unbiased, a medical monitor will be appointed at the sponsoring institution (The University of Calgary). The medical monitor will be an Emergency Department physician with expertise in clinical research. The medical monitor will review adverse event information from collaborating study sites, in lieu of the principal investigator. The principal investigator (as the sponsor) will still maintain the responsibility of reviewing any Serious Adverse Events occurring at any of the participating study sites.

**Adverse Event Coding:**
Adverse Event (AE) data will be reviewed by trained staff and coded using the Medical Dictionary for Regulatory Activity (MedDRA - [https://www.meddra.org/](https://www.meddra.org/)) system. Adverse Event data will be collected from participants at the time of the event. MedDRA coding will be assigned to each event at the end of the recruiting period.

**Health Canada (Natural Health Product Directorate) Reporting**
Adverse drug reactions (ADR) that are both serious and unexpected are subject to expedited reporting to Health Canada (NHPD) by the sponsor. These include reactions;

- Where it is fatal or life-threatening, immediately where possible and, in any event, within 7 days after becoming aware of the information
• A complete follow up report within 8 days which includes an assessment of the importance and
implication of any findings including relevant previous experience with the same or similar drugs
• Where it is neither fatal nor life-threatening within 15 days after becoming aware of the information

Each ADR which is subject to expedited reporting will be reported individually in accordance with the
data element(s) specified in Section 78 of the *NHP Regulations*, ICH Guidance Document *E2A: Clinical
Safety Data Management: Definitions and Standards for Expedited Reporting*.

**Emergency Unblinding**

Un-blinding should only occur in the event that there is clinical concern regarding the possibility of
bacteremia/sepsis or when it is felt by the treating physician that unblinding would alter the clinical
care being provided. All patients whose therapy is intentionally un-blinded will discontinue the
experimental therapy. Un-blinding should only occur when future clinical treatment of the patient will
depend on prior treatment administered. Approval from the principal investigator or designate will be
obtained prior to un-blinding. If the principal investigator cannot be reached, the un-blinding can be
performed and the principal investigator informed within 24 hours via e-mail or telephone call.
Accidental and intentional un-blinding will be documented and reported and the subject will be
withdrawn from the study.

**3.5 PREMATURE WITHDRAWL/DISCONTINUATION CRITERIA**

The subjects retain the right to withdraw from the study at any time, although withdrawal from the study
is strongly discouraged after the subject has been enrolled.

Every effort will be made to contact all subjects for follow-up as scheduled. Subjects will be withdrawn
from the study if:

1. After enrollment they are determined to meet any of the exclusion criteria
2. If the subject is admitted to an intensive care unit
3. If it is deemed by the treating physician that the child’s health may be jeopardized by continued
   participation in the study
4. The patient’s caregivers wish to withdraw their child for whatever reason

If the patient’s caregiver chooses to withdraw their child from the study, they will be provided with a
choice regarding their exit from the study:

1. The caregiver may choose to withdraw the child from the study, as well as all data collected from
   their child’s participation in the study
2. The caregiver may choose to withdraw their child from the study; however they will allow
   continued use of study data collected from their child.

**3.6 RECORD KEEPING**

The data produced from this study will be stored in a secure, locked location. Only members of the
research team will have access to the data. Following completion of the research study the data will be
stored and kept for a minimum of 25 years. The data will then be destroyed in accordance with the
University of Calgary and Tri Council destruction policy for clinical trial documentation.


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Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis: Randomized Controlled Trial


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APPENDIX 1: Pathogen-Specific Effectiveness

Knowledge of the pathogen-specific effects of probiotic consumption is lacking. A single animal study revealed that probiotics affect each viral pathogen uniquely. Amongst sows and piglets fed with or without probiotic supplementation, no differences between the groups were recorded for hepatitis E virus, encephalomyocarditis virus and norovirus. However, astrovirus was exclusively detected in the non-supplemented control group and rotavirus was shed in lower amounts in the probiotic piglet group. Analysis of immune cell antigens revealed significant differences in the B and T cell populations between the two groups. Human data is inconclusive. An unadjusted pathogen-group analysis of hospitalized Taiwanese children randomized to probiotic/placebo identified a reduction of diarrhea duration amongst children with confirmed viral infections and those without an identified etiology who received a probiotic; no benefit was seen in children with bacterial infections administered the same probiotic. A second inpatient Taiwanese study was unable to document significant pathogen-group benefits. Moreover, the applicability of both of these studies to our context is limited given the differences in population (hospitalized and pathogens, Salmonella, 16-30%) relative to our outpatient population (Salmonella causes ~1% of AGE cases; Figure 1). In fact, ~95% of children brought for ED care in North America are discharged and managed as outpatients. Lastly, in these studies, norovirus, which is likely the leading cause of AGE in North America, was under-represented. To date, no study has reported norovirus-specific probiotic effectiveness data, or any such data in relation to any pathogen in outpatient children in North America.

Several other small studies have explored probiotic effectiveness in children with rotavirus infection. In 2000, Simakachorn reported that among 35 children with rotavirus, fewer children in the probiotic arm had ongoing diarrhea after 24 hours of treatment (P=0.01). Guandalini reported that the mean Day #3 stool frequency was lower among rotavirus-positive children administered a probiotic (P<0.05). These findings are contradicted by earlier work that reported a similar time to diarrhea resolution among rotavirus-positive and rotavirus-negative participants administered a probiotic. Additionally, Costa-Ribeiro reported no difference in stool output or diarrhea duration in 62 children with rotavirus-positive AGE allocated to the probiotic arm. Thus, the limited data available regarding probiotic use in relation to rotavirus disease remains inconclusive.

Other small studies reported outcomes in patients with confirmed bacterial etiologies. Two studies reported that stool frequency was similar in the probiotic and placebo groups (P=0.42) and that the mean duration of diarrhea was similar between study arms. In contrast, amongst 21 children with E. coli enteric infections, probiotics improved stool consistency on Days #3 and 4. Thus, knowledge of probiotic efficacy in relation to bacterial infections is similarly limited, conflicting, and needed.
APPENDIX 2: Lacidofil (L. helveticus Rosell-52 and L. rhamnosus Rosell-11)

The probiotic Lacidofil® is a two-strain product which has been commercially available since 1995. It is composed of L. rhamnosus R0011 and Lactobacillus helveticus R0052 in a 95:5 ratio. L. rhamnosus R0011 was isolated from a dairy starter culture in 1976 and deposited at the Institut Pasteur Collection Nationale de Cultures de Microorganismes (CNCM, Paris, France) as I-1720. The L. helveticus R0052 was originally isolated in 1990 from a dairy culture for ‘sweet acidophilus milk’ and deposited in the Institut Pasteur CNCM as I-1722. Historically, it had been identified as a Lactobacillus acidophilus on the basis of its biochemical and metabolic activities, however, subsequent molecular investigation reclassified the identity of this strain as L. helveticus. All studies prior to April 2006 refer to R0052 as L. acidophilus. They are both Gram (+) anaerobic bacilli found in pairs or short chains.

Composition

**Active Ingredients (freeze-dried)**

| Lactobacillus rhamnosus R0011 | 3.8 x 10⁹ CFU/sachet |
| Lactobacillus helveticus R0052 | 0.2 x 10⁹ CFU/sachet |

It is available in sachets with the total weight of all ingredients = 1 gm. Placebo sachets, which are also supplied by the manufacturer, contain only the non-medicinal excipients and is identical in appearance, smell, and weight to the active sachet (verified in pilot study).

The quantities supplied in each sachet are guaranteed for 18 months after manufacture when stored at ambient temperature. Stability testing shows complete stability when stored under refrigerated conditions with 100% survival of the initial bacterial concentration. At ambient temperature the average monthly loss is 4%. To ensure that each sachet provides the desired dosage (4 x 10⁹ CFU) when stored at room temperature for 18 months, sachets are produced with overages.

Genome Sequencing

The strains in Lacidofil were sequenced at Genome Québec (Montreal, Canada) using pyrosequencing (454 Life Sciences, Branford, CT, USA) and the genomes have been uploaded to GenBank. L. rhamnosus R0011 Bioproject Genome ID: 51799 (accession: PRJNA51799). L. helveticus R0052 accession: AEIR01000000. No antibiotic resistance genes or other genes of concern were observed in the genome of either strain.

Independent Testing

Testing of sachets prepared for our pilot study, which were intended to contain 4 x 10⁹ CFU/sachet, was conducted by Dr. Denis Groleau at the Microbial and Enzymatic Technology Process Centre of the National Research Council – Biotechnology Research Institute. Sachets were provided to him for testing by the Research Support Pharmacy (Darcy Nicksy) at The Hospital for Sick Children. He found that the sachets were in the required range (maximum overage = 300%) as specified by the Natural Health Product Directorate of Canada and exceeded the anticipated shelf-life at room temperature (see Lactobacilli Enumeration Letter – page 26). He additionally conducted a molecular biologic analysis using a technique termed RAPD (Random Amplification of Polymorphic DNA – page 27) to confirm the identity of the organism. Two primers for each species were employed: Primer OPA-18 and Primer M14. The control strains were provided by Institut Rosell-Lallemand: Lactobacillus rhamnosus R0011 and Lactobacillus helveticus R0052. The following image (page 30) is a display of the results: R1, R2 and R3 were DNA originating from 3 colonies picked at random on a selective medium for L. rhamnosus; H1, H2 and H3 were DNA from 3 colonies picked at random on a selective medium for L.
**helveticus.** The electrophoresis gels were prepared, separating the pieces of DNA according to their length. The image (prepared by Dr. Groleau) demonstrates that the bacterial strains in Lacidofil match those in the controls.

**Excipients**

- Maltodextrin (carrier) 217 mg/sachet
- Magnesium stearate (lubricant) 7.5 mg/sachet
- Ascorbic acid (anti-oxidant) 1 mg/sachet

These excipients have complete chemical insensitivity to the lyophilized bacteria, are suitable for production with the technology of corporate use and ease dispersion.

**Pharmacodynamics & Pharmacokinetics**

R0052 and R0011 produce lactic acid, a pathogen inhibitory substance, and the addition of either organism to *Citrobacter rodentium* demonstrates time-dependent growth inhibition.\(^\text{16}\) Culture supernatants also inhibit *C. rodentium* growth implying that inhibitory secretory factors are present. Mouse pretreatment with Lacidofil also reduces *C. rodentium* colonization.\(^\text{16}\) Lacidofil additionally reduces bacterial colonization and gastric inflammation in *H. pylori* infected mice.\(^\text{17}\) Strains R0052 and R0011 also inhibit *H. pylori* growth and *E. coli* adhesion in a dose-dependent manner.\(^\text{18}\) These strains also resist gastric acid, bile and intestinal secretions and are capable of adhering to human intestinal epithelial cells.\(^\text{18, 19}\) There is no evidence that these microbes cross the intestinal barrier.\(^\text{20}\) The bacteria preserve the epithelial barrier by maintaining intercellular tight junctions and reducing the translocation of other bacteria.

**Stability**

Lacidofil has been found to be very stable in beverages aside from soft drinks (low pH) and hot drinks (> 70°C). Testing in apple-based fruit juices has demonstrated that consumers can expect good viability of *L. rhamnosus* R0011 over a few weeks of storage in a refrigerator, even if the bottles have been opened and cells are exposed to oxygen.\(^\text{21}\) *Stability in oral rehydration solution is optimal* because the pH and osmotic pressure are ideal for microbes (similar to phosphate buffered saline solution used in the laboratory), and this procedure has been done successfully in other studies.\(^\text{9, 22, 23}\) Stability testing has been performed by the manufacturer and is as follows:

<table>
<thead>
<tr>
<th>Solution</th>
<th>Initial Concentration/250 mL</th>
<th>Time 0 Concentration/250 mL</th>
<th>Time 0 % Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>PediaLyte-Unflavored</td>
<td>4 x 10^9</td>
<td>3.34 x 10^9</td>
<td>84%</td>
</tr>
<tr>
<td>PediaLyte-Grape</td>
<td>4 x 10^9</td>
<td>3.44 x 10^9</td>
<td>86%</td>
</tr>
<tr>
<td>Apple juice</td>
<td>4 x 10^9</td>
<td>2.97 x 10^9</td>
<td>74%</td>
</tr>
</tbody>
</table>
Montréal, 25 August 2009

Dr. Stephen Freedman, MDCM, FRCPC, FAAP, M.Sc.
Associate Scientist, Research Institute
Assistant Professor of Paediatrics
The Hospital for Sick Children,
Toronto, Canada

Re: Lactobacilli Enumeration in Sachet

Dear Dr. Freedman,

I am pleased to put in an official letter the results of the lactobacilli enumeration counts I have performed starting from one Lacidofil® sachet in early July 2009.

The work was started in the afternoon of July 10th, 2009. The content (1.0016g) was placed into 98mL of sterile phosphate buffer and the mixture shaken at 266 rpm for 10 minutes in an incubator. The sample was then serially diluted (1mL into 9mL of sterile phosphate buffer) up to dilution 10⁻⁵. The dilutions 10⁻⁵ to 10⁻² were thereafter used for counting. Counting was done as follows: 0.1mL aliquots (in triplicate) was added to each of 3 Petri dishes to which ca. 20mL of reconstituted MRS agar at 45°C was added; mixing was done by manual swirling after which the plates, once the agar had hardened, were incubated at 37°C for ca. 72 hours under anaerobic conditions (GasPak system, BBL).

The counting of colonies was done by Mr. Wayne Levadoux from my group (I was on vacation). Using the 10⁻² and 10⁻⁵ dilution plates (total of 6 plates), the following result was obtained: 11.34 x 10⁵ CFU (colony forming units) per sachet (per gram).

I hope that you will find these results satisfactory.

Sincerely yours,

Denis Groleau, PhD (Microbiology)
Group Leader
Microbial and Enzymatic Technology
Bioprocess Centre
NRC-BRI
Montréal, 25 February 2010

Dr. Stephen Freedman, MDCM, FRCP, FAAP, M.Sc.
Associate Scientist, Research Institute
Assistant Professor of Paediatrics
The Hospital for Sick Children,
Toronto, ON, Canada

Re: Identification of the bacterial population present in lactobacilli sachets using a molecular biology approach (RAPD)

Dear Dr. Freedman:

At your request, I am pleased to submit a short description of the work we did here very recently in order to identify, using a molecular biology approach named RAPD, the lactobacilli population present in one of the lactobacilli sachets used in your study.

The work was divided into two steps:

**Step 1:** Estimation of the total population of the lactobacilli (per gram of dry material) together with an estimate of the population of each one of the two lactobacilli species comprising the population.

**Step 2:** Molecular biology-based (RAPD) identification of the two lactobacilli species
RESULTS

For Step 1

The content of one sachet (1 gram of dry material) was used, as done before, for estimating the total viable lactobacilli population using the MRS medium. The total viable population was found to be between $5.767 \times 10^9$ and $6.0 \times 10^9$.

The *Lactobacillus rhamnosus* R0011 population was found to be between $6.533 \times 10^9$ and $8.0 \times 10^9$ using MRS medium containing vancomycin.

The *Lactobacillus helveticus* R0052 population was found to be $2.33 \times 10^8$, or roughly 3.5% of the total viable lactobacilli population, using MRS medium containing clindomycin.

For Step 2

For each one of the two lactobacilli populations, *L. rhamnosus* and *L. helveticus*, three (3) colonies were selected at random from the appropriate plates, their DNA extracted and processed according to protocols provided by Dr. T. Tompkins from Institut Rosell-Lallemand (Montréal, Qc). The two control strains were treated in an identical manner.

RAPD analysis was done according to a protocol also supplied by Dr. Tompkins. Two primers were used on each sample: Primer OPA-18 and Primeer M14. Please note that, in order to obtain optimal results, Dr. Tompkin's protocol was modified very slightly: The annealing temperature was $45^\circ C$ instead of $42^\circ C$.

Earlier this week, I sent you the electronic pictures of the two electrophoresis gels obtained at the end. The pictures show conclusively that all colonies examined showed a RAPD profile identical to that of the control strain.

Conclusion

The sachet contained the "announced" two lactobacilli species.
I hope that you will be very satisfied with these results.

It has been a real pleasure to interact with you in the last few months.

Best Regards,

Denis Groleau
Group Leader
Microbial and Enzymatic Technology Group
Bioprocess Centre
NRC-BRI
R0052; *L. helveticus*
R0011; *L. rhamnosus*

* Annealing temperature for PCR was modified from 42 to 45C.
Physiological Characteristics

Resistance to Gastric Acidity: The rate of survival of *L. helveticus* R0052 and *L. rhamnosus* R0011 was measured at 3 pH values (4.0, 3.0, and 2.0) after 30 minutes at 37°C. The strain demonstrated resistance to moderate acid conditions with 87% survival at pH 4.0, and 76% survival at 3.0.\(^{18}\) In physiologic terms, this is exceptional as it is estimated that every 10 minutes the stomach eliminates 50% of its fluid, thus after 30 minutes in the stomach, it is estimated that 87% of the probiotic bacteria ingested will have been evacuated towards the intestine. *L. rhamnosus* R0011 also has excellent resistance to gastric acid, with > 80% survival in a solution with a pH of 3.0.\(^{18}\) Nonetheless, we will encourage the administration of Lacidofil/placebo to occur within 30 minutes of mealtime to avoid ingestion when the stomach pH may potentially be <3.0.

Resistance to Biliary Salt: Experiments were conducted in the following fashion: 2 tubes were inoculated with the same concentration of *L. helveticus* R0052 or *L. rhamnosus* R0011 and incubated at 37°C, one contained bile at 0.3% and a control. The turbidity of the resultant solution quantified by the measure of the wavelength at 600 nm, was used to reflect growth. The results showed that the bile exercises an inhibiting effect on the growth of both *L. helveticus* R0052 and *L. rhamnosus* R0011, but both strains are capable of surviving in the presence of the high concentration of bile.\(^{18}\)

Adherence to Epithelial Cells: *L. helveticus* R0052 and *L. rhamnosus* R0011 show very strong adhesion to the surface of epithelial cells.\(^{18}\)

Action on the Intestinal Epithelial Lining and Microflora: *L. rhamnosus* R0011 has been demonstrated to stimulate the proliferation of intestinal epithelial cells.\(^{18}\) This stimulation might confer protection against potentially pathogenic agents and aid in replacing damaged epithelial cells thereby reinforcing the physical barrier which separates the intestinal pathogens from the general blood circulation.

Inhibition of Pathogen Growth: In vitro research has found that both *L. helveticus* R0052 and *L. rhamnosus* R0011 prevent injury of the epithelial cell barrier induced by attaching-effacing bacterial enteropathogens.\(^{24}\) There is also in vitro evidence that a non-viable constituent derived from *L. helveticus* R0052 is effective in interrupting the infectious process of intestinal pathogens.\(^{25}\)

Stimulation of the Immune System: *L. helveticus* R0052 and *L. rhamnosus* R0011 have the capacity to activate in vitro the multiplication of immune cells.\(^{18}\) On contact with these probiotic agents, immune cells isolated from a mouse’s spleen show a dose-dependent proliferation response. This response appears to be greatest with *L. rhamnosus* R0011. On the other hand, *L. helveticus* R0052 induces mitogenic and polyclonal activation of B lymphocytes in vitro, stimulating the release of antibodies against pathogens. *L. helveticus* R0052 additionally stimulates the production of cytokines (Tumour Necrosis Factor alpha and interleukin-6) by macrophages.

Toxicology

Animal and human safety studies were performed in 1992-93 by Drs. Smirnov and Khrecenko in the Ukraine. The results were reported directly to Institut Rosell Inc.\(^{18}\) They sacrificed 25 rats following 14 days of Lacidofil administration.\(^{18}\) Compared with 15 controls, no differences were detected. In the intestines, the Lacidofil treated rats had greater microbial counts but significantly less hemolytic and non-lactose utilizing bacteria indicating a reduction in potentially pathogenic bacteria. A 28-day toxicity study in 10 rats who received 10 times the maximal human recommended dose found no changes indicative of toxicity. When a dose 3500 times the recommended daily human dose was given for 70 days, there were no deaths, adverse reactions, or gross anatomical changes.\(^{26}\)
Lacidofil has been sold as a dietary supplement or a pharmaceutical product as a powder in capsules or in sachet format in a number of countries since 1995. During the period of March 2003 to March 2008 exposure to Lacidofil was estimated to include 12,606,701 patients.\(^{(27)}\) A total of 6 non-serious spontaneous case reports related to Lacidofil were collected by the Post-Marketing Surveillance system which included a search in “REACTIONS,” “MEDLINE,” “EMBASE” and “INIST” using the search criteria “lactobacillus.” They were:

<table>
<thead>
<tr>
<th>Country</th>
<th>Source</th>
<th>Gender</th>
<th>Age</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>Naturopath</td>
<td>F</td>
<td>56</td>
<td>Pain, swelling, redness, itching, tenderness of outer labia</td>
</tr>
<tr>
<td>USA</td>
<td>Naturopath</td>
<td>F</td>
<td>55</td>
<td>Pain, swelling, redness, itching, tenderness of outer labia</td>
</tr>
<tr>
<td>USA</td>
<td>Naturopath</td>
<td>M</td>
<td>65</td>
<td>Rash in groin area</td>
</tr>
<tr>
<td>Poland</td>
<td>Competent authority</td>
<td>M</td>
<td>Child</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>Poland</td>
<td>Consumer</td>
<td>Child</td>
<td></td>
<td>Diarrhea</td>
</tr>
<tr>
<td>Poland</td>
<td>Health professional</td>
<td></td>
<td></td>
<td>Macular rash</td>
</tr>
</tbody>
</table>

**Lacidofil – Research by Dr. Sherman’s Lab**

*Lacidofil inhibits enterohaemorrhagic Escherichia coli O157:H7:* Lacidofil administration reduces epithelial injury following exposure to Escherichia coli O157:H7 and E. coli O127:H6.\(^{(28)}\) When human epithelial cells were treated with protein extract alone, infected with E. coli O157:H7, or pretreated with surface layer protein extract from Lacidofil prior to infection,\(^{(25)}\) pre-treatment with protein extracts decreased pathogen adherence and attaching-effacing lesions in addition to preserving the barrier function of monolayers. Thus, a non-viable constituent derived from a Lacidofil may prove effective in interrupting the infectious process of an intestinal pathogen. Additionally, pretreatment with the probiotic *L. rhamnosus* reduced morphological changes and diminished the number of attaching and effacing lesions induced in response to EHEC O157:H7 infection.\(^{(24)}\) There was a corresponding attenuation of the EHEC-induced drop in electrical resistance and the increase in barrier permeability assays. In addition, *L. rhamnosus* protected epithelial monolayers against EHEC-induced redistribution of tight junction proteins. Collectively, these findings provide in vitro evidence that treatment with *L. rhamnosus* could be an effective treatment for preventing injury by enteropathogens.

**Ability of Lacidofil to prevent Campylobacter jejuni disease:** Human colon and embryonic intestine epithelial cells were pretreated with Lacidofil and then infected with two C. jejuni pathogens.\(^{(29)}\) *L. helveticus* strain R0052 reduced C. jejuni invasion by 35-41%. It also adhered to both epithelial cell types, which suggest that competitive exclusion could contribute to protection by probiotics.

**Ability of Lacidofil to ameliorate the effects of Inflammatory Cytokines:** Following *L. rhamnosus* inoculation, interferon-gamma priming, and 24 hour tumour necrosis factor-alpha stimulation, epithelial cells maintained transepithelial electrical resistance and zona occludens-1 distribution.\(^{(30)}\) *L. rhamnosus* diminished the nuclear translocation of p65. These findings indicate that *L. rhamnosus* ameliorates the effects of pro-inflammatory cytokines on epithelial barrier integrity and inflammation mediated, at least in part, through inhibition of tumour necrosis factor signaling.

**Animal Studies**
Ability of *Lacidofil* to colonize and reduce gastric inflammation in *H. pylori*-infected mice: Mouse pretreatment with *Lacidofil* suppresses colonization by *H. pylori*. Thirty mice were divided into four groups: Group A was fed sterile water, group B received probiotics in sterile drinking water, group C was challenged orogastrically with *H. pylori*, and group D was pretreated with probiotics in drinking water prior to and following challenge with *H. pylori*. Probiotics in drinking water did not affect the overall well-being of mice (Group B). Pretreatment with probiotics reduced the number of mice with *H. pylori* growth from stomach homogenates and the percentage of mice with moderate-severe *H. pylori*-induced inflammation in the gastric antrum was reduced (71% to 29%).

*Lacidofil* reduces the effects of *Citrobacter rodentium* infection in mice: Mouse pretreatment with *Lacidofil* attenuates *C. rodentium*-induced colonic disease. Mice were administered sterile drinking water, *Lacidofil* in sterile drinking water, maltodextrin in sterile drinking water, orogastric *C. rodentium*, or maltodextrin in sterile drinking water for 1 week before *C. rodentium* infection, or they were pretreated with *Lacidofil* for 1 week before challenge with *C. rodentium*. Mice that received *Lacidofil* remained healthy. Dr. Sherman subsequently found that *C. rodentium* infection in newborn mice causes death and that probiotics promote survival. Infection with *C. rodentium* was induced at postnatal day 14 however a subset of mice were pretreated orally with *Lacidofil* starting at 7 days. Survival was maintained by daily treatment with Lactobacilli. Weight loss, colonic epithelial cell hyperplasia, mucosal barrier dysfunction, and elevated serum corticosterone levels in *C. rodentium*-infected mice were ameliorated by *Lacidofil*.

*Lacidofil* prevents bacterial translocation and improves intestinal barrier function: Rats were subjected to either water avoidance stress or sham stress for one hour per day for 10 consecutive days. Additional animals received seven days of *Lacidofil* in the drinking water prior to stress and remained on these probiotics for the duration of the study. 70% of rats subjected to water avoidance stress had bacterial translocation to mesenteric lymph nodes while there was no bacterial translocation in controls. Water avoidance stress induced increased ileal short circuit current was reduced with *Lacidofil* whereas there was no impact on altered conductance. Pretreatment with *Lacidofil* completely abrogated water avoidance stress induced bacterial adhesion and prevented translocation of bacteria to mesenteric lymph nodes. Thus, *Lacidofil* prevents stress induced intestinal abnormalities.

*Lacidofil* normalizes corticosterone release and improves colonic dysfunction: Maternal separation pups were separated from the dam for 3 hours per day from days 4 to 19; non-separated pups served as controls. Twice per day during the separation period, *Lacidofil* was administered to maternal separation and non-separated pups; vehicle-treated pups received saline. There was increased adhesion and penetration of total bacteria in maternal separation pups, but a significant reduction in Lactobacillus species. Probiotic administration ameliorated the maternal separation-induced gut functional abnormalities and bacterial adhesion and penetration at both day 20 and 60, and reduced the elevated corticosterone levels at day 20. Thus altered enteric flora is responsible for colonic pathophysiology. *Lacidofil* improves gut dysfunction in part by normalization of the hypothalamic-pituitary-axis activity.

**Human Clinical Trials**
In a randomized trial in Prague of 113 outpatients aged 1-6 years with diarrhea, 1 capsule of *Lacidofil* (2 x 10⁹ CFU) administered daily resulted in normalization of stool consistency more rapidly than placebo (4.0 vs 5.5 days). There was no change in stool frequency. Rotavirus accounted for 49% of the cases. In children treated with *Lacidofil* there was a significant reduction in abdominal gas causing severe distention, P=0.03. In a non-randomized study of 75 children in 2 hospitals in Prague, 33 children were administered 1-3 capsules (2 – 6 x 10⁹ CFU) of *Lacidofil* per day for 10-14 days and 42 received clay and smectite. *Lacidofil* resulted in more rapid resolution of diarrhea (3.1 vs 6.8 days).
In a recent open label clinical study, the ability of *L. rhamnosus* R0011 and *L. acidophilus* R0052 to survive during the passage through the human digestive tract was evaluated.\(^{(35)}\) Fourteen healthy patients were administered Lacidofil in addition to bacterial spores of *B. stearothermophilus* as a transit marker. Quantification of both strains and the bacterial groups in the feces were performed in a pre-treatment exclusion period, the consumption period and during a 12 day wash-out period. The mean population of *L. rhamnosus* R0011 found in the feces after the consumption period was high. The dominant microbiota of the stool (*Clostridium cocoides*, *Bifidobacterium* sp., *Bacteroides* sp., and *Clostridium leptum* groups) was not significantly altered by the consumption of Lacidofil. In a prospective study, 57 children aged 1 – 16 years were randomly assigned to receive Lacidofil while they took a course of antibiotics.\(^{(36)}\) Diarrhea occurred in 7% of children taking Lacidofil vs. 37% of children in the control group. Clostridial toxins were detected in the stool of 7% in the Lacidofil group vs. 43% of the control group.

A controlled study of 248 children with AGE demonstrated that Lacidofil supplementation (1 capsule, 3 times daily for 14 days) increased phagocytosis, as well as levels of IgA (0.59 ± 0.12 g/l before treatment, 0.68 ± 0.08 g/l after treatment) and IgG (6.34 ± 0.31 g/l prior to treatment, 7.62 ± 0.43 g/l post treatment).\(^{(37)}\) Conversely, a controlled study showed no significant increase in saliva or stool IgA concentrations after a lower dose of Lacidofil supplementation (1 capsule, once daily for 10 days) in infants with AGE.\(^{(33)}\)
A summary of human studies with gastrointestinal disorders is provided:\(^{(38)}\):

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study type</th>
<th>Study size</th>
<th>Dosing regime</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antibiotic-associated diarrhoea</strong>&lt;br&gt;Aryayev and Kononenko, 2009</td>
<td>randomised controlled</td>
<td>36 children with mucoviscidosis (18 conventional therapy with Lacidofil + 18 conventional therapy alone)</td>
<td>age &lt;12 months: 1 capsule qd; 1-3 yrs: 1 capsule bid, 3-12 yrs: 1 capsule tid, &gt;12 yrs: 2 capsules tid</td>
<td>decreased AAD development in children with Lacidofil; improved intestinal microbial balance</td>
</tr>
<tr>
<td>Marushko et al., 2007</td>
<td>randomised controlled</td>
<td>34 children aged 10 months-3 yrs with respiratory pathology (16 antibacterial therapy with Lacidofil + 16 antibacterial therapy alone)</td>
<td>age &lt;1 yr: 1 capsule qd, 1-3 yrs: 1 capsule bid for 2-4 weeks</td>
<td>decreased incidence and duration of AAD; more normalised intestinal microbiome following therapy</td>
</tr>
<tr>
<td>Maydannik et al., 2010</td>
<td>randomised controlled</td>
<td>244 children aged 0-17 yrs with acute respiratory, urinary, or digestive exacerbations (117 antibiotic therapy with Lacidofil + 127 antibiotic therapy alone)</td>
<td>age &lt;1 yr: 1 capsule qd, 1-3 yrs: 1 capsule bid or tid, &gt;12 yrs: 1-2 capsules tid for 14-21 days</td>
<td>lower incidence/decreased duration of AAD with Lacidofil; decreased Clostridium difficile/toxin carriage with Lacidofil</td>
</tr>
<tr>
<td>Patbera et al., 2010</td>
<td>randomised controlled</td>
<td>59 children aged 3-18 yrs with pulmonary TB (23 anti-TB chemotherapy with Lacidofil + 36 anti-TB chemotherapy alone)</td>
<td>3 capsules/day for 1 month; 6 capsules/day for 1 month</td>
<td>reduced C. difficile toxins in stool with 6 capsules/day; alleviated gastrointestinal symptoms; normalised stool; disappearance of glossitis</td>
</tr>
<tr>
<td>Song et al., 2010</td>
<td>randomised controlled double-blind</td>
<td>214 adults with respiratory tract infections on antibiotics (103 Lacidofil + 111 placebo)</td>
<td>1 capsule bid for 14 days vs. placebo (1 capsule bid for 14 days)</td>
<td>improved bowel frequency and consistency with Lacidofil; no difference in AAD occurrence (possibly too small dose of Lacidofil given)</td>
</tr>
</tbody>
</table>

**Helicobacter pylori eradication**

Babak, 2007 | randomised controlled | 35 adults with *H. pylori* associated duodenal ulcer; (20 RAB/AMO/CLA with Lacidofil + 15 RAB/AMO/CLA alone) | 2 capsules tid for 20 days | increased eradication of *H. pylori* with Lacidofil; faster alleviation of dyspeptic symptoms; improved duodenal mucous coat; improved restoration of intestinal microbiota; decreased incidence of AAD with Lacidofil |

Gnyntenko et al., 2009 | non-randomised controlled | 45 children with *H. pylori* (25 anti-*Helicobacter* therapy with Lacidofil + 20 anti-*Helicobacter* therapy alone) | 1 capsule bid for 20 days | |

Vdovychenko et al., 2008 | controlled (randomised?) | 49 adults with *H. pylori* (25 triple therapy with Lacidofil + 24 triple therapy alone) | 2 capsules bid for 10 days | increased eradication of *H. pylori*; increased healing of ulcers |

Ziemniak, 2006 | open label | 641 adults with *H. pylori* (192 PPI/CLA/AMO + 241 PPI/TET/TNZ/bismuth salts + 53 according to antibiogram + 102 according to antibiogram + 53 PPI/CLA/AMO with Lacidofil) | 2 capsules bid for 20 days | increased eradication of *H. pylori* with Lacidofil |

**Paediatric gastrointestinal diseases**

Skorodumova et al., 2007 | controlled, matched for age, sex, duration of disease, aetiology and severity of acute intestinal infections | 248 paediatric patients with acute intestinal infection (225 conventional treatment + 23 conventional treatment with Lacidofil) | 1 capsule tid for 14 days | restored intestinal microbiome with probiotic; improved immune status (increased phagocytosis), increased IgA/IgG with probiotic |
Health Canada Issues: A Clinical Trial Application has already been approved by Health Canada (CTA #136815) for the pilot study on which the current protocol is based.

Pediatric Dosing: The optimal pediatric dose is unknown but the minimum effective dose for therapeutic purposes has been reported to be a daily intake of $10^6 - 10^9$ CFU. While a meta-analysis concluded that a positive linear association exists between the log of the probiotic dose and the reduction in diarrhea duration, no optimal CFU/kg dose has ever been determined. Comparative studies to determine the minimum effective and optimal doses, frequency of administration, delivery vehicles, and duration of treatment are needed. Our three arm (placebo, low dose, high dose) probiotic clinical trial has found no trend towards increased side effects with the higher ($8 \times 10^9$ CFU/day) dose. Thus, to ensure that a sufficient dose is administered, the high dose ($8 \times 10^9$ CFU/day) will be employed.
APPENDIX 3: Reduction in Nucleic Acid Content/gm of Stool Homogenate

The reduction in stool pathogen load will be quantified as the difference in the number of copies of pathogen-specific NA/gm between Days #0, 5, and 28 (i.e. Day #0 – Day #5 – Day #28 = stool pathogen load reduction). The anticipated distribution of viral load is based on our quantification of norovirus in 244 randomly collected stool samples from individuals with AGE (Figure 3).

Although the change in viral load in an individual patient over time is relatively unknown our data infers that there is variability during the course of disease. Aim #2 will directly address the relationship between pathogen load and disease severity by quantifying the absolute reduction in 278 children in whom we anticipate (1) identifying a pathogen and (2) collecting Days #0, #5, and #28 stool samples. We will determine if pathogen (group and specific) and probiotic exposure have an effect on the change in pathogen load employing regression modeling adjusting for interaction. Separate models will determine if (1) the Day #0 pathogen load correlates with baseline disease severity and (2) the Day #5 pathogen load correlates with ongoing symptoms at Day #5.

Figure 3. Norovirus Viral Load N=244

![Histogram of Norovirus Viral Load](image)

Mean = 4.90E8
Std. Dev. = 3.033E9
N = 244
APPENDIX 4: Discharge Instructions

**Medication – Probiotic (Lacidofil) or Placebo**
Please administer the medication we have provided you with **TWICE DAILY** for the **next 5 DAYS**.
- The medication should be given, mixed with 1 ounce (30 mLs) of an unfrozen beverage (containing no ice crystals), at mealtime in the morning and again in the evening. It is also recommended that you do not mix the medication with carbonated or highly acidic beverages.
- Each medication dose should be separated by 12 hours (minimum of 8 hours) and taken within 30 minutes of food/drink.
- It is very important that **ALL 10 MEDICATION DOSES are administered**. The nurse will have given the first dose while you and your child were still in the Emergency Department. There should be 9 doses remaining, as well as 5 extra doses provided (in case one is spilled or vomited).
- If your child vomits the medication within 15 minutes of administration, please repeat the dose. If he/she vomits a second time during the same dose, do not repeat again; wait until the next dose is scheduled.

When your child has completed all 10 study doses of the medication, **please return all of the unused study medication to the research team in the postage paid envelope provided**.

**Follow Up Phone Calls/Email Surveys**
Every day until your child’s symptoms resolve, you will receive a phone call or email survey asking questions related to your child’s health and progress. If possible, please try to provide your best answer to all of the questions asked. You will also receive an email survey/phone call **5 days and 14 days after your visit to the Emergency Department**, regardless of your child’s current symptoms.

**Stool Sample Collection – If you did not consent to provide stool samples at home, please disregard this section of the instructions.**
- **If your child already gave a bulk stool (poop) sample while still in the Emergency Department**, you only need to collect samples, using the supplies provided, from your child on Day 5 and Day 28.
- **If your child did not provide a bulk stool sample while still in the Emergency Department**, please use the supplies provided to collect the first available sample from your child (after leaving the hospital). You will then collect the additional samples at the regularly scheduled times (Day 5 and Day 28).

Remember to collect the sample in the appropriately labeled container (Day 0, Day 5, or Day 28). Instructions for sample collection and courier pick up have been provided with the collection kits.

**You will receive a reminder email/phone call on Day 4 and Day 27 to remind you to collect the stool samples on Day 5 and Day 28, as well as to return the unused study medication.**

**Below are Several Indications You May Want to Take Your Child to See a Health Care Provider**
- a. Blood in vomit
- b. Green vomit
- c. Constant vomiting or diarrhea
- d. Blood in stools (poop)
- e. Signs of dehydration
f. Dry tongue or mouth (dry lips do not indicate dehydration)
g. Sunken eyes
h. Decreased urine (pee) frequency and volume
i. Decreased tears
j. Rapid, deep breathing
k. Very sleepy, hard to arouse, little interaction with surroundings
l. A very bad headache
m. A very sore neck
n. Worsening stomach pain

For additional information, please review the handouts you have been provided on “diarrhea”, “dehydration”, or “gastroenteritis”.

Questions?
If you have any questions related to this study and your child’s participation, you may contact the project coordinator at: (XXX) XXX-XXXX.
APPENDIX 5: Instructions for Stool Collection (Infant and Child)

Instructions for Infant Stool Collection at Home

Pick up available DAILY between _______ and _______ by calling (XXX) XXX-XXXX.

Sample Collection Checklist (please check the box once you have collected the sample)

☐ Day 0 Sample (Only collect if you are given the Day 0 pre-labeled collection kit. If your child gave a bulk stool while still in the Emergency Department, you do not need to collect a Day 0 sample at home)

☐ Day 5 (Collect 5 days after visiting the Emergency Department)

☐ Day 28 (Collect 28 days after visiting the Emergency Department)

STEP 1 – Put on the gloves provided. Scoop the baby's stool (poop) from the diaper using the lid provided from the Stool Sample Container (Figure 1). If possible, try to scoop at least a "pea size" amount into the container.

STEP 2 – Replace the lid on the Stool Sample Container and secure tightly (Figure 2).

STEP 3 – WASH hands with soap and water. Label the Stool Sample Container with date and time of collection (label provided). Write the date and time of collection at the bottom of the blue piece of paper provided with the collection supplies (Figure 3).

STEP 4 – Put the Stool Sample Container back in to the small plastic bag provided (labeled with the appropriate Day # (0, 5, or 28)) and seal (Figure 4). WASH hands with soap and water.

STEP 5 – IMMEDIATELY call the courier service ("name of courier") at (XXX)XXX-XXXX and use reference ________ for sample pickup. The courier service will come to your home within __________ after calling.

For all other study instructions, please reference the discharge instruction sheet in the envelope provided during your visit to the Emergency Department.

THANK YOU FOR YOUR PARTICIPATION

For more information, please contact the study coordinator ("name") at (XXX)XXX-XXXX.
Instructions for Child Stool Collection at Home

Pick up available DAILY between _______ and ______ by calling (XXX) XXX-XXXX

**Sample Collection Checklist (please check the box once you have collected the sample):**

- **Day 0 Sample:** (Only collect if you are given the Day 0 pre-labeled collection kit. If your child gave a bulk stool while still in the Emergency Department, you do not need to collect a Day 0 sample at home)
- **Day 5:** (Collect 5 days after visiting the Emergency Department)
- **Day 28:** (Collect 28 days after visiting the Emergency Department)

**STEP 1** – Raise the toilet seat. Place the stool (poop) collection bucket on the toilet bowl. Placement Instructions can be found on the right side of the bucket (Figure 1).

**STEP 2** – Lower the toilet seat back down to secure the collection bucket (Figure 2). The bucket should be centered under the bowl, if possible.

**STEP 3** – Have the child “poop” directly into the collection bucket. **No urine (pee)** should be collected in the collection bucket, if possible.

**STEP 4** – Put on the gloves provided.

**STEP 5** – Scoop stool/poop from the collection bucket, using the lid provided from the Stool Sample Container (Figure 3).

**STEP 6** – Replace the lid on the Stool Sample Container and store tightly (Figure 4).

**STEP 7** – Remove gloves, and WASH hands with soap and water. Label the Stool Sample Container with date and time of collection (label provided). Write the date and time of collection at the bottom of the blue piece of paper provided with the collection supplies (Figure 5).

**STEP 8** – Put the Stool Sample Container in the plastic bag provided, labeled with the appropriate Day # (0, 5, or 28) and seal (Figure 6). WASH hands with soap and water.

**Instructions for Child Stool Collection at Home**

**STEP 9** – IMMEDIATELY call the courier service (“name of courier”) at (XXX) XXX-XXXX and use reference __________ for sample pickup. The courier service will come to your home within __________ after calling.

For all other study instructions, please reference the discharge instruction sheet in the envelope provided during your visit to the Emergency Department.

**THANK YOU FOR YOUR PARTICIPATION!**

For more information, please contact the study coordinator (“name”) at (XXX) XXX-XXXX.
APPENDIX 6: Fecal Secretory IgA Procedures

**Fecal Secretory IgA:** sIgA testing will be performed employing the Eagle Biosciences Secretory IgA ELISA kits (catalog #: SGA35-K01) in accordance with the manufacturer’s instructions.
### APPENDIX 7: Study Subject Timeline

| Day #0 | - Present to ED  
|        |   - Triaged χ potentially eligible children identified  
|        |   - Clinical Research Nurse Coordinator/Assistant applies inclusion/exclusion criteria  
|        |   - If eligible, consent will be requested  
|        |   - Consent obtained  
|        |   - Data collection form completed  
|        |   - Randomization performed ([www.randomize.net](http://www.randomize.net))  
|        |   - Medication administered in ED  
|        |   - Study drug kit provided to family  
|        |   - Study drug return envelope provided  
|        |   - Discharge teaching and instructions provided  
|        |   - Stool sample obtained  
|        |   - Bulk stool sample obtained at home, if unavailable in ED (optional)  |
| Day #1 | - Administer study medication twice daily  
|        |   - Contacted by study team (method to be selected by family: telephone or e-mail survey) for data collection  |
| Day #2 | - Administer study medication twice daily  
|        |   - Contacted by study team for data collection  |
| Day #3 | - Administer study medication twice daily  
|        |   - Contacted by study team for data collection  |
| Day #4 | - Administer study medication twice daily  
|        |   - Contacted by study team for data collection  
|        |   - Reminder telephone call/email to collect bulk stool on Day #5 (if applicable)  |
| Day #5 | - Administer last 2 doses of study medication  
|        |   - Contacted by study team for data collection & compliance assessment  
|        |   - Contact continues daily if still symptomatic  
|        |   - Bulk stool sample obtained at home (optional)  |
| Day #6-13 | - If no longer symptomatic on Day 5, contact ceases until final data point (Day #14)  
|           |   - Contact continues daily while still symptomatic  |
| Day #14 | - Contacted by study team regarding final survey data collection and the need to return unused medication  |
| Day #21 | - Chart reviewed to confirm caregiver report  |
| Day #27 | - Reminder telephone call/email to collect bulk stool on Day #28 (if applicable)  |
| Day #28 | - Bulk stool sample obtained at home (optional)  |
APPENDIX 8: Modified Vesikari Score

The manuscript describing the development, evaluation and validation of the Modified Vesikari Score (MVS) which is being employed as the primary outcome measure of this study (MVS) has been published in Pediatrics (see Publications Section of the grant).\(^{(42)}\) Below is a summary of the rationale for the development of a Modified Vesikari Score.

The primary outcome measure, the Modified Vesikari Score is a composite measure which includes several aspects which represent the severity of disease. The original score was first described by Drs. Ruuska and Vesikari in 1990\(^{(43)}\) Since then the score has been extensively employed in clinical research, particularly those evaluating the effectiveness of rotavirus vaccines.\(^{(43-54)}\) The score incorporates features of gastroenteritis that are of importance to a variety of stakeholders including parents, children and physicians. It includes the duration of diarrhea and vomiting, the frequency of diarrhea and vomiting, the maximal height of fever, the presence of dehydration and the medical interventions required. The maximal score is 20 points. Most studies have defined severe disease as a score \(\geq 11\).\(^{(45, 48-50, 53, 55-57)}\) Perhaps the best known use of the Vesikari score was in the rotavirus vaccine safety and efficacy evaluation study, published in the New England Journal of Medicine, whose primary outcome, severe rotavirus gastroenteritis, was defined as a score of \(\geq 11\).\(^{(51)}\) In keeping with previous research, we will define moderate gastroenteritis as a score of \(\geq 9\) points.\(^{(58)}\)

### Original Vesikari Score

<table>
<thead>
<tr>
<th>Points</th>
<th>0</th>
<th>1-4 days</th>
<th>5 days</th>
<th>(\geq 6) days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea Duration (days)</td>
<td>0</td>
<td>1-3</td>
<td>4-5</td>
<td>(\geq 6)</td>
</tr>
<tr>
<td>Maximum # of diarrheal stools/24 hr period</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>(\geq 3)</td>
</tr>
<tr>
<td>Vomiting Duration (days)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>(\geq 3)</td>
</tr>
<tr>
<td>Maximum # of vomiting episodes/24 hr period</td>
<td>0</td>
<td>1</td>
<td>2-4</td>
<td>(\geq 5)</td>
</tr>
</tbody>
</table>

**Maximal Recorded Fever**

| < 37.0°C Rectal | 37.1-38.4 °C Rectal | 38.5-38.9°C Rectal | \(\geq 39.0°C\) Rectal |

**Percent Dehydration**

| 0% | - | 1-5% | \(\geq 6\)% |

**Treatment**

| None | Rehydration | Hospitalization | - |

*Temperatures will be adjusted for location of measurement: 1.1°C will be added to axillary temperatures and 0.6°C will be added to oral temperatures.\(^{(59)}\)

Unfortunately, one element of the score, percent dehydration is very difficult to assess accurately, particularly in the ED. It is well known that the clinical assessment of dehydration is inaccurate;\(^{(60)}\) thus the difference between the baseline and fully rehydrated weights is considered the gold standard.\(^{(61)}\) However, even this is of limited value in the outpatient setting due to difficulties in determining when full rehydration has occurred, the variations that occur due to timing of voiding and stooling, and the difficulties in obtaining repeat weights. Thus, we have elected to capture another very important outcome measure – the need for future health care use - which can easily be done in the outpatient setting. **We have modified the Vesikari score by replacing percent dehydration by the need for an unscheduled future health care visit within 2 weeks.** This modification is supported by evidence that
(1) the demand for professional medical care correlates with clinical severity - 41% with severe versus only 5% of mild cases require hospitalization.(62) (2) Modifications of the original Vesikari score have been routinely performed when the outcome of dehydration has been unavailable.(62, 63) (3) In the original dataset, the outcome of ≥ 6% dehydration occurred in 6% of the sample. This is similar to gastroenteritis ED revisit data in children 0-4 years of age provided to us by the Pediatric Emergency Care Applied Research Network (PECARN; courtesy of Dr. Marc Gorelick). Of a total of 12,694 visits to 4 PECARN sites, 682 revisits occurred within 2 weeks (5.4%). Based on the above, we have replaced the outcome of percent dehydration with need for future health care provider visits. The original score structure for this variable (0, 2, 3 points) has been maintained.

0 Points: No future health care provider visit related to vomiting, diarrhea, fever or fluid refusal
2 Points: Community based health care provider visit related to vomiting, diarrhea, fever or fluid refusal
3 Points: ED health care provider visit related to vomiting, diarrhea, fever or fluid refusal

The calculation of the MVS will be based on data collected via daily telephone or email/survey follow-up during the 2 weeks following the index visit with all data points employing the ED visit as Time 0 (i.e. the score prior to administration of the first dose – the pre-enrollment score – will not be included in the score calculated for assessment of the study’s primary outcome). Interventions that occur at the index visit (i.e. following randomization) will only be counted if the child remains in the hospital for greater than 2 evenings (i.e. past midnight on 2 consecutive days). Should the latter occur, the child will be deemed to have required both hospitalization and an ED revisit as that is evidence that the child’s disease severity requires hospitalization. This is based on the expectation of an early onset of benefit as a meta-analysis has reported a day #2 reduction of 1.6 stools/day (95% CI: 07, 2.6). (40) Thus, the primary outcome will be the presence of moderate-severe disease, as defined by a maximum MVS of ≥ 2 during the 2 week follow-up period.

Our estimate for the development of moderate to severe gastroenteritis in controls is based our 2009 evaluation of the MVS in 455 children aged 3 – 48 months who presented to one of 11 Canadian EDs with < 72 hours of gastroenteritis symptoms.(42) We calculated the MVS employing symptoms that occurred following the ED visit (i.e. using the ED visit as time zero and not including anything that occurred up to and including the ED visit – these symptoms will be employed to calculate a pre-enrollment score) and found that 25% of children had scores consistent with moderate to severe disease following discharge. This point estimate is lower than previously reported in ED(50, 57) and community populations,(49, 56, 58) as we did not include the symptoms that brought them to the ED (pre-enrollment symptoms). Since the estimate was derived in the same population as the proposed clinical trial and was calculated in a manner identical to that proposed, 25% is likely a very accurate point estimate for the outcome in our control population.

The following is a description of the distribution of the MVS in our prospective cohort.
Examples Describing Calculation of the Modified Vesikari Score
(Including handling of baseline score – i.e. the pre-enrollment score)

Example #1
A 2 year old child who presents with the following symptom complex:

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea for 36 hours</td>
<td>1</td>
</tr>
<tr>
<td>Maximal stools in a 24 hour period = 8</td>
<td>3</td>
</tr>
<tr>
<td>Vomiting for 36 hours</td>
<td>2</td>
</tr>
<tr>
<td>Maximal vomiting episodes in a 24 hour period = 7</td>
<td>2</td>
</tr>
<tr>
<td>Maximal recorded fever = 39.5 axillary</td>
<td>3</td>
</tr>
<tr>
<td>Health care provider visit = emergency department</td>
<td>3</td>
</tr>
<tr>
<td>Treatment = rehydration</td>
<td>1</td>
</tr>
</tbody>
</table>

TOTAL SCORE at ENROLLMENT (i.e. pre-enrollment score) = 15

Following enrollment the caregiver is contacted as per the protocol and we record the following information:

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea for 72 more hours</td>
<td>1</td>
</tr>
<tr>
<td>Maximal stools in a 24 hour period = 2</td>
<td>1</td>
</tr>
<tr>
<td>Vomiting = none</td>
<td>0</td>
</tr>
<tr>
<td>Maximal vomiting episodes in a 24 hour period = 0</td>
<td>0</td>
</tr>
<tr>
<td>Maximal recorded fever = 37.5 axillary (= 38.6 rectal)</td>
<td>2</td>
</tr>
<tr>
<td>Health care provider visit = none</td>
<td>0</td>
</tr>
<tr>
<td>Treatment = none</td>
<td>0</td>
</tr>
</tbody>
</table>

TOTAL MODIFIED VESIKARI SCORE (MVS) for STUDY PERIOD (PRIMARY OUTCOME) = 4
For subject #1, the score of 4 implies that the primary outcome did NOT occur (i.e. did not develop moderate to severe disease following enrollment as the MVS is < 9) despite having a baseline score of ≥ 9. The baseline score of 15 however will be employed as a covariate to enable us to adjust the primary analysis should that be necessary. Moreover a subgroup analysis will be conducted comparing outcomes amongst those with moderate to severe disease at enrollment to those with mild disease at enrollment.

**Example #2**

A 6 month old child who presents with the following symptom complex:

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea for 26 hours</td>
<td>1</td>
</tr>
<tr>
<td>Maximal stools in a 24 hour period = 3</td>
<td>1</td>
</tr>
<tr>
<td>Vomiting = none</td>
<td>0</td>
</tr>
<tr>
<td>Maximal vomiting episodes in a 24 hour period = 0</td>
<td>0</td>
</tr>
<tr>
<td>Maximal recorded fever = 37.5 rectal</td>
<td>1</td>
</tr>
<tr>
<td>Health care provider visit = emergency department</td>
<td>3</td>
</tr>
<tr>
<td>Treatment = rehydration</td>
<td>1</td>
</tr>
</tbody>
</table>

TOTAL SCORE at ENROLLMENT (i.e. pre-enrollment score) = 7

Following enrollment the caregiver is contacted as per the protocol and we record the following information:

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea for 7 more days</td>
<td>3</td>
</tr>
<tr>
<td>Maximal stools in a 24 hour period = 12</td>
<td>3</td>
</tr>
<tr>
<td>Vomiting for 36 hours</td>
<td>2</td>
</tr>
<tr>
<td>Maximal vomiting episodes in a 24 hour period = 10</td>
<td>3</td>
</tr>
<tr>
<td>Maximal recorded fever = 38.5 axillary (= 39.6 rectal)</td>
<td>3</td>
</tr>
<tr>
<td>Health care provider visit = emergency department</td>
<td>3</td>
</tr>
<tr>
<td>Treatment = hospitalization</td>
<td>2</td>
</tr>
</tbody>
</table>

TOTAL MODIFIED VESIKARI SCORE (MVS) for STUDY PERIOD (PRIMARY OUTCOME) = 19

For subject #2, the score of 19 implies that the primary outcome DID occur (i.e. the child developed moderate to severe disease following enrollment as the MVS is ≥ 9) despite having a baseline score of < 9. The baseline score of 7 however will be employed as a covariate to enable us to adjust the primary analysis should that be necessary. Moreover a subgroup analysis will be conducted comparing outcomes amongst those with moderate to severe disease at enrollment to those with mild disease at enrollment.
APPENDIX 9: Economic Analysis Plan

Prior Experience - Economic Analyses
The research team includes experts in the conduct of economic analyses. Dr. Willan and Mr. Goeree, MA, have successfully collaborated on many studies including economic evaluations in the context of clinical trials and multinational studies. Moreover, Dr. Willan is an expert on the conduct of cost-effectiveness analyses of RCTs with binary measures of effectiveness employing an interacting covariate on methods to account for between-country differences in the analysis of cost-effectiveness data, on the value of information, and cost-effectiveness thresholds. The extensive involvement of these co-investigators will ensure that the cost analysis is successfully completed employing innovative methodology.

Preliminary Data - AGE
Dr. Freedman, the Co-PI recently published an economic analysis evaluating the financial impact of ED ondansetron administration to children with AGE. This analysis, which was conducted from both the Canadian and American perspectives, demonstrated that there can be significant clinical and economic benefits when an effective intervention is administered to children with AGE. In fact, we concluded that the administration of ondansetron to eligible children would prevent approximately 4,065 intravenous insertions and 1,003 hospitalizations annually in Canada alone. Moreover, at the current average wholesale price, and employing cost data, its routine administration to eligible children would annually save society US$1.7 million and healthcare payers US$1.2 million. Thus, there is evidence that the implementation of a simple intervention in children with AGE can result in significant economic benefits.

METHODS
Cost-Effectiveness
This specific aim is designed to prospectively collect and analyze data on resource utilization. All children enrolled will participate in this analysis. An economic analysis will be conducted to evaluate the overall cost-effectiveness of routinely administering Lacidofil to eligible children. This analysis will be performed in keeping with current recommendations on the conduct of cost-effectiveness analyses conducted as part of a clinical trial. In this analysis, all costs will be expressed in monetary terms. In our model, the strategies compared will be (1) administering Lacidofil in addition to routine care versus (2) routine care alone. The measure of effectiveness will be avoidance of the primary outcome (i.e. avoiding an episode of moderate-severe disease). Discounting will not be considered given the 14-day time horizon of the trial.

Measurement of Resource Utilization
The study viewpoints will be those of society and the healthcare payer. We will collect patient-specific data on healthcare resource utilization during the child’s condition, namely, the administration of interventions and treatments. The minimum amount of information that is required to address our proposed analysis will be collected. Data forms will integrate the economic questions to minimize repetition, simplify the questionnaires, and maximize follow-up while minimizing missing data. These forms were included in our pilot study. Price weight estimates will be based on national standards and will be performed by staff under the supervision of Mr. Goeree at the Program for Assessment of Technology in Health (PATH) Research Institute at McMaster University. All cost data will be reported adjusted for use of 2013 as the base year. Conversions will be conducted using indices commonly employed to adjust for inflation and the Statistics Canada Health and Personal Care Consumer Price Index. Direct costs will include medical expenses: hospitalization, ED visits,
physician office visits, medications, intravenous solutions, radiographs, and laboratory tests in addition to private out-of-pocket costs. Also included will be professional services, supplies, and equipment associated with the use of these resources. Nonmedical costs will include caregiver-foregone earnings, travel time, parking costs, extra diapers, special foods, ORS, and childcare. Data related to nonmedical costs will be supplied by the parent. When the caregiver misses workdays, foregone earnings will be calculated. The cost of work lost can be calculated directly when hourly wage information is available. If individual hourly wages are unavailable, the annual income and the number of hours worked weekly will be used to determine an average hourly wage. If income information is unavailable, then the value of lost wages will be estimated by applying a postal code adjusted mean wage rate to missed work time, obtained from Statistics Canada. The point estimate charge for LactoBact administration will be the average wholesale price (AWP). The AWP has been selected as it is almost always greater than the wholesale acquisition cost and lists prices that are much higher than public or private payers are likely to pay, reflecting the opportunity cost.\(^{(84)}\)

**Cost Analysis**

An average cost per patient will be calculated for overall, direct and indirect costs per treatment group. The analysis will first determine if one treatment dominates another (i.e. lower costs and better clinical outcomes). If a trade-off exists between costs and outcomes we will estimate the incremental cost-effectiveness ratio (ICER) together with the 90% confidence interval. The ICER is the additional cost at which probiotics provides one additional unit of health benefit (i.e. moderate or severe disease episode avoided), relative to routine care.\(^{(85)}\) The incremental net benefit (INB)\(^{(86)}\) and the corresponding 90% confidence intervals will be plotted as functions of the threshold value for avoiding a moderate or severe disease episode. Expressing INB in units of cost will allow us to examine cost minimization, incremental cost-effectiveness and INB in a single graph, complete with the corresponding statistical inferences.\(^{(87)}\) Because the tests of hypothesis for cost-effectiveness are one-sided (i.e. INB ≤ 0 vs. INB > 0), if the lower limit of the 90% confidence interval is > 0, then you can reject the null hypothesis and limit the type I error probability to 5%. A cost-effectiveness acceptability curve (CEAC) will be calculated. The CEAC, which is the probability that the INB is positive, as a function of the threshold value, provides an excellent means of quantifying the cost-effectiveness of a new healthcare intervention and its uncertainty. A gamma model for cost will be used to account for right skewing.\(^{(76)}\) Key cost drivers will be identified.

**Value of Information Analysis**

Value of Information (VOI) methods are a systematic approach to inform optimal research design and prioritization. In health technology assessment, decision makers face situations where, having reviewed the best available evidence of the costs and effectiveness of a new healthcare intervention (i.e. probiotics) relative to existing practice, the new intervention has positive but uncertain net benefit at a decision maker’s threshold value for health effects. Assuming no further evidence from other jurisdictions is expected, decision makers then have 3 options: (1) adopt the new intervention without further research; (2) adopt the new intervention and undertake another trial; or (3) delay the decision and undertake a trial.\(^{(88)}\) We will use VOI at the completion of our trial to determine if further research is potentially worthwhile.\(^{(89)}\) This will be achieved by determining if the information from our RCT is sufficient for decision making.\(^{(90)}\) A sensitivity analysis will be conducted with respect to the threshold willingness-to-pay and the time horizon.

**Willingness to Pay**

This method of quantifying the valuation of clinical outcomes to the patient/caregiver provides a measure of how much an individual values the particular clinical benefit of treatment. This methodology is gaining popularity.\(^{(91,92)}\) Moreover, because the analysis of INB requires the
specification of the willingness-to-pay (WTP) for a unit of effectiveness, we will conduct a willingness to pay evaluation in the ED of The Alberta Children’s Hospital. Formal study design and pilot testing will be performed as has been done previously by Ron Goeree and staff at the PATH Research Institute at McMaster University. Caregivers surveyed will be those who bring their child to the ED for symptoms unrelated to AGE. Given their broad demographics this will allow for an accurate representation of society’s willingness to pay for the use of probiotics given a variety of postulated clinical benefits. Surveys will be performed by select groups of trained research volunteers who sole roles are to support research in their respective EDs. This crucial evaluation will allow policy makers to evaluate whether they should provide a supply of probiotic to eligible children upon discharge from the ED. Otherwise caregivers will have to pay out of pocket for the cost of the probiotic agent. At present, insurance companies do not reimburse such expenses. Thus, it is important to know if the valuation of benefits of probiotics outweighs the costs.
APPENDIX 10: Sample Size/Power Analysis

Sample Size
The following formula\(^{(100)}\) was used to calculate the required sample size \((2N)\) to compare proportions between two different groups where \(N\) is the size of each treatment group:

\[
P_C = \text{Probability in the control group} = 0.25 \\
P_I = \text{Probability in the treatment group} = 0.15 \\
\Delta = P_C - P_I \\
N = \frac{2(Z_{\alpha/2} \sqrt{2PQ} + Z_\beta \sqrt{P_C Q_C + P_I Q_I})^2 + \frac{2}{\Delta \mid}}{\Delta^2}
\]

\(2N = 670\) participants
\(N=335\)

Power Analysis
Based on a sample size of 670, we obtain the following results for the primary outcome:

<table>
<thead>
<tr>
<th>(P_C)</th>
<th>(P_I)</th>
<th>(\Delta)</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.30</td>
<td>0.21</td>
<td>9%</td>
<td>0.76</td>
</tr>
<tr>
<td>0.30</td>
<td>0.20</td>
<td>10%</td>
<td>0.85</td>
</tr>
<tr>
<td>0.25</td>
<td>0.15</td>
<td>10%</td>
<td>0.90</td>
</tr>
<tr>
<td>0.25</td>
<td>0.16</td>
<td>9%</td>
<td>0.82</td>
</tr>
<tr>
<td>0.25</td>
<td>0.17</td>
<td>8%</td>
<td>0.72</td>
</tr>
<tr>
<td>0.20</td>
<td>0.10</td>
<td>10%</td>
<td>0.95</td>
</tr>
<tr>
<td>0.20</td>
<td>0.12</td>
<td>8%</td>
<td>0.81</td>
</tr>
<tr>
<td>0.20</td>
<td>0.13</td>
<td>7%</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Sample Size Adjustment
Since daily follow-up will be required for 2 weeks, based on previous data\(^{(101)}\) and to err on the conservative side, our calculations assumed:

A- 9% loss to follow-up,
\[= 670/(1-0.10) = 744\]

B- 5% drop out and 2.5% drop in rate = 7.5% non-compliance
\[= 744/(1-0.075)^2 = 868\]

C- O’Brien-Fleming
\[= 868 \times 1.02 = 886\]
APPENDIX 11: Recruitment

**Site Requirements:** Initially five eligible sites were identified within the PERC network. Sites were selected based on volume and success with participation in prior PERC clinical trials. Specifically, we required a sufficient number of annual visits by children aged 0 - 4 years with gastroenteritis, adequate geographic diversity to ensure generalizability, and a Site Investigator willing and capable of acting as a “champion” for the study at their respective institution.

A sixth and final site has been added to the study due to low enrolment at one of the previously selected sites. The sixth site is part of the PERC network and has been successful in conducting PERC endorsed clinical trials in the past. Emergency Department volumes and success with study participation rates are currently unavailable for this site.

The data listed below is for the original proposed 5 study sites. Data for the sixth and final site is not included in the data listed.

Number of ED visits for gastroenteritis in children 3 month and 48 months of age at the 5 participating sites:

- 2009: **8,855**
- 2010: **9,596**
- 2011: **10,399**

We have set the minimum accrual estimate at 90% of the data points presented above (9,359). This is likely a low estimate as 2009 to 2011 data have in fact identified a 17% increase.

We have included the IWK Health Centre (Halifax) for several reasons: (1) They enable us to have geographic diversity across Canada (only Maritime site); (2) they have a track record of excellent recruitment, retention and high quality data performance, (3) financial support will be in large proportional to recruitment. Thus, our study Steering Committee has agreed that the selection of IWK Health Centre would represent an excellent study site.

**Percent Presenting November 1 – May 31 (2009 – 2011 data)**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Alberta Children’s Hospital:</td>
<td>74%</td>
</tr>
<tr>
<td>The Hospital for Sick Children:</td>
<td>76%</td>
</tr>
<tr>
<td>The Children’s Hospital of Eastern Ontario:</td>
<td>78%</td>
</tr>
<tr>
<td>Sainte-Justine:</td>
<td>67%</td>
</tr>
<tr>
<td>IWK Health Centre</td>
<td>75%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>74%</strong></td>
</tr>
</tbody>
</table>

**Percent Meeting Definition of Diarrhea**

Data from The Children’s Hospital and Regional Medical Center ED in Seattle, Washington, reported that of children who presented with diarrhea (N=1,626), the mean number of episodes in the 24 hours prior to presentation was 8 (median = 6).\(^\text{(102)}\) Thus, we anticipate that the majority (95%) of children with diarrhea will meet our definition of 3 stools in the preceding 24 hours. However, it is unknown what proportion of children do not have any diarrhea at the time of presentation (i.e. have isolated vomiting). In a 5 year chart review of children diagnosed with gastroenteritis at The Hospital for Sick Children we found that 51% had ≥ 3 diarrheal episodes in a 24 hour period. This is in large part due to
the diagnosis of gastroenteritis often being assigned to children who have isolated vomiting. Thus we will use a point estimate of 50%.

Percent with < 72 hours of Symptoms at Presentation
Data from Children’s Hospital and Regional Medical Center ED in Seattle, Washington, reported that of children who presented with diarrhea, the median duration at the time of presentation was 3 days.\(^{(102)}\) Thus, to err on the conservative side, we will assume that 55% of children will have had diarrhea for > 72 hours at the time of ED assessment. In our 5 year chart review we found that 58% had symptoms for <72 hours. For accrual analysis purposes, we will assume that only 45% of children evaluated will have had symptoms for < 72 hours.

Presence of Hematochezia
Data recently published by the PI (S.F.) from The Hospital for Sick Children\(^{(5)}\) reported that over 5 years, only 4% of children had hematochezia, hence a conservative estimate of 5% will be employed.

Other Eligibility Requirements (telephone access, indwelling vascular access line, congenital heart disease, immunosuppressive therapy, immunodeficiency, inflammatory bowel disease, exclusively breastfed, bilious or bloody vomitus, probiotic use, language barrier, soy allergy, pre-existing pancreatic dysfunction, oral or gastrointestinal surgery)
These features are uncommon as individual entities, however, when combined they may have an effect on recruitment, particularly as enrollment will be occurring at tertiary care centers. This may represent up to 20% of children with gastroenteritis.

Percent Presenting Between 8:00 – 24:00
In a database of 3508 AGE visits at The Hospital for Sick Children, 82% of children were triaged between 8:00 – 24:00. As presentation patterns and coverage may vary between institutions and we must account for sick days, our point estimate will be 75%.

Consent
Data from a recently published PERC multi-centre RCT (CanBEST) reported an overall consent rate of 52% (N=1540).\(^{(103)}\) The consent rate in our pilot study was 56%. Thus we will estimate a 50% consent rate.

Summary: Taking all the above factors into account, our best point estimate is that we will be able to enroll approximately 494 children/year. However, slight alterations in our best estimates result in a reduction to approximately 294 children/year. Our collective research expertise is that recruitment never follows the best point estimates and hence we anticipate that we will require three gastroenteritis recruiting seasons (winter/spring) to achieve our necessary sample size (886).

<table>
<thead>
<tr>
<th></th>
<th>Minimum Estimates</th>
<th>Best Point Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total # visits</td>
<td>9,359</td>
<td>10,399</td>
</tr>
<tr>
<td>% November 1 – May 31</td>
<td>70</td>
<td>74</td>
</tr>
<tr>
<td>% Meeting Definition of Diarrhea</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td>% &lt; 72 hours of Symptoms</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td>% Absence of Hematochezia</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>% Absence of other exclusion criteria</td>
<td>75</td>
<td>80</td>
</tr>
<tr>
<td>% Presenting Between 8-24</td>
<td>70</td>
<td>75</td>
</tr>
<tr>
<td>% Consenting</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>----------------</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>TOTAL ANNUAL ACRUAL/Year</td>
<td>294</td>
<td>494</td>
</tr>
</tbody>
</table>

**Estimates from the Probiotic Pilot RCT:** The probiotic pilot RCT that we conducting has screening log data available for analysis. The overall number of children enrolled 50/2235 (2.2% of all children screened) is slightly lower that the estimates above (minimum = 3.1%; best point = 4.7%). This difference is likely due to the requirement of daycare attendance in children included in the pilot. Daycare attendance was the only identifiable exclusion criteria in 187 children screened. Given the 56% consent rate in our pilot (50/90), if these additional 187 patients were eligible, as would be the case with the proposed RCT, then the overall number of enrolled children would be 155, yielding an eligibility rate of 6.9%.
APPENDIX 12: Feasibility

886 Children Enrolled in Probiotic RCT

Day #0

665 (886 x 0.75) Rectal Swabs Performed in ED

221 (886 x 0.25) Bulk Stool Obtained in ED

*133 Children Enrolled at Sites in Year #1 Lacking Funding to Collect Bulk Stool Samples

399 (532 x 0.75) with Day #0 Bulk Stool Sample Collected from Home

160 Bulk Stools Tested to Confirm Rectal Swab Negative Result

620 Children with Day #0 Bulk Stool Samples Collected

Day #5

465 (620 x 0.75) Children with Day #5 Bulk Stool Samples Collected

Immune Outcome (sIgA)

278 (465 x 0.60) Children with Days #0 and 5 Bulk Stool Samples & Pathogen Identified (~60% of all Luminex xTAG GPP)

Biomarker Outcome (Pathogen Load)

1046 Samples from 866 children for Pathogen Identification via Luminex xTAG GPP

10% Lost to Follow-Up (i.e. no Modified Vesikari Scale score)

Clinical Outcome

*Limited funds to collect bulk stool samples in year #1 and thus some of the children enrolled in the RCT in Year #1 will not provide bulk samples.
‡ Projected bulk stool collection success from home 75% at Day #0 and Day #5.
**APPENDIX 13: Data Management, Training, and Site Monitoring**

**Data Management**

Data management will be performed by the Women & Children’s Health Research Institute (WCHRI), located at the University of Alberta in Edmonton, Alberta. WCHRI uses a state-of-the-art computer and network infrastructure housed within a dedicated server room with fully redundant power and cooling systems. Access to the server room is restricted to authorized staff. Servers are backed up daily and tapes are stored in a fireproof safe in the Faculty of Medicine and Dentistry at the University of Alberta. Communication over public networks is encrypted using 128 bit SSL encryption and direct access to the servers is only available to users located within the WCHRI office or via the Faculty’s virtual private network (VPN).

In accordance with the Tri-Council Policy Statement, direct subject identifiers will not be stored in the study database. A REDCap data collection application will be employed. It is protected by appropriate security measures which include password protection, enforced password complexity, enforced password changes, inactivity timeout and user lockout following failed login attempts. Inactive user accounts are automatically suspended by the system. Defined user roles ensure that data access is appropriate to the user’s job function. A detailed audit trail monitoring system will be instituted to document the person, time, and nature of data access, with flags for aberrant use and "abort" algorithms to end questionable or inappropriate access.

A complete data management plan will be designed in conjunction with the Women & Children’s Health Research Institute (WCHRI) to ensure that the information collected will enable us to conduct all required analyses. This management plan will be incorporated into the Manual of Operations. Case report forms (CRFs) will be developed for each data recording step (e.g., ED visit data, telephone follow-up data, stool specimen collection data). Data collected by study personnel, as well as from participating families, will be entered into a Web-accessible database (REDCap\(^{104}\)). Study sites will maintain the option of collecting data directly into the web based database via tablet, or collecting data using paper CRFs (later transcribing to the REDCap database). The data entry interface will include range and logic checks to enable the conduct of exploratory data analysis as data entry is ongoing (and will be repeated as the first stage of the analysis) to minimize data entry errors.\(^{105}\) Following data entry a data quality audit will be performed to ensure that data is complete and accurate. Data will be entered in a timely fashion, with paper CRFs being entered within 7 days of completion. Automatic alerts will be triggered by incomplete, out-of-range, or logically inconsistent data. Paper CRFs will be stored in a secure fashion at participating sites. They will be available for review by site monitors (see below).

A complete quality assurance and site monitoring plan will be designed in conjunction with WCHRI when the Manual of Operations is developed.

**Training**

A training module will be developed for all study personnel, including Site Investigators, Site Physicians, and Clinical Research Assistants/Nurses. The specific training will be tailored to the role of the individual in the study. All Clinical Research Assistants/Nurses will be familiar with Good Clinical Practice guidelines prior to collecting any study related data, and all study personnel must meet human subjects and research ethics training requirements at their home institution. Prior to the start of the study, there will be an in-person training session for all Site Investigators, Clinical Research
Nurses, and Research Assistants. Site Investigators and Coordinators will then be responsible for ensuring the training of other personnel involved in study procedures at their sites. Written documentation of such training will be required. Refresher training sessions will be conducted a month prior to each enrollment season. Monthly conference calls during the enrollment seasons will be conducted for site investigators, coordinators, and research assistants, during which study procedures will be reviewed and issues discussed. Standardization of data collection procedures will be developed and reinforced during personnel training sessions.

Site Monitoring Plan
Site monitoring visits will be coordinated by the Clinical Research Project Manager to ensure that all regulatory requirements are being met and to monitor the quality of the data collected. Each site will have a remote monitoring visit within 2 months of the start of patient enrollment during which, patient forms and original source documents will be inspected. In addition, on-site visits will be performed based on financial and human resource availability during which essential document binders, CRFs, 100% of informed consent documentation, 100% of inclusion/exclusion criteria, and 100% of the serious adverse events will be reviewed. A minimum of 10% of the subjects will have CRFs reviewed (remotely or on site) thereafter. Additionally, site monitors will assess and verify adequate patient accrual and review regulatory documents.

Remote site monitoring will also be performed by the Clinical Research Project Manager to verify selected data elements. This will require the clinical site staff to locate the relevant source document, make a copy, de-identify the material, and send the copy to ACH where the information will be compared with data stored in the REDCap system. Remote monitoring reports will be distributed to the Study Investigators and Coordinators. The documents will be retained in accordance with all applicable regulatory requirements.

Ongoing data monitoring will be performed throughout the course of the study by the Clinical Research Project Manager utilizing data quality tools in the REDCap online data collection system.
APPENDIX 14: Specimen Collection & Processing

Bulk Stool Sample Collected if Possible → Day #0 in ED → Rectal Swab Performed if Bulk Stool not Collected in ED

Pathogen Identification: Luminex xTAG Gastrointestinal Pathogen Panel (GPP)

If rectal swab test is negative

†Bulk Stool Sample Collected if not Done in ED → Day #0 at Home

If Day #0 Luminex Positive and Paired Day #5 Sample Obtained

Stool Pathogen-Load Quantification

If Day #0 Luminex Positive

If Day #0 and Paired Day #5 Sample Obtained

Fecal Secretory IgA

Day #5 at Home

*Bulk Stool Sample Collected If Day #0 Obtained

*Projected bulk stool collection success rate of 75% at Day #5 amongst those with Day #0 bulk stool. As samples will be batch processed, we will not know who had a positive Day #0 sample until following collection of Day #5 samples.
†If rectal swab Luminex negative, will repeat with Day #0 at home bulk stool and estimate ~10% positive.
June 9, 2006

Re : Probiotics and timing of antibiotics

We performed a study where we gave hospitalized patients on antibiotics, capsules of Lacidofil provided by Rosell Institut Lallemand containing Lactobacillus acidophilus and Lactobacillus rhamnosus. The patients had all been prescribed antibiotics both oral and intravenous formulations particularly those to which the lactobacilli was sensitive. The study was performed to determine whether the organisms could survive in this setting. The probiotic capsules or sachets were given to the patients with their meals to try to protect them from destruction in the stomach by the otherwise acidic conditions of the stomach. The antibiotics were given on differing schedules and there was no attempt to time the probiotic doses related to the receipt of antibiotics.

We discovered that we could recover viable Lactobacilli in the stool in the patients and that the recovery appeared to depend mainly on the initial dose of probiotic given and did not appear to be correlated with the timing of the antibiotic doses.

Should you require any further information do not hesitate to contact me.

Yours truly,

Sandra Dial, MD,MSc
Assistant Professor, McGill University
Department of Medicine.
APPENDIX REFERENCES


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Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis: 
Randomized Controlled Trial

Protocol

Summary of Changes

1. Addition of Study Investigators
   a. Dr. Katrina Hurley replaced Dr. Karen Black after she left the study site and relocated to an alternate institution.
   b. Following the award of funding for additional microbial analyses Drs. Finkelstein, Chui, Lee, Pang, Louise, and Nettel-Aguirre were added to the protocol.
   c. Due to the need to add an additional recruiting site, Dr. Naveen Poonai joined the study team as the site investigator at the London Health Sciences Centre, London, ON.

2. Additional Hypothesis Added
   a. Additional funding was obtained to also address the hypothesis that that the therapeutic benefits of probiotics in children with AGE vary by infecting pathogen. This hypothesis will be evaluated employing clinical and bio-specimens collected as part of the PROGUT study but the laboratory analyses will be conducted following the reporting of the clinical trial and will be reported in a separate manuscript.
   b. Thus, an additional secondary clinical efficacy question was added (Is there a difference in the effectiveness of treatment based on the infecting pathogen?) but it will be addressed and analyzed at a future time. This question will be answered in connection to the microbiologic question – “In this group of patients, is there a difference in the pathogen specific reduction in stool pathogen load in those who receive Lacidofil compared to those who receive placebo?”
   c. To enable the conduct of the microbial analyses the stool/rectal swab collection and testing protocol was revised to the following:
      i. “Two swabs will be performed. One sample will be collected for bacterial culture according to site specific practices. The second sample will be collected using a flocked tipped sterile swab (FLOQSwabs™ Flocked Swabs, Copan) and will be stored and frozen (-80°C) in Universal Transport Media (UTM; Copan). This approach allows us to obtain a specimen for molecular pathogen identification prior to discharge (i.e. prior to probiotic administration altering the accuracy of pathogen identification) on all study participants and will only be tested if an ED bulk stool is not obtained. Viral testing will be performed in batches. We will also attempt to collect a bulk stool sample from all RCT participants in the ED prior to discharge. This specimen is the preferred specimen for pathogen identification testing.

Bulk Stool from Home (Pathogen Identification): Patients enrolled at all sites will be asked to provide additional bulk samples at home. Patients may decline, when obtaining informed consent, to collect bulk stool at home. The need to provide bulk stool samples will be stressed as these samples are
required to perform pathogen-specific load quantification (i.e. cannot be performed on rectal swabs).

DAY #0: We will collect a bulk stool sample from all study participants who do not provide specimens in the ED prior to discharge.

DAY #5: We will collect a bulk stool sample from all study participants who provided a Day #0 bulk stool sample.

DAY #28: We will collect a Day #28 bulk stool sample from all study participants who consent to provide a Day #0 and #5 bulk stool sample.

To collect specimens, caregivers will be provided with instructions along with stool collection containers.

Initial pathogen identification testing will employ the sample (either bulk stool or rectal swab) obtained in the ED to minimize the impact of probiotic administration on test results. The specimen will be tested using the Luminex xTAG GPP. Day #0 bulk stool specimens collected at home will only undergo pathogen identification testing if the ED rectal swab test does not identify a pathogen. This will ensure that negative rectal swab test results do not reflect inadequate sampling (i.e. rectal swab performed but insufficient stool obtained thereby yielding a false negative test). Day #5 and Day #28 specimens will only be tested if the Day #0 specimen identifies a pathogen. The pathogen identification data is required to assign an etiology to all study participants; this information will be employed to determine the pathogen-specific response across all study aims.

Bulk Stool from Home (secretory IgA): In addition to pathogen identification and quantification, bulk specimens provided by participants on Days #0, #5, #28 will be sent to the Hospital for Sick Children (HSC) to the lab of Dr. Philip Sherman for slgA testing (Appendix 6-slgA Procedures). Samples will be stored at -80°C and will be sent to the Hospital for Sick Children (HSC) in bulk shipments from the labs of Dr. Linda Chui and Dr. Xiao-Li Pang. Fecal slgA analysis will be performed by Dr. Sherman’s laboratory which is certified to handle human specimens.

If a sample is unable to be provided at enrolment, on Days 5 and 28, the first sample provided after enrolment, the Day 5, and Day 28 time points respectively will be accepted.

Patients/caregivers will receive a reminder telephone call or email correspondence, based on preferred method of follow up, one day prior to the scheduled sample return date (i.e. on Day 4 and Day 27).
All specimens will be labeled with the date and time of collection and the subject’s study identification number. Once a sample is obtained, caregivers will contact a contracted biomedical courier service who will transport the specimens to the enrolment site with shipment costs covered by study funds. Upon receipt at the laboratory, each sample will be frozen and split appropriately for future testing. This procedure is based on work by the Centers for Disease Control, and our colleagues.

Sites will batch ship all frozen stool samples to the Alberta Provincial Laboratory (ProvLab) and the lab of Dr. Xiao-li Pang in Edmonton, Alberta on a regular basis to enable interim laboratory analyses to verify collection and processing procedures. Regular shipments will minimize shipping costs and is acceptable given the stability of nucleic acid in frozen stool samples. All the analyses will be conducted blinded to patient allocation.

d. We also clarified future sub-studies related to fecal sIgA levels (Specifically, we will determine, at a pathogen-specific level, if fecal sIgA levels are higher in children treated with a probiotic agent compared with placebo, and if higher fecal sIgA levels are associated with improved clinical outcomes.) and described how we would conduct pathogen load quantification analyses (Pathogen Load Quantification: To determine if a 5-day probiotic treatment course administered to children with AGE results in pathogen-specific reductions in stool pathogen load. Our team, which includes experts in molecular diagnostics, virology and bacteriology, has the capacity to quantify the impact of probiotic administration on stool pathogen infectious loads. These measures represent disease severity in individuals with AGE; higher stool loads are associated with more severe symptoms, prolonged shedding, hospitalization, and the presence of virus in the blood (i.e. viremia). In children with AGE, stool viral loads correlate (r = 0.80, P<0.001) with the Vesikari Score. Bacterial loads, analyzed from other biological specimens, also have clinical relevance – for example, sputum *Pseudomonas aeruginosa* loads correlate with clinical status and those of *Neisseria meningitides* in serum are associated with death and permanent sequelae. All of this work builds on the model of human immunodeficiency disease, where serum viral load has been a key prognostic marker for decades. Consequently, stool infectious load quantification is increasingly encouraged. Our team, which has led many key advances in molecular virology, has developed a standardized approach to quantify stool viral and bacterial loads, enabling us to quantify an objective marker of disease severity.)

e. Future studies will also address several sub-studies including:
   i. The classification of children who have multiple pathogens identified. Initial classification will be based on Day #0 load (i.e. classified based on higher load); re-classification will evaluate the impact of classification according to the agent with the lower pathogen load.
   ii. The clinical benefits will be explored, separately, in relation to:
      1. Pathogen-group: virus vs. bacteria vs. not identified
      2. Viral agent: rotavirus vs. norovirus vs. adenovirus
3. Bacterial agent: *Campylobacter* vs. *Salmonella* (only ones anticipated to have sufficient numbers)
4. All analyses will first employ 2-way ANOVA to assess main effects and interactions of treatment assignment and pathogen group. To assess for other covariates and potential confounders, multivariable regression models including treatment, pathogen and other key covariates (e.g. age, sex, Modified Vesikari Scale score at enrollment, hospitalization, antibiotic use) will be constructed.

3. A data sharing plan was described:
   a. Participant data will be stored in an online electronic data capture system (REDCap). Collected data will be downloaded at the coordinating centre in Calgary, Alberta Canada. In order to complete the planned subgroup and economic analyses, a de-identified dataset containing only the variables required will be shared with collaborating institutions. The planned economic analyses will be performed by the Program for Assessment of Technology in Health (PATH) Research Institute at McMaster University located in Hamilton, Ontario Canada. Data will also be shared with the University of Utah Data Coordinating Center (DCC) located in Salt Lake City, Utah USA. The DCC will integrate our study data with those from a companion clinical trial taking place in the United States (co-PIs Dr. Stephen Freedman and Dr. David Schnadower). Integration of data will allow for additional analyses to be performed that would be underpowered for either study to perform them in isolation.

   Pathogen Load Quantification Data: Specimens are received de-identified by the processing labs. Results of the pathogen load testing performed by Drs. Xiao-Li Pang, Linda Chui, and Bonita Lee will be compiled and entered into a simple database. The de-identified database will be sent to the coordinating centre in Calgary, Alberta using a secure email service (Alberta Health Services). These results may be shared with the DCC in Salt Lake City, Utah.

   Fecal Secretory IgA Data: Fecal sIgA results will be entered into a simple database. The database will be encrypted and sent to the Principal Investigator at the coordinating centre via institutional email. All participant results will be de-identified. De-identified specimens are received at the lab of Dr. Sherman located at the Hospital for Sick Children. These results may be shared with the DCC in Salt Lake City, Utah.

4. A medical monitor was appointed:
   a. To ensure that data remains confidential and unbiased, a medical monitor will be appointed at the sponsoring institution (The University of Calgary). The medical monitor will be an Emergency Department physician with expertise in clinical research. The medical monitor will review adverse event information from collaborating study sites, in lieu of the principal investigator. The principal investigator (as the sponsor) will still maintain the responsibility of reviewing any Serious Adverse Events occurring at any of the participating study sites.
5. The approach to adverse event coding was specified:
   a. Adverse Event (AE) data will be reviewed by trained staff and coded using the Medical Dictionary for Regulatory Activity (MedDRA - https://www.meddra.org/) system. Adverse Event data will be collected from participants at the time of the event. MedDRA coding will be assigned to each event at the end of the recruiting period.
Statistical Analysis Plan

Protocol Title: Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis: Randomized Controlled Trial

Protocol Version and Date: 1.00; August 14, 2013
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<tr>
<td>AGE</td>
<td>Acute Gastroenteritis</td>
</tr>
<tr>
<td>DSMC</td>
<td>Data and Safety Monitoring Committee</td>
</tr>
<tr>
<td>ITT</td>
<td>Intent-To-Treat</td>
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<tr>
<td>MCID</td>
<td>Minimal Clinically-Important Difference</td>
</tr>
<tr>
<td>MVS</td>
<td>Modified Vesikari Score</td>
</tr>
<tr>
<td>PERC</td>
<td>Pediatric Emergency Research Canada</td>
</tr>
<tr>
<td>PROGUT</td>
<td>Probiotic Regimen for Outpatient Gastroenteritis Utility of Treatment</td>
</tr>
<tr>
<td>(S)AE</td>
<td>(Serious) Adverse Event</td>
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<td>SAP</td>
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*August 14, 2013*
1  Preface

1.1  Purpose of SAP
This Statistical Analysis Plan (SAP) describes the planned analysis and reporting for the PROGUT (Probiotic Regimen for Outpatient Gastroenteritis Utility of Treatment) Protocol: Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis: Randomized Controlled Trial (PROGUT).

The purpose of this study is to determine if probiotic administration reduces the severity of acute gastroenteritis (AGE) 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 Pediatric Emergency Research Canada (PERC) Network. 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:
PROGUT Protocol: Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis: Randomized Controlled Trial (PROGUT Study).
- Case Report Forms for the PROGUT 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 PROGUT 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, pre-specified statistical analysis approach is completely followed in the revised technical specifications.

2  Study Objectives and Outcomes
2.1  Study Objectives
2.1.1  Primary Objective
To determine if in previously healthy children, ages 3-48 months, who present to an ED with less than 72 hours of AGE like symptoms, is the proportion who develop moderate to severe disease
[Modified Vesikari Score (MVS) ≥ 9] following ED evaluation, significantly different in those who receive a probiotic agent (Lacidofil) compared to those who receive placebo.

2.2 Study Outcomes

2.2.1 Primary Outcome

The primary outcome is the development of moderate-severe disease in the 2 weeks after the index ED visit as measured by the MVS.¹

2.2.2 Secondary Outcomes

Clinical

A) The duration of diarrhea: Time from treatment initiation until the appearance of the last watery stool²⁴ as reported during daily phone conversations.

B) The duration of vomiting: Limited data indicate that probiotic administration may reduce vomiting.⁵⁶ Recovery will be evaluated in children who vomit ≥ 3 times over the 24 hours prior to the ED visit and defined as “time from treatment initiation until last vomiting episode.” We have previously reported that vomiting frequency predicts outcomes in AGE.⁷

C) Return visits for unscheduled care to a health care provider related to vomiting, diarrhea, dehydration, fever, or fluid refusal, within two weeks: Not included will be scheduled visits (e.g. re-assessment, vaccinations). This outcome is important as > 50% of children have a follow-up office visit,⁸ 8-18% require an ED visit,⁹ and 5-8% are hospitalized.⁸

2.2.3 Adverse Events

To determine if short course probiotic administration to young children with AGE is associated with an increase in adverse events. As stated by the NIH, probiotic safety needs to be studied scientifically.¹⁰ Groups will be compared regarding the development of any adverse events with particular attention paid to bloating, abdominal distention, duration of fever, and buttock rash. The importance of evaluating adverse events has been highlighted by a recent adult pancreatitis study which found an unexpected increase in mortality in probiotic treated patients.¹¹

2.2.4 Tertiary Outcomes

Tertiary outcomes are work and daycare absenteeism.

Mechanism of Action: The results of the fecal sIgA analyses will not be included in manuscript reporting on primary results.

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 usage during the 14 days prior to ED visit
- Infective agent at baseline (e.g., norovirus, rotavirus)
- Severity of baseline diarrhea and vomiting
- Hydration assessment
- Need for hospitalization at index visit

3 Study Design and Methods

3.1 Overall Study Design

The PROGUT 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.

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. Permuted blocks of lengths 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: www.randomize.net, an internet based randomization service, will produce a randomization list stratified by study site, using random-number generating software. The lists will be sent to the central pharmacy (ACH) who will prepare consecutively numbered study kits according to the randomization schedule. These will be couriered to the clinical sites, using proper shipment containers and temperature monitors, where they will be stored in the Research Support Pharmacies.

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 PROGUT 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 DSMC 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.

Only the research pharmacy at the coordinating center and www.randomize.net will retain the randomization code. The DSMC may request to be unblinded to treatment assignment at any time.

3.3 Sample Size and Power Determination
Clinical Outcome: The sample size is based on the assessment of the between-group difference in proportions of children with a post-randomization score ≥ 9 on the MVS. This is a superiority study in which the adoption of probiotic use can be recommended if the rate of the primary outcome is significantly lower amongst those who receive the probiotic medication. Calculations are based on a two-sided type I error (α) of 0.05 and power (1-β) of 0.90. The null hypothesis is $H_0: P_T - P_C = 0$, where $P_T$ and $P_C$ are the event rates in the intervention and control groups respectively. The alternative hypothesis is $H_A: |P_T - P_C| > 0.10$ (i.e. the event rates will differ by at least 10 percentage points).

Minimal Clinically Important Difference (MCID): Ten content experts from the US and Canada were surveyed regarding the MCID. Absolute risk differences ranging from 7.5-15% were suggested. We chose a conservative estimate of 10% for the primary outcome (number needed to treat of 10).

Outcome in Control Group: Our estimate for the development of moderate to severe AGE in the controls is based on data collected as part of our 2009 evaluation of the MVS in 455 children aged 3 – 48 months, with < 72 hours of symptoms, who presented to one of 11 Canadian EDs. Using the ED visit as time 0, 25% of eligible children had scores consistent with moderate to severe disease following discharge. This is lower than previous reports of ED and community populations because we did not include symptoms that existed prior to the visit. However, Dr. Schnadower’s group in the United States has just completed data collection on 282 children enrolled at 6 sites in the United States and they found that 24% of children in their sample had scores consistent with moderate to severe disease following discharge (personnel communication September 6, 2012). Since our study population and method of MVS calculation in the derivation and recent validation studies and the current proposal are the same, 25% is a very accurate estimate. Given the above, the required sample size to compare proportions between two different groups is 670.
Sample Size Adjustment Calculation: Based on previous work by our group with similar follow-up designs18-20 and extensive reviewer feedback, we have assumed a 10% loss to follow-up (670/0.9=744), 5% drop out (744/(0.95)^2=825), and 2.5% drop in (caregivers who decide to buy a probiotic agent at a pharmacy to administer to their child) rate (825/(0.975)^2=868). Adjustment for O’Brien-Fleming monitoring boundaries requires a further 2% increase. Thus, the total number randomized (final sample size) will be 886.

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 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 ≥ 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 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 must meet all of the following inclusion criteria:
1. Presence of diarrhea: defined as ≥ 3 watery stools in a 24-hour period.21
2. Duration of vomiting or diarrhea < 72 hours: Early administration = greater efficacy.6,22,23
3. Age 3 to < 48 months: AGE severity and frequency are greatest amongst young children.24

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Patients meeting any of the following criteria will be excluded from the study:
1. Presence of an indwelling vascular access line or structural heart disease (bacteremia risk).\textsuperscript{25}
2. Taking immunosuppressive therapy, or known history of immunodeficiency (bacteremia risk).\textsuperscript{26}
3. Hematochezia in the preceding 72 hours, underlying significant chronic gastrointestinal problem or inflammatory bowel disease: Not including constipation, gastroesophageal reflux or chronic pain.
4. Family member with an indwelling vascular access line, on immunosuppressive therapy, or with a known immunodeficiency: Does not include use of short course oral (<7 days) or inhaled steroids.
5. Bilious vomitus: May indicate a diagnosis other than AGE is possible.
6. Probiotic use (supplement) in the preceding 2 weeks: However, consumption of foods containing probiotics will not result in exclusion as they are ubiquitous.
7. Previously enrolled in this trial (to ensure that the observations on trial patients are independent).
8. Daily telephone follow-up will not be possible while symptomatic (travel plans or language barrier).
9. Allergy to soy: Lactofill, as well as the placebo product have come in contact with soy during the manufacturing process.
10. Pre-existing, or known, pancreatic dysfunction or insufficiency\textsuperscript{11}
11. Oral or Gastrointestinal surgery within the preceding 7 days: theoretical wound infection risk.

5 General Analysis Issues
5.1 Analysis Software
Analyses were performed with SPSS, version 24.0.0.1 (SPSS Inc) and Stata version 15.0 (StataCorp LLC).

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 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.
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**

1. The presence of a MVS ≥ 9 will be analyzed by
   a. age < 1 year,
   b. breast-feeding status,
   c. antibiotic usage and
   d. protocol compliance.

2. Duration of vomiting will be analyzed only in those patients who have ≥ 3 episodes of vomiting in the 24 hours prior to enrollment.

3. Daycare and work absenteeism will only be analyzed for children who attend daycare and caregivers who work.

4. Presence of rotavirus infection will be evaluated by adding an interaction term between treatment and rotavirus positivity in a logistic regression model. The independent variables in the model will be
   a. treatment group,
   b. rotavirus positivity (yes/no) and
   c. the interaction between treatment group and rotavirus positivity.

Universal rotavirus vaccination does not exist in Canada with the decision being made individually by each province based on the expense as well as feasibility. At present it is included in the provincial schedules in Quebec and Ontario but not in Nova Scotia or Alberta. The varying use of the vaccine and our goal to identify etiologic agents and to conduct sub-
analyses will yield very important information related to probiotic use in the presence/absence of rotavirus vaccination.

5.6 Derived and Computed Variables
All derived and computed variables will be outlined in the analysis dataset specifications for this study.

6 Overview of Planned Analyses
6.1 Schedule of Interim Analyses
The Data Safety Monitoring Committee (DSMC) will meet after 200 and 500 patients to review enrollment, study procedures, form completion, data quality, 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 AGE in the probiotic arm is equal to that in the placebo arm. Based on trends and adverse events, the DSMC may decide to meet sooner than planned using boundaries adjusted accordingly. Because this trial involves children under the age of 6 months, the DSMC has approved a plan to complete an interim safety analysis on the first 20 subjects enrolled under 6 months of age. All serious adverse events will be reported within 24 hours to the DSMC and based on these reports; the DSMC may decide to conduct a safety analysis before the full 20 subjects have been enrolled in this age group. Otherwise, a blinded analysis will be conducted after the 20 subjects < 6 months of age have been enrolled. This data will be unblinded if the DSMC deems it necessary to conduct an unblinded interim safety analysis. The results of this analysis will be communicated to the NHPD branch of Health Canada at the discretion of the DSMC chair should any concerns be identified.

6.2 Stopping Rules for Interim Analyses
Two-sided O'Brien-Fleming boundaries, implemented using the alpha-spending function approach will be used to ensure that the total Type I error rate will be less than or equal to 0.05.

6.3 Blinding in the Interim Analyses
The DSMC will be partially blinded, aware of results by treatment arm, but not of arm identities. Other research team members will be blinded to treatment assignments, but the study coordinator will have access to individual safety and efficacy data. Study personnel 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 the DSMC report. The DSMC 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
- Age
- Season
- Weight
- Primary care physician access
- Daycare attendance
- Ondansetron administration in the ED
- Receipt of rotavirus vaccine
- Vomiting and diarrhea duration and frequency
- Presence of fever
- Prior ED visits, IV rehydration, hospitalization
- Baseline MVS
- Duration of symptoms prior to enrollment (<48 hours vs. 48 hours or more)
- Breast-feeding status
- Antibiotic usage during the 14 days prior to ED visit
- Infective agent at baseline (e.g., norovirus, rotavirus)
- Hydration assessment (Clinical Dehydration Scale 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 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 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.

<table>
<thead>
<tr>
<th>Points</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea duration</td>
<td>0</td>
<td>1–96 hrs</td>
<td>97–120 hrs</td>
<td>≥ 121 hrs</td>
</tr>
<tr>
<td>Max # of diarrheal stools/24 hrs</td>
<td>0</td>
<td>1–3</td>
<td>4–5</td>
<td>≥ 6</td>
</tr>
<tr>
<td>Vomiting duration</td>
<td>0</td>
<td>1–24 hrs</td>
<td>25–48 hrs</td>
<td>≥ 49 hrs</td>
</tr>
<tr>
<td>Max # of vomiting episodes/24 hrs</td>
<td>0</td>
<td>1</td>
<td>2–4</td>
<td>≥ 5</td>
</tr>
<tr>
<td>Max recorded fever</td>
<td>≤ 37°C</td>
<td>37.1–38.4°C</td>
<td>38.5–38.9°C</td>
<td>≥ 39°C</td>
</tr>
<tr>
<td>Unscheduled healthcare visit</td>
<td>0</td>
<td>—</td>
<td>Primary Care</td>
<td>Emergency Department</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----</td>
<td>----</td>
<td>--------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Treatment</td>
<td>None</td>
<td>Rehydration</td>
<td>Hospital Admission</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 1: Modified Vesikari Score

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 diarrhea is considered to have continued throughout that time period. The diarrhea episode would thus be calculated from randomization date/time until the final 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 calculated from randomization date/time.

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”. All ED visits to the participating site were verified by database review.

7. **Treatment**: Treatment will be classified as “none”, “intravenous 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 proportion of children with moderate to severe disease (i.e. MVS ≥ 9) will be analyzed by comparing proportions utilizing a Mantel-Haenszel test, stratified by clinical centre. **Significance for the primary outcome measure will be determined using a two-sided 0.05 level.** The pre-enrollment MVS will not be included in the primary analysis as we do not anticipate the baseline and post-intervention scores to be correlated.

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. When the variable was a computed variable (e.g. diarrhea duration) it was not imputed directly. To minimize the use of imputation, only the missing components were imputed (e.g. if the end date of diarrhea was reported, but the end time was missing, only the end time will be imputed).

Variables were imputed in a selective pre-defined order to maximize logic. For example, in instances where both the end date and end time were missing, the maximum number of diarrheal stools/24 hours and the maximum number of vomiting episodes/24 hours were first imputed (if needed) to determine the day that participant met then study end point (i.e. the earliest day that both diarrhea and vomiting stopped), then the diarrhea duration was calculated from randomization until the day of the study end point.

In the case where no MVS outcome data are available, the subject will not be included in imputations and the outcomes will remain missing.

**Imputation Method:** We will use the Multiple Imputation-Automatic method with SPSS 24.0. The Automatic method scans the data and uses the monotone method (noniterative method) if the data show a monotone pattern of missing values; otherwise, fully conditional specification (iterative Markov chain Monte Carlo method) is used. We will use logistic regression methods to model unscheduled ED visit, unscheduled primary care visits, rehydration, and hospital admission. We will use linear regression methods to model diarrhea end time, maximum number of diarrheal stools/24 hours, vomiting end time, maximum number of vomiting episodes/24 hours, and maximum fever.
Variables to include in imputation models
Minimum and maximum possible values will be computed and used as bounds for imputing MVS components: diarrhea end time, maximum number of diarrheal stools/24 hours, vomiting end time, maximum number of vomiting episodes/24 hours, maximum fever.
General model variables: treatment assigned, age, hospital/site
Baseline model variables: maximum number of diarrheal stools/24 hours, maximum number of vomiting episodes/24 hours, presence of fever
Post-randomization model variables: primary care visits, ED visits, rehydration, hospital admissions

Number of imputations: We will impute and analyze 20 datasets. We will increase that number if the efficiency of the imputed datasets is less than 0.99.

Multiple Regression with Multiple Imputed Data Sets: For analyses using multiple regression (e.g. logistic regression, linear regression, negative binomial regression), the pooled of the 20 imputed datasets will be analyzed and the pooled analysis results will be provided by SPSS 24.0.

7.2.2 Additional Analyses of Primary Outcome
Secondary analyses of the primary outcome will employ logistic regression methods to adjust for covariates that may be imbalanced between groups (e.g. age, pre-enrollment MVS, severity of baseline diarrhea and vomiting, hydration assessment, need for hospitalization at index visit). We will also analyze the MVS as a continuous variable through a stratified Wilcoxon rank-sum test.

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. 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. Sensitivity analyses will also be performed to assess the possibility and consequences of losses to follow-up not occurring at random.

7.3 Analysis of Secondary Outcomes
7.3.1 Duration and Frequency of Symptoms
The continuous variables of (1) duration of diarrhea and (2) vomiting will be measured in hours and analyzed with a Van Elteren test, stratified by clinical centre.

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 using a Mantel-Haenszel test, stratified by clinical center.

7.3.3 Adverse Events

Adverse Event (AE): An adverse event is any unfavorable or unintended clinical or other occurrence during the study period that may or may not be the result of participation in the research study.

Expected Adverse Drug Reactions/Events
These include the following as they are part of the natural history of the underlying disease process:

- Hospitalization
- Future health care provider visit, ED return visit
- IV rehydration
- Abdominal pain, distension
- Vomiting, diarrhea, fever, flatulence

Because expected adverse events are part of the natural history of AGE and diarrheal illness in children, they will not need to be reported as Adverse Events. This information will be recorded in normal study data collection processes.

AEs will be recorded from the time of randomization through 14 days. 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 DSMC may request to see more detailed tables. The proportions of children experiencing any adverse event, as reported by the caregivers, will be compared between groups using using the Mantel-Haenszel test, stratified by site. The analysis will evaluate the presence/absence of side effects, as an aggregate outcome variable.

Serious Adverse Events
A SAE is defined as:

- Results in death
- Is life-threatening. This refers to an event in which the patient was at immediate risk of death; it does not refer to an event that might have caused death had it been more severe.
- Results in a persistent or significant disability/incapacity
- Results in a congenital anomaly/birth defect
• Is medically significant. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

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

7.4 Analysis of Tertiary Outcomes
Missed Daycare and Missed Work: Tertiary outcomes will be analyzed in a manner similar to the primary and secondary outcomes. These continuous outcomes will be compared using an appropriate model with robust estimates for standard errors.

Additional analyses involving these outcomes will include linear and logistic regression models that adjust for possible effects of baseline characteristics.

7.5 Analysis of Subgroups
There are 4 subgroup factors pre-specified for formal analysis in this trial:
(i) Age < 1 year
(ii) Breast-feeding status,
(iii) Antibiotic usage
(iv) Protocol compliance

Rates of the primary study outcome will be reported by treatment arm for all pre-specified 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 0.05/4 = 0.0125. 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


Statistical Analysis Plan

Protocol Title: Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis: Randomized Controlled Trial

Protocol Version and Date: 2.00; November 1, 2017
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### Abbreviations

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<th>Abbreviation</th>
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<tr>
<td>AGE</td>
<td>Acute Gastroenteritis</td>
</tr>
<tr>
<td>DSMC</td>
<td>Data and Safety Monitoring Committee</td>
</tr>
<tr>
<td>ITT</td>
<td>Intent-To-Treat</td>
</tr>
<tr>
<td>MCID</td>
<td>Minimal Clinically-Important Difference</td>
</tr>
<tr>
<td>MVS</td>
<td>Modified Vesikari Score</td>
</tr>
<tr>
<td>PERC</td>
<td>Pediatric Emergency Research Canada</td>
</tr>
<tr>
<td>PROGUT</td>
<td>Probiotic Regimen for Outpatient Gastroenteritis Utility of Treatment</td>
</tr>
<tr>
<td>(S)AE</td>
<td>(Serious) Adverse Event</td>
</tr>
<tr>
<td>SAP</td>
<td>Statistical Analysis Plan</td>
</tr>
</tbody>
</table>
1 Preface

1.1 Purpose of SAP

This Statistical Analysis Plan (SAP) describes the planned analysis and reporting for the PROGUT (Probiotic Regimen for Outpatient Gastroenteritis Utility of Treatment) Protocol: Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis: Randomized Controlled Trial (PROGUT).

The purpose of this study is to determine if probiotic administration reduces the severity of acute gastroenteritis (AGE) 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 Pediatric Emergency Research Canada (PERC) Network. 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:

- PROGUT Protocol: Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis: Randomized Controlled Trial (PROGUT Study).
- Case Report Forms for the PROGUT 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 PROGUT 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, pre-specified statistical analysis approach is completely followed in the revised technical specifications.

2 Study Objectives and Outcomes

2.1 Study Objectives

2.1.1 Primary Objective

To determine if in previously healthy children, ages 3-48 months, who present to an ED with less than 72 hours of AGE like symptoms, is the proportion who develop moderate to severe disease...
[Modified Vesikari Score (MVS) ≥ 9] following ED evaluation, significantly different in those who receive a probiotic agent (Lacidofil) compared to those who receive placebo.

2.2 Study Outcomes
2.2.1 Primary Outcome
The primary outcome is the development of moderate-severe disease in the 2 weeks after the index ED visit as measured by the MVS.\(^1\)

2.2.2 Secondary Outcomes
Clinical
A) The duration of diarrhea: Time from treatment initiation until the appearance of the last watery stool\(^2\) as reported during daily phone conversations.

B) The duration of vomiting: Limited data indicate that probiotic administration may reduce vomiting.\(^5,6\) Recovery will be evaluated in children who vomit ≥ 3 times over the 24 hours prior to the ED visit and defined as “time from treatment initiation until last vomiting episode.” We have previously reported that vomiting frequency predicts outcomes in AGE.\(^7\)

C) The frequency of diarrhea and vomiting: The total number of episodes of vomiting and diarrhea related to the presenting illness will be compared between groups.

D) Return visits for unscheduled care to a health care provider related to vomiting, diarrhea, dehydration, fever, or fluid refusal, within two weeks: Not included will be scheduled visits (e.g. re-assessment, vaccinations). This outcome is important as > 50% of children have a follow-up office visit,\(^8\) 8-18% require an ED visit,\(^9\) and 5-8% are hospitalized.\(^8\)

2.2.3 Adverse Events
To determine if short course probiotic administration to young children with AGE is associated with an increase in adverse events. As stated by the NIH, probiotic safety needs to be studied scientifically.\(^10\) Groups will be compared regarding the development of any adverse events with particular attention paid to bloating, abdominal distention, duration of fever, and buttock rash. The importance of evaluating adverse events has been highlighted by a recent adult pancreatitis study which found an unexpected increase in mortality in probiotic treated patients.\(^11\)

2.2.4 Tertiary Outcomes
ED revisits, IV rehydration, hospitalization, days of missed work and daycare absenteeism.

Mechanism of Action: The results of the fecal sIgA analyses will not be included in manuscript reporting on primary results.
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 usage during the 14 days prior to ED visit
- Infective agent at baseline (e.g., norovirus, rotavirus)
- Severity of baseline diarrhea and vomiting
- Hydration assessment
- Need for hospitalization at index visit

3 Study Design and Methods
3.1 Overall Study Design
The PROGUT 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.

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. Permuted blocks of lengths 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: www.randomize.net, an internet based randomization service, will produce a randomization list stratified by study site, using random-number generating software. The lists will be sent to the central pharmacy (ACH) who will prepare consecutively numbered study kits according to the randomization schedule. These will be couriered to the clinical sites, using proper shipment containers and temperature monitors, where they will be stored in the Research Support Pharmacies.

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 PROGUT 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 DSMC 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.

Only the research pharmacy at the coordinating center and www.randomize.net will retain the randomization code. The DSMC may request to be unblinded to treatment assignment at any time.

3.3 Sample Size and Power Determination
Clinical Outcome: The sample size is based on the assessment of the between-group difference in proportions of children with a post-randomization score ≥ 9 on the MVS. This is a superiority study in which the adoption of probiotic use can be recommended if the rate of the primary outcome is significantly lower amongst those who receive the probiotic medication. Calculations are based on a two-sided type I error (α) of 0.05 and power (1-β) of 0.90. The null hypothesis is \( H_0: P_I - P_C = 0 \), where \( P_I \) and \( P_C \) are the event rates in the intervention and control groups respectively. The alternative hypothesis is \( H_A: |P_I - P_C| > 0.10 \) (i.e. the event rates will differ by at least 10 percentage points).

Minimal Clinically Important Difference (MCID): Ten content experts from the US and Canada were surveyed regarding the MCID. Absolute risk differences ranging from 7.5-15% were suggested. We chose a conservative estimate of 10% for the primary outcome (number needed to treat of 10).

Outcome in Control Group: Our estimate for the development of moderate to severe AGE in the controls is based on data collected as part of our 2009 evaluation of the MVS in 455 children aged 3 – 48 months, with < 72 hours of symptoms, who presented to one of 11 Canadian EDs.\(^1\) Using the ED visit as time 0, 25% of eligible children had scores consistent with moderate to severe disease following discharge. This is lower than previous reports of ED\(^{12,13}\) and community populations\(^{14-16}\) because we did not include symptoms that existed prior to the visit. However, Dr. Schnadower’s group in the United States has just completed data collection on 282 children enrolled at 6 sites in the United States and they found that 24% of children in their sample had scores consistent with moderate to severe disease following discharge (personnel communication September 6, 2012). Since our study population and method of MVS calculation in the derivation and recent validation studies and the current proposal are the same, 25% is a very accurate estimate. Given the above, the required sample size to compare proportions between two different groups is 670.\(^{17}\)
Sample Size Adjustment Calculation: Based on previous work by our group with similar follow-up designs\textsuperscript{18-20} and extensive reviewer feedback, we have assumed a 10\% loss to follow-up (670/0.9\textsuperscript{2}=744), 5\% drop out (744/(0.95)\textsuperscript{2}=825), and 2.5\% drop in (caregivers who decide to buy a probiotic agent at a pharmacy to administer to their child) rate (825/(0.975)\textsuperscript{2}=868). Adjustment for O'Brien-Fleming monitoring boundaries requires a further 2\% increase. Thus, the total number randomized (final sample size) will be 886.

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 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 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 must meet all of the following inclusion criteria:
1. Presence of diarrhea: defined as \( \geq 3 \) watery stools in a 24-hour period.\textsuperscript{21}
2. Duration of vomiting or diarrhea < 72 hours: Early administration = greater efficacy.\textsuperscript{6,22,23}
3. Age 3 to < 48 months: AGE severity and frequency are greatest amongst young children.\textsuperscript{24}
Patients meeting any of the following criteria will be excluded from the study:
1. Presence of an indwelling vascular access line or structural heart disease (bacteremia risk).\textsuperscript{25}
2. Taking immunosuppressive therapy, or known history of immunodeficiency (bacteremia risk).\textsuperscript{26}
3. Hemoatochezia in the preceding 72 hours, underlying significant chronic gastrointestinal problem or inflammatory bowel disease: Not including constipation, gastroesophageal reflux or chronic pain.
4. Family member with an indwelling vascular access line, on immunosuppressive therapy, or with a known immunodeficiency: Does not include use of short course oral (<7 days) or inhaled steroids.
5. Bilious vomitus: May indicate a diagnosis other than AGE is possible.
6. Probiotic use (supplement) in the preceding 2 weeks: However, consumption of foods containing probiotics will not result in exclusion as they are ubiquitous.
7. Previously enrolled in this trial (to ensure that the observations on trial patients are independent).
8. Daily telephone follow-up will not be possible while symptomatic (travel plans or language barrier).
9. Allergy to soy: Lacidofil, as well as the placebo product have come in contact with soy during the manufacturing process.
10. Pre-existing, or known, pancreatic dysfunction or insufficiency\textsuperscript{11}
11. Oral or Gastrointestinal surgery within the preceding 7 days: theoretical wound infection risk.

5 General Analysis Issues
5.1 Analysis Software
Analyses were performed with SPSS, version 24.0.0.1 (SPSS Inc) and Stata version 15.0 (StataCorp LLC).

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 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.
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
(1) The presence of a MVS ≥ 9 will be analyzed by
   (i) age < 1 year,
   (ii) breast-feeding status,
   (iii) antibiotic usage and
   (iv) protocol compliance.

(2) Duration of vomiting will be analyzed only in those patients who have ≥ 3 episodes of vomiting in the 24 hours prior to enrollment.

(3) Daycare and work absenteeism will only be analyzed for children who attend daycare and caregivers who work.

(4) Presence of rotavirus infection will be evaluated by adding an interaction term between treatment and rotavirus positivity in a logistic regression model. The independent variables in the model will be
   (i) treatment group,
   (ii) rotavirus positivity (yes/no) and
   (iii) the interaction between treatment group and rotavirus positivity.

Universal rotavirus vaccination does not exist in Canada with the decision being made individually by each province based on the expense as well as feasibility.2728 At present it is included in the provincial schedules in Quebec and Ontario but not in Nova Scotia or Alberta. The varying use of the vaccine and our goal to identify etiologic agents and to conduct sub-
analyses will yield very important information related to probiotic use in the presence/absence of rotavirus vaccination.

5.6 Derived and Computed Variables
All derived and computed variables will be outlined in the analysis dataset specifications for this study.

6 Overview of Planned Analyses
6.1 Schedule of Interim Analyses
The Data Safety Monitoring Committee (DSMC) will meet after 200 and 500 patients to review enrollment, study procedures, form completion, data quality, 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 AGE in the probiotic arm is equal to that in the placebo arm. Based on trends and adverse events, the DSMC may decide to meet sooner than planned using boundaries adjusted accordingly. Because this trial involves children under the age of 6 months, the DSMC has approved a plan to complete an interim safety analysis on the first 20 subjects enrolled under 6 months of age. All serious adverse events will be reported within 24 hours to the DSMC and based on these reports; the DSMC may decide to conduct a safety analysis before the full 20 subjects have been enrolled in this age group. Otherwise, a blinded analysis will be conducted after the 20 subjects < 6 months of age have been enrolled. This data will be unblinded if the DSMC deems it necessary to conduct an unblinded interim safety analysis. The results of this analysis will be communicated to the NHPD branch of Health Canada at the discretion of the DSMC chair should any concerns be identified.

6.2 Stopping Rules for Interim Analyses
Two-sided O'Brien-Fleming boundaries, implemented using the alpha-spending function approach will be used to ensure that the total Type I error rate will be less than or equal to 0.05.

6.3 Blinding in the Interim Analyses
The DSMC will be partially blinded, aware of results by treatment arm, but not of arm identities. Other research team members will be blinded to treatment assignments, but the study coordinator will have access to individual safety and efficacy data. Study personnel 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 the DSMC report. The DSMC 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
- Age
- Season
- Weight
- Primary care physician access
- Daycare attendance
- Ondansetron administration in the ED
- Receipt of rotavirus vaccine
- Vomiting and diarrhea duration and frequency
- Presence of fever
- Prior ED visits, IV rehydration, hospitalization
- Baseline MVS
- Duration of symptoms prior to enrollment (<48 hours vs. 48 hours or more)
- Breast-feeding status
- Antibiotic usage during the 14 days prior to ED visit
- Infective agent at baseline (e.g., norovirus, rotavirus)
- Hydration assessment (Clinical Dehydration Scale 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 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 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.

<table>
<thead>
<tr>
<th>Points</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea duration</td>
<td>0</td>
<td>1–96 hrs</td>
<td>97–120 hrs</td>
<td>≥ 121 hrs</td>
</tr>
<tr>
<td>Max # of diarrheal stools/24 hrs</td>
<td>0</td>
<td>1–3</td>
<td>4–5</td>
<td>≥ 6</td>
</tr>
<tr>
<td>Vomiting duration</td>
<td>0</td>
<td>1–24 hrs</td>
<td>25–48 hrs</td>
<td>≥ 49 hrs</td>
</tr>
<tr>
<td>Max # of vomiting episodes/24 hrs</td>
<td>0</td>
<td>1</td>
<td>2–4</td>
<td>≥ 5</td>
</tr>
<tr>
<td>Max recorded fever</td>
<td>≤ 37°C</td>
<td>37.1–38.4°C</td>
<td>38.5–38.9°C</td>
<td>≥ 39°C</td>
</tr>
</tbody>
</table>
Table 1: Modified Vesikari Score

<table>
<thead>
<tr>
<th>Unscheduled healthcare visit</th>
<th>0</th>
<th>—</th>
<th>Primary Care</th>
<th>Emergency Department</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>None</td>
<td>Rehydration</td>
<td>Hospital Admission</td>
<td>—</td>
</tr>
</tbody>
</table>

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 diarrhea is considered to have continued throughout that time period. The diarrhea episode would thus be calculated from randomization date/time until the final 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 calculated from randomization date/time.

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”. All ED visits to the participating site were verified by database review.

7. **Treatment**: Treatment will be classified as “none”, “intravenous 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 proportion of children with moderate to severe disease (i.e. MVS ≥ 9) will be analyzed employing a logistic regression model, stratified by study site, to provide odds ratios and 95% confidence intervals for the risk of moderate-severe disease in the probiotic versus placebo groups. **Significance for the primary outcome measure will be determined using a two-sided 0.05 level.** The pre-enrollment MVS will not be included in the primary analysis as we do not anticipate the baseline and post-intervention scores to be correlated.

### 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. When the variable was a computed variable (e.g. diarrhea duration) it was not imputed directly. To minimize the use of imputation, only the missing components were imputed (e.g. if the end date of diarrhea was reported, but the end time was missing, only the end time will be imputed).

Variables were imputed in a selective pre-defined order to maximize logic. For example, in instances where both the end date and end time were missing, the maximum number of diarrheal stools/24 hours and the maximum number of vomiting episodes/24 hours were first imputed (if needed) to determine the day that participant met then study end point (i.e. the earliest day that both diarrhea and vomiting stopped), then the diarrhea duration was calculated from randomization until the day of the study end point.

In the case where no MVS outcome data are available, the subject will not be included in imputations and the outcomes will remain missing.

**Imputation Method:** We will use the Multiple Imputation-Automatic method with SPSS 24.0. The Automatic method scans the data and uses the monotone method (noniterative method) if the data show a monotone pattern of missing values; otherwise, fully conditional specification (iterative Markov chain Monte Carlo method) is used. We will use logistic regression methods to model unscheduled ED visit, unscheduled primary care visits, rehydration, and hospital admission. We will use linear regression methods to model diarrhea end time, maximum number of diarrheal stools/24 hours, vomiting end time, maximum number of vomiting episodes/24 hours, and maximum fever.
Variables to include in imputation models
Minimum and maximum possible values will be computed and used as bounds for imputing MVS components: diarrhea end time, maximum number of diarrheal stools/24 hours, vomiting end time, maximum number of vomiting episodes/24 hours, maximum fever.
General model variables: treatment assigned, age, hospital/site
Baseline model variables: maximum number of diarrheal stools/24 hours, maximum number of vomiting episodes/24 hours, presence of fever
Post-randomization model variables: primary care visits, ED visits, rehydration, hospital admissions

Number of imputations: We will impute and analyze 20 datasets. We will increase that number if the efficiency of the imputed datasets is less than 0.99.

Multiple Regression with Multiple Imputed Data Sets: For analyses using multiple regression (e.g. logistic regression, linear regression, negative binomial regression), the pooled of the 20 imputed datasets will be analyzed and the pooled analysis results will be provided by SPSS 24.0.

7.2.2 Additional Analyses of Primary Outcome
Secondary analyses of the primary outcome adjust for covariates that may be imbalanced between groups (e.g. age, frequency of vomiting and diarrhea in 24 hours preceding randomization, site, and detection of rotavirus infection). We will also analyze the MVS as a continuous variable through a employing a linear regression model adjusted for site.

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. 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. Sensitivity analyses will also be performed to assess the possibility and consequences of losses to follow-up not occurring at random.

7.3 Analysis of Secondary Outcomes
7.3.1 Duration and Frequency of Symptoms
The continuous variables of (1) duration of diarrhea and (2) vomiting will be measured in hours and analyzed with a using a linear regression model stratified by clinical center. Incident rate ratios were analyzed to compare the number of episodes of (3) diarrhea and (4) vomiting following randomization employing a negative binomial model that included terms for treatment, site and number of episodes of diarrhea and/or vomiting, respectively, in the 24-hours pre-randomization.
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 logistic regression models, stratified by clinical center.

7.3.3 Adverse Events
**Adverse Event (AE):** An adverse event is any unfavorable or unintended clinical or other occurrence during the study period that may or may not be the result of participation in the research study.

**Expected Adverse Drug Reactions/Events**
These include the following as they are part of the natural history of the underlying disease process:
- Hospitalization
- Future health care provider visit, ED return visit
- IV rehydration
- Abdominal pain, distension
- Vomiting, diarrhea, fever, flatulence

Because expected adverse events are part of the natural history of AGE and diarrheal illness in children, they will not need to be reported as Adverse Events. This information will be recorded in normal study data collection processes.

AEs will be recorded from the time of randomization through 14 days. 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 DSMC may request to see more detailed tables. The proportions of children experiencing any adverse event, as reported by the caregivers, will be compared between groups using using logistic regression models, stratified by site. The analysis will evaluate the presence/absence of side effects, as an aggregate outcome variable.

**Serious Adverse Events**
A SAE is defined as:
- Results in death
• Is life-threatening. This refers to an event in which the patient was at immediate risk of
death; it does not refer to an event that might have caused death had it been more severe.
• Results in a persistent or significant disability/incapacity
• Results in a congenital anomaly/birth defect
• Is medically significant. Important medical events that may not result in death, be life-
threatening, or require hospitalization may be considered SAEs when, based upon
appropriate medical judgment, may jeopardize the patient and may require medical or
surgical intervention to prevent one of the outcomes listed in this definition

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

7.4 Analysis of Tertiary Outcomes
Missed Daycare and Missed Work: Tertiary outcomes will be analyzed in a manner similar to
the primary and secondary outcomes. These continuous outcomes will be compared using the
van Elteren test.

Analyses of ED revisits, IV rehydration, and hospitalization employing logistic regression with
adjustment for participant enrollment site.

Additional analyses involving these outcomes will include linear and logistic regression models
that adjust for possible effects of baseline characteristics.

7.5 Analysis of Subgroups
There are 4 subgroup factors pre-specified for formal analysis in this trial:
(i) Age < 1 year
(ii) Breast-feeding status,
(iii) Antibiotic usage
(iv) Protocol compliance

Rates of the primary study outcome will be reported by treatment arm for all pre-specified
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 $0.05/4 = 0.0125$. 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


Impact of Emergency Department Probiotic Treatment of Pediatric Gastroenteritis: Randomized Controlled Trial

Statistical Analysis Plan

Summary of Changes

1. Additional tertiary outcomes specified:
   a. ED revisits, IV rehydration, hospitalization.

2. Analysis of primary outcome changes to logistic regression model.

3. Covariates included in model of primary outcome specified to include frequency of vomiting and diarrhea in 24 hours preceding randomization, site, and detection of rotavirus infection.

4. Additional analysis of primary outcome (MVS as a continuous variable) altered to employ a linear regression model adjusted for site.

5. Analysis of the continuous variables of (1) duration of diarrhea and (2) vomiting will employ a linear regression model.

6. Analysis of the number of episodes of (3) diarrhea and (4) vomiting following randomization specified to employing a negative binomial model that included terms for treatment, site and number of episodes of diarrhea and/or vomiting, respectively, in the 24-hours pre-randomization to generate incident rate ratios.

7. Analyses of return visits and adverse events specified to employ logistic regression models.

8. Missed daycare and work analyses specified to employ the van Elteren test.

9. Analyses of ED revisits, IV rehydration, and hospitalization employing logistic regression with adjustment for participant enrollment site.