# Protective Efficacy of Orally Delivered Bovine Serum Immunoglobulin (BSIgG) Specific for the Colonization Factor CS6 Following Challenge With the CS6-expressing Enterotoxigenic E. Coli (ETEC) Strain B7A

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# Protective efficacy of orally delivered bovine serum immunoglobulin (BSIgG) specific for the colonization factor CS6 following challenge with the CS6-expressing Enterotoxigenic *E. coli* (ETEC) strain B7A


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Investigator's Agreement

Protective efficacy of orally delivered bovine serum immunoglobulin (BSIgG) specific for the colonization factor CS6 following challenge with the CS6-expressing Enterotoxigenic *E. coli* (ETEC) strain B7A

"I have read this protocol and agree to conduct the study as outlined herein in accordance with International Conference on Harmonization Good Clinical Practice Guideline and FDA and DoD Regulations."

_____ _____

Kawsar R. Talaat, MD Date

Principal Investigator

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## GLOSSARY OF ABBREVIATIONS

| Abbreviation | Definition |
|---|---|
| AE | Adverse event |
| ALS | Antibody Lymphocyte Supernatant |
| B7A | CS6- expressing ETEC strain |
| BIgG | Bovine Immunoglobulin |
| BP | Blood Pressure |
| BSIgG | Bovine serum Immunoglobulin |
| C | Celsius |
| CFR | Code of Federal Regulations |
| CBC | Complete blood count |
| CF | Colonization Factor |
| CFA | Colonization factor antigen |
| CfaB | The major rod-forming subunit of CFA/I fimbriae |
| CfaE | Minor subunit (tip adhesin) of CFA/I fimbriae |
| cfu | Colony forming unit |
| cGMP | Current Good Manufacturing Practice |
| CIR | Center for Immunization Research |
| CS | Coli surface antigen |
| CsbD | Minor subunit (tip adhesin) of CS17 fimbriae |
| CS17 | Coli surface antigen 17 |
| CS17-ETEC | ETEC which have fimbriae composed of colonization factor CS17 |
| CsbA | The major rod-forming subunit of CS17 fimbriae |
| DoD | Department of Defense |
| DS | Double strength |
| eCRF | Electronic case report form |
| EDC | Electronic data capture |
| ELISA | Enzyme-linked immunosorbent assay |
| E. coli | *Escherichia coli* |
| ETEC | Enterotoxigenic *E. coli* |
| F | Fahrenheit |
| FDA | US Food and Drug Administration |
| FUP | Fimbrial usher proteins |
| F/U | Follow Up |
| GCP | Good Clinical Practice |
| GM1 | Anti-ganglioside M1 |
| H10407 | *Escherichia coli* strain H10407 |
| HBsAg | Hepatitis B surface antigen |
| HCV | Hepatitis C virus |
| HIV | Human immunodeficiency virus |
| ICH | International Conference on Harmonization |
| IgA | Immunoglobulin A |
| IgG | Immunoglobulin G |
| IND | Investigational New Drug |
| IP | Investigational product |
| IRB | Institutional Review Board |

| | |
|---|---|
| JHBSPH | Johns Hopkins Bloomberg School of Public Health |
| JHH | Johns Hopkins Hospital |
| JHU | Johns Hopkins University |
| LPS | Lipopolysaccharide |
| LT | Labile toxin |
| CT | Cholera toxin |
| NMRC | Naval Medical Research Center |
| ORS | Oral Rehydration Solution |
| PBF | WRAIR Pilot Bioproduction Facility |
| PCB | Production Cell Bank |
| PBMC | Peripheral blood mononuclear cell |
| PVT | Psychomotor vigilance testing |
| PI | Principal Investigator |
| SAE | Serious adverse event |
| SAS | Statistical Analysis System |
| SOP | Standard Operating Procedure |
| SSP | Study Specific Procedure |
| tid | Three times a day |
| WBC | White blood cell |
| WRAIR | Walter Reed Army Institute of Research |

## CLINICAL PROTOCOL SYNOPSIS

| | |
|---|---|
| PROTOCOL TITLE | Protective efficacy of orally delivered bovine serum immunoglobulin (BSIgG) specific for the colonization factor CS6 following challenge with the CS6-expressing Enterotoxigenic *E. coli* (ETEC) strain B7A |
| IND NUMBER | TBD |
| INVESTIGATIONAL PRODUCTS | 1. Anti-CS6 BSIgG (lot PD1601105CS)<br>2. Anti-B7A whole cell killed BSIgG (positive control) (PD1601132ET)<br>3. Non-hyperimmune BSIgG (negative control/placebo) (lot PD1601071NC)<br>4. CS6-expressing ETEC strain (B7A) (O148:H28- $CS6^+$ $LT^+ST^+$) (Lot 0481) |
| SPONSOR | A. Louis Bourgeois, PhD, MPH |
| MANUFACTURERS | ETEC strain B7A: Pilot Bioproduction Facility, Walter Reed Army Institute of Research, Silver Spring, MD.<br><br>anti-CS6 and anti-B7A whole cell killed hyperimmune BSIgG products and non-hyperimmune BSIgG: SAB Biotherapeutics, Sioux Falls, SD. |
| PRINCIPAL INVESTIGATOR | Kawsar Talaat, MD |
| STUDY SITE | Center for Immunization Research (CIR) Isolation Unit<br>301 Building<br>301 Mason Lord Drive Suite 4300<br>Baltimore, MD 21224<br><br>CIR Outpatient Clinic<br>624 N. Broadway, Hampton House Rm. 117<br>Baltimore, MD 21205 |
| LABORATORIES | Quest Diagnostics Incorporated, Baltimore, MD 21227<br>Johns Hopkins Hospital, Baltimore, MD 21287<br>Johns Hopkins University School of Public Heath, Baltimore, MD 21205<br>Johns Hopkins University School of Public Health, Baltimore, MD 21287<br>Johns Hopkins Biological Repository, Baltimore, MD 21205<br>Core Lab of Johns Hopkins School of Public Health, Baltimore, MD 21287<br>Naval Medical Research Center, Silver Spring, MD 20910 |
| STUDY OBJECTIVES | The primary objectives of this study are to assess the safety of serum-derived bovine immunoglobulins in healthy adult subjects when orally administered three times a day over 7 days and to estimate protective efficacy of those preparations against moderate-severe diarrhea upon challenge with B7A. The secondary objectives include assessments of a variety of clinical endpoints, measuring mucosal and systemic immune responses and obtaining and archiving samples for future proteomics and/or systems biology efforts. There are a variety of other exploratory clinical, immunological, and microbiological endpoints. |
| STUDY DESIGN | The study is a randomized, double-blind, placebo-controlled clinical trial in which up to 60 subjects (two inpatient periods of approximately 30 subjects) will receive one of the three investigational products (IP) three times daily following meals beginning 2 days prior to experimental challenge with B7A. Randomization and blinding will be utilized for the clinical study team. Subjects will be assigned to groups as per the Table below. The test articles/placebo will be administered for a total of 7 days, or until antibiotic treatment has been initiated. Subjects will be |

<table>
<tr><td></td><td>assessed daily for adverse events and all stools will be collected to assess for the primary endpoint of moderate (4-5 loose stools in 24 hours or 401-800 g of loose stools in 24 hours) to severe (≥ 6 loose stools in 24 hours or >800g of loose stools in 24 hours) diarrhea post-inoculation. Any subject passing a grade 3-5 stool will be encouraged to start drinking oral rehydration solution (ORS) (an oral glucose/electrolyte solution to prevent dehydration) or Gatorade at a rate equal to their stool output. IV rehydration will be provided if pre-specified criteria are met. All subjects will be treated with ciprofloxacin (500 mg by mouth twice daily for three days) five days after ingesting the B7A unless early treatment criteria are met. Alternate antibiotic treatment to which the strain is susceptible may also be considered as clinically appropriate. Subjects will be discharged from the inpatient facility when clinical symptoms are resolved or resolving AND two consecutive stool cultures are negative for ETEC.<br><br>
<table>
<tr><td>Product[a]</td><td>N</td><td>Dose (approximate)</td></tr>
<tr><td>Anti-CS6 BSIgG</td><td>20</td><td>1.0g three times daily (tid)</td></tr>
<tr><td>Anti-B7A whole cell killed BSIgG</td><td>20</td><td>1.0 g tid</td></tr>
<tr><td>Negative Control (Non-hyperimmune) BSIgG</td><td>20</td><td>1.1 g protein total (equivalent) tid</td></tr>
</table>
[a] All products will be given 3 times daily</td></tr>
<tr><td>PRIMARY ENDPOINT</td><td>Moderate to severe diarrhea defined as<br><br>• Moderate diarrhea: 4 to 5 loose/liquid stools or 401-800 g of loose/liquid stool in any 24 hour period<br>• Severe diarrhea: ≥6 loose/liquid stools or > 800 g of loose/liquid stool in any 24 hour period</td></tr>
<tr><td>STUDY DURATION</td><td>About thirty subjects per cohort: screening (85 days); inpatient (12 days); outpatient (28 days); immunologic assays (3 months); six-month phone check; Entire study, considering serial scheduling of cohorts of about 30 subjects, analysis and reporting after last clinic visit (2 months) – 1 to 1 ½ years</td></tr>
<tr><td>ELIGIBILITY CRITERIA</td><td>Potential subjects will be recruited by responding to IRB-approved advertisements, telephone calls, emails, and word of mouth. Subjects will be screened at the CIR. Up to 6 alternates per inpatient period will be recruited to replace anyone who does not report or is unable to participate at time of inpatient unit admission.<br><br><u>Inclusion Criteria</u>:<br>1. Male or female between 18 and 50 years of age, inclusive.<br>2. General good health, without significant medical illness, abnormal physical examination findings or clinical laboratory abnormalities as determined by principal investigator (PI) or PI in consultation with the research monitor and sponsor.<br>3. Demonstrate comprehension of the protocol procedures and knowledge of ETEC illness by passing a written examination (pass grade ≥ 70%)<br>4. Willing to participate after informed consent obtained.<br>5. Available for all planned follow-up visits.<br>6. Negative serum pregnancy test at screening and negative serum and/or</td></tr>
</table>

urine pregnancy test on the day of admittance to the inpatient phase for female subjects of childbearing potential. Females of childbearing potential must agree to use an efficacious hormonal or barrier method of birth control during the study. Abstinence is acceptable. Female subjects unable to bear children must have this documented (e.g., tubal ligation or hysterectomy).

Exclusion Criteria:

General health criteria

1. Presence of a significant medical condition, (e.g. psychiatric conditions or gastrointestinal disease, such as peptic ulcer, symptoms or evidence of active gastritis or gastroesophageal reflux disease, inflammatory bowel disease, alcohol or illicit drug abuse/dependency, or other laboratory abnormalities which in the opinion of the investigator precludes participation in the study.
2. Immunosuppressive illness or IgA deficiency (serum IgA < 7 mg/dL or below the limit of detection of assay)
3. Evidence of confirmed infection with HIV, HBsAg, or HCV, with confirmatory assays.
4. Use of any investigational product within 30 days preceding the receipt of the investigational products, or planned use during the active study period
5. Significant abnormalities in screening lab hematology or serum chemistries, as determined by PI or PI in consultation with the research monitor and sponsor.
6. Lactation or breastfeeding.

Research-related exclusions applicable to challenge

7. History of microbiologically confirmed ETEC or cholera infection in last 3 years.
8. Occupation involving handling of ETEC or *Vibrio cholerae* currently, or in the past 3 years.
9. Travel to countries where ETEC or cholera infection is endemic (most of the developing world) within 3 years prior to dosing.
10. Symptoms consistent with Travelers' Diarrhea concurrent with travel to countries where ETEC infection is endemic (most of the developing world) within 3 years prior to dosing, OR planned travel to endemic countries during the length of the study.
11. Vaccination for or ingestion of ETEC, cholera, or E coli heat labile toxin within 3 years prior to dosing.
12. Any prior experimental infection with ETEC strain B7A.

Study-specific Exclusion Criteria (potential increased risk or complicating outcome ascertainment)

13. Abnormal stool pattern (fewer than 3 per week or more than 3 per day).
14. History of diarrhea in the 2 weeks prior to planned inpatient phase.
15. Regular use of laxatives, antacids, or other agents to lower stomach acidity (regular defined as at least weekly).
16. Use of antibiotics during the 7 days before receipt of any investigational

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|---|---|
| | product or proton pump inhibitors, $H_2$ blockers, or antacids within 48 hours of receipt of any investigational product.<br>17. Use of any medication known to affect the immune function (eg, systemic corticosteroids and others) within 30 days preceding the administration of challenge or planned use during the active study period.<br>18. Known allergy to fluoroquinolones.<br>19. Inability to tolerate 150 mL sodium bicarbonate buffer (based on requirement for frequent dosing). |
| STUDY PROCEDURES | |
| RANDOMIZATION | Subjects will be randomized in a 1:1:1 ratio to one of 3 treatment groups. An analyst at NMRC will prepare a randomization list, allocating volunteer identification numbers to the study groups using the PROC PLAN function of SAS v9.2 (Cary, NC). The randomization scheme will utilize block sizes of 6 in order to ensure comparable group sizes in the event that the targeted number of 60 subjects is not reached. NMRC staff will print the code, log and output of the SAS procedure, sign them, and store them under lock and key. A photocopy of the signed output will be e-mailed to the research pharmacist prior to the first BSIgG administration day. |
| GROUP ASSIGNMENT | Prior to the first dose of test article/placebo, subjects will be assigned a study number determining what IP they receive. Subjects will receive the test article/placebo in containers bearing their assigned identification numbers. This number will be linked to the randomization code list securely maintained throughout the clinical phase of the study by an unblinded NMRC analyst and the Johns Hopkins University (JHU) research pharmacist. Study identification numbers will identify all samples for laboratory analyses. |
| BLINDING | Investigators and subjects will remain blinded to group assignments until completion of the clinical phase of the trial and validation of the clinical and immunological data. Investigators may be unblinded prior to the 6 month follow up phone call. Each multi-dose test article/placebo bottle will be labeled with an open label. The research pharmacist will use the randomization list to prepare the IP. All mixing and administration of the test articles/placebo will be performed per formulation and product administration study specific procedures (SSPs). Administration will occur in a separate room from where the doses are formulated.<br><br>Only in a medical emergency, when knowledge of the study treatment is essential for further management of subjects, will the randomization code be broken. In the event that this is necessary, the PI will provide the study identification number to the research pharmacist, who in turn will provide the investigator with the broken code for that subject. The investigator will notify the Sponsor immediately and document the event on the appropriate study documents. |
| TEST ARTICLE DOSING | BSIgG products will be administered starting day -2 and then continued for 6 days.<br><br>On Day 0, subjects will eat breakfast and then take their morning dose of IP about 15 minutes later. Subjects will fast for 90 minutes and will drink 120 ml of sodium bicarbonate just prior to ingesting 30 ml of sodium bicarbonate containing the ETEC inoculum on day 0 of the study. Subjects will take the second daily dose of IP approximately 15 minutes after drinking the inoculum, and subjects will otherwise fast for 90 minutes after drinking the inoculum. Subjects will then be allowed to eat and will take the third daily dose of IP approximately 15 minutes |

<table>
<tr><td></td><td>after dinner.<br><br>Order of events on day of challenge
<table>
<tr><th>Event</th><th>Volume (approximate)</th></tr>
<tr><td>Breakfast</td><td>-</td></tr>
<tr><td>1st daily dose of test article/placebo (range 10-25 min)</td><td>150 ml</td></tr>
<tr><td>90 minutes fast</td><td>-</td></tr>
<tr><td>Bicarbonate buffer</td><td>120 ml</td></tr>
<tr><td>1 minute interval (up to 2 minutes)</td><td>-</td></tr>
<tr><td>Bicarbonate buffer + Challenge</td><td>30 ml</td></tr>
<tr><td>Interval of 15 minutes (range 10-25 min)</td><td>-</td></tr>
<tr><td>2nd daily dose of test article/placebo</td><td>150 ml</td></tr>
<tr><td>Fast at least 90 minutes from challenge</td><td>-</td></tr>
<tr><td>Lunch</td><td>-</td></tr>
<tr><td>Dinner</td><td>-</td></tr>
<tr><td>15 minutes after dinner complete (range 10-25 min)</td><td>-</td></tr>
<tr><td>3rd daily dose of test article/placebo</td><td>150mL</td></tr>
</table></td></tr>
<tr><td>CLINICAL MONITORING</td><td>Daily medical assessments with adverse event determination, vital signs three times daily, examination and weighing of all stools, stool culture work-up for ETEC study strain (at least once daily), and safety laboratory tests (refer to Time and Events Schedule).</td></tr>
<tr><td>CLINICAL IMMUNOLOGY</td><td>Immunology will be evaluated by measuring serum IgG and IgA responses to CS6, LPS type O148, and LT, and IgG and IgA antibody in lymphocyte supernatant (ALS) responses to CS6, LPS, and LT antigens. All immunological assessments to be carried out at NMRC Laboratories.</td></tr>
<tr><td>CLINICAL MANAGEMENT</td><td><u>Fluid Management</u><br>Oral: Any subject passing a grade 3-5 stool will be encouraged to start drinking Gatorade or ORS at a rate equal to their stool output.<br><br>Intravenous: A subject may be administered IV fluids (clinician discretion) for the following reasons:<br><br>• Subject experiences abrupt onset of diarrhea defined by passage of an initial loose/liquid stool of > 300g or passage of > 400 g of loose/liquid stools over 2 hours.<br>• Subject becomes hypovolemic.<br>• It is determined necessary by the study physician, i.e., diarrhea with nausea/vomiting and unable to drink enough to keep up with output, or other reason.<br><br><u>Antibiotic Treatment</u><br>All subjects will be treated with ciprofloxacin (500 mg by mouth twice daily for three days). Alternate antibiotic treatment to which the strain is susceptible may also be considered as clinically appropriate. This ETEC strain is susceptible to ciprofloxacin and other common antibiotics. Administration of IV antibiotic treatment may be performed if warranted by the PI. The test article/placebo administration will be discontinued with initiation of treatment.</td></tr>
</table>

| | |
|---|---|
| | Early antibiotic treatment after challenge may commence when any of the following criteria are identified and a study physician considers it to be warranted:<br>• Severe diarrhea (based on volume, 800 g in 24 hours)<br>• Stool output consistent with moderate diarrhea for 48 hours<br>• Mild or moderate diarrhea and 2 or more of the following symptoms: severe abdominal pain, severe abdominal cramps, severe nausea, severe headache, severe myalgias, any fever (≥ 38.0°C), or any vomiting.<br>• A study physician determines that early treatment is warranted for any other reason. |
| DISCHARGE PROCEDURES | All subjects are scheduled for discharge from the inpatient ward approximately 8 days after receipt of the challenge inoculum. The day of discharge may be earlier if the subject meets the criteria for discharge prior to day 8. Subjects will be discharged from the inpatient phase of the study when clinical symptoms are resolved or resolving AND two consecutive stool cultures are negative for ETEC. |
| SAMPLE SIZE ESTIMATE/ ANALYSIS | The hypotheses are that (1) anti-CS6 BSIgG confers ≥ 60% protective efficacy against moderate to severe diarrhea upon challenge with B7A (in comparison to the placebo group); and (2) anti-B7A whole cell killed BSIgG confers ≥ 60% protective efficacy against moderate to severe diarrhea upon challenge with B7A (in comparison to the placebo group).<br><br>Assuming a two-sided alpha = 0.05 and an attack rate of 80% in the placebo group, the power (two group continuity adjusted chi-square) to detect a preliminary efficacy of ≥60% in the immunoprophylaxis groups is over 80% when each group contains 20 subjects. There will be no adjustment for multiple comparisons. |

## Table 1. Time and Events Schedule

| | **Screening (1-2 visits[a])** | | **Test Article Dosing and Challenge Phase (Inpatient)** | | | | | | | | | | | | **F/U Visit and Call** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Event** | -30 | | -3[b] | -2 | -1 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8[c] | Outpatient f/u: D28 | 180[d] |
| Compliance Range (study day) | -90 to -31 | -30 to -5 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 6-8 | +/- 1d | 26-30 | +/- 1 month |
| Outpatient | X | X | | | | | | | | | | | | | X | |
| Inpatient stay | | | X | X | X | X | X | X | X | X | X | X | X | X | | |
| Informed Consent | X | | | | | | | | | | | | | | | |
| Comprehension test | X | | | | | | | | | | | | | | | |
| Medical interview[e] | X | (X) | X | X | X | X | X | X | X | X | X | X | X | (X) | X | |
| Focused physical exam[f] | (X) | X | X | X | X | X | X | X | X | X | X | X | X | (X) | (X) | |
| Vital signs[g] | X | X | X | X | X | X | X | X | X | X | X | X | X | (X) | X | |
| Serology (HIV, HBsAg, HCV), IgA level and blood typing | | X | | | | | | | | | | | | | | |
| CBC with differential | X | | X | | | | | | | | X | | | | | |
| Serum chemistry[h] | X | | X | | | | | | | | X | | | | | |
| Serum pregnancy test (females) | X | | X | | | | | | | | | | | | | |
| Urine pregnancy test (females) | | | X | | | | | | | | | | | | X | |
| Drug screen (urine)[i] | X | (X) | | | | | | | | | | | | | | |
| Test article | | | | X | X | X | X | X | X | X | | | | | | |
| Challenge | | | | | | X | | | | | | | | | | |
| Start Antibiotic therapy[j] | | | | | | | | | | | X | | | | | |
| Stool weighing/grading[k] | | | | X | X | X | X | X | X | X | X | X | X | | | |
| Stool bacteriology (CS6 ETEC detection)[l] | | | | | | X | X | X | X | X | X | X | X | (X) | | |
| Stool collection for Microbiome | | | | X | X | X | X | X | X | X | X | X | X | (X) | X | |
| Stool Transcriptomics/ PCR | | | | X | X | X | X | X | X | X | X | X | X | | X | |

| | **Screening (1-2 visits[a])** | | **Test Article Dosing and Challenge Phase (Inpatient)** | | | | | | | | | | | | **F/U Visit and Call** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Event** | -30 | | -3[b] | -2 | -1 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8[c] | Outpatient f/u: D28 | 180[d] |
| Serology: IgG, IgA anti-CS6, LT, LPS antigens[m] | | | X | | | | | | | | | | | | X | |
| ALS: IgA, IgG anti-CS6, LT, LPS antigens[m] | | | X | | | | | | | | | | X | | | |
| Saliva and Fecal Immunology | | | X | | | | | | | | | | X | | X | |
| Memory B Cells | | | X | | | | | | | | | | | | X | |
| Flow Cytometry | | | X | | | | | | | | | | | | X | |
| Systems Biology | | X | X | | | | X | X | | | | | X | | | |
| Cytokines PBMC | | | X | | | | | | | | | | | | X | |
| Cognitive Study (PVT)[n] | | | | | X | X | X | X | X | X | X | X | X | | | |
| Functional Bowel Disorder Survey | X | | | | | | | | | | | | | | | X |
| Discharge from inpatient phase[b] | | | | | | | | | | | | | X | | | |
| Study completion[o] | | | | | | | | | | | | | | | X | |
| 6 Month phone follow-up | | | | | | | | | | | | | | | | X |
| Approximate blood volume (mL) by study day[p] | 10 | 49 | 95 | 0 | 0 | 0 | 24 | 8 | 0 | 0 | 10 | 0 | 32 | 0 | 66 | 0 |

Note: (X) denotes optional event or procedure

[a] Screening may consist of 1 to 2 visits. If within day -30 window, all screening activities may take place at one visit. After screening, subject continuing eligibility must be confirmed by reassessing relevant inclusion and exclusion criteria prior to first dose of IP, on either day of admission or day -2.

[b] Baseline immunology specimens may be collected on Day-3 or Day-2..

[c] Criteria for discharge from the unit: Subjects will be discharged from the inpatient phase of the study when they feel well enough, clinical symptoms have resolved or are resolving, have completed at least two doses of antibiotics, and have 2 consecutive negative stool cultures. Subjects will be required to complete their antibiotics as outpatients. It is expected that most subjects will be discharged on days seven or eight. If a subject does not fulfill criteria for discharge he/she may be required to stay on the unit until all criteria have been fulfilled.

[d] Six-month (+/- 1 month) phone call will also be completed to inquire about new onset serious health events or hospitalizations.

[e] The medical interview occurring at baseline is to establish eligibility. During the inpatient and outpatient phases of the study, the interview will be used to update baseline medical history, monitor safety, and to confirm ongoing eligibility.

[f] Physical Examination (PE) will include: HEENT (Head; Ears; Eyes; Nose; Throat), skin, respiratory (lung), cardiovascular (heart), abdomen, neurological and musculoskeletal system. PE will be done at screening and on admission. During the inpatient period a symptom focused PE will be completed.

[g] Vital Signs (VS) will include heart rate, blood pressure, and oral temperature. If a VS needs to be repeated, standard practice will be to repeat the VS within approximately 20 minutes of the original reading. Only the VS that needs to be repeated will be repeated. Both the original and repeat measurements will be recorded in the study source documents, however, only the repeat measurement will be recorded in the CRF field for that measurement if the PI or designee determines the repeat measurement to be more accurate, even though it may have been obtained several minutes later than the original VS.

The following VS are obtained and documented in the source documents:

- During the screening visit
- At least 3 times daily during in-patient period
- Before and after challenge
- At the day 28 visit

A grade 1 bradycardia, or other grade 1 abnormalities will not be considered to be exclusionary at screening, unless judged to be clinically significant by the PI. Clinically relevant and concurrent medical conditions or surgical procedures will be recorded as medical history if the onset is prior to administration of IP. This includes pre-existing lab abnormalities, VS abnormalities, and symptoms associated with menses (e.g. cramps, headaches, etc.). Grade 2 abnormalities recorded after screening but prior to challenge administration will be determined on a case-by-case basis at PI discretion. Clinically significant abnormalities not on the toxicology table can be recorded on the MH if deemed necessary by the PI.

The following VS will be captured in the electronic CRF:

- Screening
- Admission
- Before and after challenge
- At discharge
- At visit day 7
- At visit day 28
- In addition, any abnormal VS deemed to be clinically significant or clinically relevant may also be entered into the eCRF.

[h] Serum chemistry will include: electrolytes (Na, K, creatinine, random glucose, and ALT (SGPT),. Follow-up samples may be taken if clinically significant abnormalities are seen. Clinically relevant laboratory abnormalities will be recorded as medical history if obtained before day -2.

[i] Urine Drug Screen will test for the presence of amphetamine, barbiturates, opiates, phencyclidine, cocaine, and benzodiazepine, methadone, and propoxyphene at screening and at the discretion of the study clinician. In addition, the study clinician may ask for a sample to test for the presence of antibiotics.

[j] Subjects may begin antibiotic treatment early if one or more criteria are met.

[k] During the inpatient period all stool samples are collected, weighed and graded. If a subject meets discharge criteria prior to day 7, no further stool samples will be collected.

[l] Stool sample for bacteriology will begin the day after challenge, or prior to institution of early antibiotic therapy (whichever is sooner). If a stool sample is not obtained before 1300 hours, a rectal swab will be obtained. Swabs will be used only for bacteriology. Stool samples will be collected for assays as specified in the laboratory study of event schedule and as per written SSPs. A subset of these samples, during high shedding points, will be reserved for the later validation and development of bacteriological assays for shedding of ETEC and other organisms.

[m] Blood for immunology endpoints will be collected as specified in the laboratory study of event schedule and as per written SSPs. Total approximate predefined blood volumes can be found in the laboratory study event schedule.

[n] Exploratory Cognitive Assessment will be performed on all individuals during the inpatient phase (thrice daily) using PVT evaluation.

[o] Study completion is defined as a subject completing all clinic visits.

[p] Approximate total blood volume to be collected is 294 mL.

# 1.0 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

## 1.1 Background Information

Diarrhea is a significant medical problem globally yielding an estimated 1.3- 4.6 billion annual cases [1, 2]. Infectious diarrhea causes significant acute morbidity (negatively impacting growth and cognitive development) and mortality in infants, young children, and vulnerable populations in resource-limited countries, and civilian and military travelers to these areas [3, 4]. According to the World Health Organization (WHO) diarrheal illness is the second leading cause of death in children under five years of age, accounting for 760,000 deaths per year in this age group attributed in part to malnutrition [1].

Travelers' diarrhea (TD) affects up to 60% of travelers [5, 6]. TD incidence rates reach 0.5 episodes per person over 1 - 2 weeks of initial exposure in developing regions [7-11]. TD is commonly self-limiting, lasts 2-6 days [12], and resolves after a week in 90% of cases, with a minority of patients experiencing persistent or chronic diarrhea. Although generally a self-limiting illness, about 20% of travelers who experience diarrhea are bedridden for some period and approximately 40% change their itinerary in some way because of the illness [6]. Diarrhea can vary in severity from mild discomfort to severe dehydration and dysentery. Personal hygiene and field sanitation measures have been unsuccessful in eliminating the risk of TD [13-15]. For example, pre-travel education and counseling of individuals on reducing risk behaviors (e.g. avoid ice/tap water, undercooked meat, unwashed/unpeeled fruits/vegetables) is common practice, however, while this intuitively makes sense, multiple studies have failed to show any consistent reduction in disease incidence [16, 17]. Safe, efficacious preventive modalities are critically needed to minimize the impact of this common infectious disease threat.

Bacterial enteropathogens comprise the majority of the pathogens identified in TD (civilian and military) encompassing upwards 80% of identified cases [18], with Enterotoxigenic *Escherichia coli* (ETEC) consistently the most identified. Additionally, ETEC is the most common bacterial etiology of infectious diarrhea in endemic pediatric populations accounting for 30 - 50% of diarrheal episodes [9-11]. ETEC is culpable in an estimated 400 million cases and 160,000 deaths annually among infants and young children [19]. ETEC may be the first enteric illness encountered by infants [20] and the heavy burden of illness early in life contributes to malnutrition, which can then lead to growth stunting and diminished cognitive development [8]. In 2010 ETEC associated Disability-Adjusted Life Years (DALYs) were estimated at 8.5 million (10 percent of all diarrhea DALYs), and Years Lived with Disability (YLDs) were estimated at one million (13 percent of all diarrhea YLDs) [21, 22].

ETEC exposures occur through ingestion of contaminated food and water, typically producing non-invasive, watery diarrhea, although the diarrhea may manifest with a spectrum of disease presentations (based on strain virulence characteristics), ranging from mild diarrheal episodes to severe, cholera-like purging (even in immunocompetent hosts). Until vaccines become available, there is an urgent need for development of effective diarrhea prevention modalities suitable for use in different contingencies. A number of approaches have been taken to develop an ETEC vaccine, including killed whole-cell, live attenuated, and protein subunit vaccine strategies [23-28]. However, a licensed product is not expected in the near term, and this has revitalized interest in other approaches to ETEC diarrhea prevention. Development of an effective prophylactic agent to control ETEC diarrhea would offer a useful product for travelers and military personnel going to high-risk areas in Latin America, Africa and Asia. This study is part of an effort to fill this void by developing and advancing bovine serum immunoglobulins (BSIgG), targeting fimbriae (and their respective fimbrial tip adhesins) an investigational modality that has shown proof of principle as a safe, food-based anti-diarrheal supplement when constituted from bovine derived hyper-immunized serum targeting protective ETEC antigens.

In an effort to develop a product that will abrogate the effects of enterotoxigenic *E. coli* (ETEC)-mediated diarrhea in military and civilian traveler populations, investigators at the Naval Medical Research Center (NMRC), Silver Spring MD had spearheaded a development program funded by the Peer-Reviewed Medical Research Program, and the U.S. Army Medical Material Development Activity. The objective of this program was to develop a passive oral immunoprophylaxis product composed of anti-adhesive hyperimmune BSIgG specific for the most prevalent class of ETEC colonization factors that would confer protection against ETEC diarrhea. The proposed clinical studies within this program will be conducted at the CIR, Johns Hopkins Bloomberg School of Public Health (JHBSPH).

### 1.1.1 Diarrhea in the Military

A unique subset of vulnerable travellers is the military. Military associated diarrheal illness (essentially TD occurring in deployed military) has consistently been reported in deployed military personnel and remains the leading cause of disease non-battle injury (DNBI) accounting for a significant reduction in operational readiness, and mission capability [29] particularly for deployments to the developing world. Among military personnel mortality has decreased (compared to historical controls), however, there remains significant morbidity, and a clear impact on operational readiness [30]. For historical perspective, data suggests that during the U.S. Civil War, 21,000 military deaths were attributable directly to dysentery. During the Korean War, approximately 80,000 duty-days were lost due to diarrhea and dysentery. During the Vietnam War, hospital admission rates or confinement to quarters due to diarrheal illness was higher than malaria by a 4:1 ratio, making diarrhea the most burdensome disease of that conflict [31]. Up to 70% of deployed U.S. personnel in support of Operations Enduring Freedom and Iraqi Freedom reported diarrheal episodes and 30% had three or more episodes with some units experiencing a monthly incident rate of up to 60% [32, 33]. Forty percent of UK forces in Afghanistan suffered at least one episode of diarrhea during their tour contributing to significant operational impact [34] with up to 43,000 man-days lost to 'no duty' or 'reduced performance' during the six months between April and October 2009 [34]. UK military data from Kenya has shown up to a 60% attack rate over a 6 week exercising period [34]. Diarrheal disease continues to be of significant military relevance as large numbers of young service members are deployed to areas with high TD rates [29]. From a military public health standpoint, its acute impact on troop health is larger than any other infectious disease syndrome and is compounded by the chronic risk of significant post-infectious sequelae [33, 35-37]. The most cost-effective response to this military readiness threat is to prevent the exposure leading to diarrhea. The military has developed extensive capabilities for the provision of sanitation and hygiene, and clean food and water. This strategy is reasonably effective when it is possible to develop the proper infrastructure, but it is often undermined during rapid deployments and during small scale and brief operations. In large scale deployments conducted under strict security measures that prohibit routine exposure to indigenous food and water, diarrhea remains a serious problem. During the joint multinational military exercise conducted in Egypt (Operation Bright Star '01) under stringent security conditions, 9% of personnel reported developing diarrhea [37]. Controlling the base area infrastructure may be possible but patrolling patterns in high-risk areas often involves exposure to local pathogens.

Therefore, development of countermeasures including a safe and effective vaccine is needed to reduce the impact of ETEC disease on deployed military personnel and has been deemed a high priority by the U.S. military, as ETEC diarrhea has the potential to curtail critical overseas missions.

### 1.1.2 Pathogenicity of ETEC

The pathogenesis of ETEC diarrhea involves the sequential steps of colonization (via colonization factors (CF) promoting intestinal adherence) followed by secretogenic toxin production. CFs are surface-exposed polymeric protein appendages that are vital to ETEC pathogenesis. Colonization ensues via the proteinaceous adhesive fimbrial surface-exposed polymeric protein appendages (the CF) potentiating

microorganism attachment to the human intestinal epithelial cell contributing to infectivity and pathogenicity (interfering with intestinal physiology including motility) [38]. Upon colonization, ETEC secretes one or both of two enterotoxins that induce fluid and electrolyte secretion (by differing pathways) resulting in watery diarrhea. The two enterotoxins produced by ETEC are heat-stable enterotoxin (ST) and heat-labile enterotoxin (LT).

### 1.1.3 ETEC Colonization Factors

To date, more than two dozen serologically distinct CFs associated with ETEC and culpable in human disease have been identified. The majority of CFs are fimbrial structures (bacterial surface appendages) composed of major and minor protein subunits, and some of these CF types are more prevalent in disease-associated ETEC than others. Based on sequence analysis, ETEC CFs of relevance to human disease can be divided into 7 genetically distinct types, and more than a third of these (8) have been grouped as Class 5 fimbriae. Many of the most prevalent ETEC CFs fall into either the α- or γ3-clade [39].

CS3 and CS6 are both atypical fimbriae in the fimbrial usher proteins (FUP) γ3-clade, each with two major subunits and no tip-localized adhesin, and no sequence similarities between the CS3 and CS6 major subunits. Based on meta-analyses of all available reports on ETEC CF prevalence and distribution, very conservative estimates indicate that ETEC Class 5 fimbriae in the FUP α-clade along with CS3 and CS6 (γ3-clade) are expressed by at least 70% of ETEC causing human disease [40]. The most commonly detected CFs of the α- and γ3-clade are CFA/I and CS6, respectively, which account for ~26% of all ETEC in travelers based on current evidence [40] (Figure 1).

**Figure 1. Conservative, Cumulative Estimates of Expected Global Coverage for an ETEC Vaccine or Immunoprophylactic**


CFA/IV includes the structurally indistinct CS6 expressed alone or with CS4 or CS5. While human challenge studies with ETEC strains expressing CFA/I, CFA/II, CFA/IV as well as other CFs have demonstrated disease and induced CF-specific immune responses, the role of each CF in disease pathogenesis is less clear and rests largely on epidemiologic data showing their relative prevalence, distribution, and in some cases association with disease in case-control comparisons [41].

### 1.1.4 Class 5 Tip Adhesins

The tip-adhesin-based approach to ETEC prophylaxis is designed to grapple with the issue of multivalency using the epidemiologically important group of ETEC expressing Class 5 fimbriae. Tip adhesion antibodies may mediate homologous and heterologous protection (cross reactivity) to Class 5 subclass fimbriae. For example, anti-CfaE antibodies may confer passive protection against ETEC that express any of the 3 subclass 5a fimbriae (i.e., CFA/I, CS4, and CS14). Thus, a multivalent vaccine containing three CF tip-adhesins could protect against a total of 8 CF types in class 5, thus greatly reducing the valency needed for an effective ETEC vaccine. A final bovine IgG (BIgG) product with Class 5 fimbriae-wide protective coverage may afford clinically significant protection against ETEC diarrhea in real-life travel settings.

### 1.1.5 Optimizing Protective Efficacy Exploiting Passive Oral Prophylaxis with BIgG Raised Against Tip Adhesins

If antibodies to the fimbrial tip adhesins are responsible for inhibiting ETEC binding, then the drawback with administering CFA/I to cows to raise a BIgG product is that the tip adhesion accounts for only a small portion of the immunizing antigen, and the majority of antibodies are raised to the major subunit instead of the tip adhesion. If the formulation and dose of the product is not optimal and the degradation in the harsh environment of the stomach is high, the low concentrations of the important anti-CfaE antibodies may be reduced to below a crucial level for protection. One way to address this problem would be to immunize the cows with CfaE only, and to administer BIgG significantly enriched for antibodies to CfaE. A second and equally compelling potential advantage to using CfaE as the immunizing antigen would be that anti-CfaE antibodies would be more effective than antibodies to CFA/I in the inhibition of colonization against ETEC strains that express CFs other than CFA/I (i.e., tip adhesion antibodies potentiate superior heterologous cross-protection to that of the whole CF fimbriae or its stalk). If fewer bovine antigens are needed to produce a product with broadened coverage against ETEC, it would make such a multivalent product more practical and cost-efficient. A prior clinical study (NCT00435526) has compared the efficacy of anti-CfaE BIgG to that of anti-CFA/I BIgG as part of a larger product development plan to develop a multivalent BIgG anti-ETEC product finding them to be comparable thus supporting the assertion that a multivalent product is feasible.

### 1.1.6 Evidence for anti-CF Immunity

CFs are surface-exposed polymeric protein appendages that are vital to ETEC pathogenesis. Accumulated evidence suggests that protective immunity to ETEC may occur, attributable in part to B-cell responses against the surface-exposed CFs and labile toxin (LT) enterotoxin most probably in the gut mucosal compartment [42, 43]. In endemically exposed populations, there is an inverse relationship between age and ETEC disease incidence, consistent with the notion that protective immunity develops from repeated exposure to infection [19, 20, 44].

The role of CFs and LT enterotoxins as protective antigens has been substantiated by a number of studies in populations naturally exposed to ETEC diarrhea as well as volunteer studies of experimentally induced diarrhea [42, 45-48]. Additionally, passive administration of bovine milk antibodies with activity against ETEC [expressing the CFs (CFA/I, CS17) and the respective tip-localized adhesins of CFA (CfaE)], has been shown to confer significant protection against diarrhea in controlled human challenge trials [49-54].

Based on this dual proof-of-principle indicating that two different α-clade ETEC fimbrial CFs are protective antigens as measured by passive protection conferred by anti-fimbrial BIgG, it is reasonable to extrapolate that bovine immunization with a cocktail of α-fimbriae selected based on epidemiological

prevalence and cross-reactivities could be produced to cover a substantial proportion of naturally occurring, pathogenic ETEC.

### 1.1.7 Understanding and Knowledge Gaps of CS6 as a Virulence Factor and Protective Antigen

Despite the robust evidence supporting the class 5 CFs as protective antigens, there is a dearth of evidence on other CF types, such as CS6. Since its first description in 1985 [55], CS6 has been the focus of considerable research, yet the generation of incontrovertible evidence as to its specific role in diarrhea pathogenesis and the role of antibodies towards it in protection against infection is lacking. One clear and consistent finding is that CS6, expressed most often alone but also with CS5 or CS4, is one of the most common CFs associated with symptomatic ETEC infection in both endemically exposed populations, as well as travelers [40]. This has driven the focus on CS6 as a target for many groups working in vaccine development [23, 56-59]. The crystal structure of the two CS6 major subunits have been solved [60], yet we have not defined its ultrastructural appearance on the bacterial surface [61] and have a limited understanding as to how CS6 might interact with the human intestinal surface as a CF [62-65].

The majority of individuals naturally infected with CS6-expressing ETEC exhibited mucosal and serological responses against CS6 [66] as well as CS6-specific B-cell memory responses [67], while naive subjects experimentally infected with CS6-expressing ETEC showed less robust mucosal and serological responses [68]. In limited investigations, however, serum anti-CS6 antibody titers did not show a protective relationship for subsequent CS6-expresssing ETEC diarrhea [69]. While these findings indicate that CS6 is recognized by the host during infection, we have little understanding regarding bacterial regulation of CS6 expression *in vitro* or *in vivo* [70]. Considering our current body of knowledge, the epidemiological importance of CS6 stands in sharp contrast with the absence of consistent, credible proof that CS6 serves as a virulent antigen--proof that is urgently needed to facilitate advances in vaccine development. The overarching goal of this study is to begin assessing the protective capacity of CS6 in passive prophylaxis studies by making use of the experimental human challenge model for ETEC strain B7A, which expresses this epidemiologically important CF. The study proposed herein would serve the dual purpose of expanding the scientific basis for development of a multivalent anti-ETEC BIgG product while also putting a proven model to use in directly defining the role of CS6 as a virulent antigen.

### 1.1.8 B7A Challenge Strain: CS6-expressing, O148:H28 (LT+/ST+)

The one CS6-expressing ETEC strain that has been established as causing diarrhea in volunteer challenge studies is B7A, which expresses CS6, both LT and ST toxins, and is serotype O148:H28 [68, 71-74]. The B7A strain was originally isolated from a US military adult serving in Vietnam suffering from acute diarrhea. In the most recent volunteer challenge study for which the inoculum was prepared from a cell bank produced under current Good Manufacturing Practices (cGMP) conditions at the Walter Reed Army Institute of Research (WRAIR), this strain induced moderate-severe diarrhea attack rates of 37.5 and 100% at doses of $10^9$ and $10^{10}$ colony forming units (cfu) respectively. One concern regarding this and other ETEC challenge models is the high dose of inoculum required to induce high enough attack rates to facilitate evaluation of a vaccine in reasonable numbers of subjects. A B7A inoculum of $10^{10}$ cfu may not be reflective of the average inoculum in naturally acquired infection and may in turn skew efficacy results towards the null in an assessment of a protective intervention. In addressing this concern with another ETEC challenge strain, H10407 (CFA/I-expressing ETEC), a refinement of the model was instituted whereby implementation of an overnight fast (in place of the typical 90 minute fast before challenge) resulted in reproducible attack rates among subjects with inoculum doses 2 logs below previously required doses [75]. Prior to executing the investigation, under NMRC.2015.0007 we optimized the experimental infection model with B7A to identify the optimal fasting duration and dose required to achieve sufficient moderate-severe diarrhea attack rates.

### 1.1.9 History of the ETEC Human Challenge Model

In a challenge model, a well characterized strain that has been associated with diarrhea and related gastrointestinal symptoms but is not resistant to antibiotics is selected. The strain is fed to inpatient subjects under supervision in a hospital at a dose that induces diarrhea. Once illness is induced the subject is treated with antibiotics, which has been universally effective in curing the infection in 1 to 2 days. In the last four decades hundreds of subjects have participated in these challenge studies. There have been no deaths or long term side effects associated with these studies. Dehydration is the most serious potential side-effect, and that is routinely treated with proper oral rehydration therapy, and the occasional need for intravenous rehydration. Models for a number of different bacterial enteropathogens have been developed, including types of diarrheagenic *E. coli* [72, 76, 77], *Shigella* [78-80], *Vibrio's* [81-83], and *Campylobacter* [84].

Over the past 40 years, the enterotoxigenic *E. coli* (ETEC) human challenge model has been used to elucidate the pathogenesis and immune responses associated with ETEC infection as well as to test the safety and efficacy of ETEC specific investigational drugs and vaccines. The initial experimental infection, published in 1971, was a landmark study establishing ETEC as the organism responsible for causing acute, cholera-like illness in a U.S. soldier in Vietnam [72]. In this classic paper, researchers demonstrated that while porcine and human isolates of disease-causing *E. coli* were both capable of inducing fluid excretion in rabbit ileal loops, only human isolates were capable of causing disease in human subjects. It was later discovered that the difference in the two strains was the species-specific tropism of the intestinal CF fimbriae. One of the strains in that original study was B7A, a CS6-expressing, LT+, ST+ strain of ETEC.

Since that landmark study, over 700 naïve subjects have been administered ETEC in an experimental infection. The majority of experience with ETEC strains is with a handful of challenge strains, including the B7A strain. In all prior studies, there have been no 'related' serious adverse events and all 'related' adverse events have been consistent with the acute diarrheal illness (with associated signs and symptoms) anticipated from an experimental infection with ETEC. At least in one prior study, it was observed that initial experimental infection with the B7A strain protected subjects against re-challenge with the same organism approximately 9 weeks later [43]. The B7A strain is sensitive to most commonly used antibiotics, including ciprofloxacin, and is readily cleared following a routine 3-day course of antibiotics.

### 1.1.10 Active and Passive Immunoprophylactic Approaches

There are a number of approaches currently being taken to develop an active ETEC vaccine, including killed whole-cell, live attenuated, and adhesin based, protein subunit vaccine strategies [85-88]. While an increasingly robust effort has been mounted to develop an active ETEC vaccine over the past decade, none has yet to achieve licensure in the U.S., and the prospects for achievement of this goal in the next decade remains uncertain.

A modality that has shown some success in the prevention of diarrhea is passive, oral administration of bovine milk IgG with specific activity against viral, bacterial and parasitic enteropathogens. As ETEC infects the small intestine, however, the protective antibodies in colostrum must traverse the acid environment of the stomach intact and reach their site of action in adequate concentrations. For this reason, the effectiveness of passive immunization with bovine antibodies requires the ingestion of large quantities of antibodies or the co-administration of buffering agents, which paradoxically may increase susceptibility to various infections by reducing the efficacy of the gastric acid barrier. Evidence for passive administration of bovine colostral antibodies in prophylaxis and treatment against a host of enteropathogens is discussed in section 1.3. As expected for a food-based product, the safety record of BIgG preparations is excellent [89, 90]. While there is no anti-diarrheal BIgG product yet licensed in the

U.S., there are comparable veterinary products on the market indicated for protection of newborn farm animals against ETEC diarrhea [89]. Additionally, some evidence of protection was observed in randomized trials involving bovine colostrum extract from cows immunized against 14 ETEC strains, which is now commercially available in the U.S. as a dietary supplement (Travelan®) to prevent ETEC-attributable travelers' diarrhea [51].

### 1.1.11 Preclinical Model for Passive Prophylaxis

There are no preclinical models in which the efficacy of passive prophylaxis for enterotoxigenic *Escherichia coli* (ETEC) can be assessed. While there has been an effort to develop the *Aotus nancymaae* model, modifying the model to enable passive oral prophylaxis before, during, and after challenge has proved difficult (S Savarino, personal communication). The human challenge model has been (and remains) the most suitable model to assess the efficacy of active and passive prophylaxis [91].

### 1.1.12 Summary

Given the limited success of bovine milk IgG products, passive oral administration of BSIgG may protect against ETEC-mediated infectious diarrhea. The hypothesized mechanism of protection stems from the passive administration of bovine anti-(tip adhesin or fimbriae) antibodies preventing their adherence in the human small intestine (the initial step in pathogenesis), thereby preventing downstream pathogenic processes and symptomatic illness. Advances in technology and shifts in industry focus have led to a transition from the manufacturing of bovine colostral to serum antibody products for human use. One potential goal is the development of a safe and efficacious commercially viable multi-valent anti-diarrheal BSIgG supplement [encompassing a multivalent cocktail of representative anti- fimbriae (and tip adhesin) antibodies] conferring broad protection against both homologous and heterologous populations of ETEC pathogens. The study described herein will establish the foundation for evaluating BSIgG products against numerous ETEC CFs.

Based on conclusive evidence that BIgG preparations against Class 5 fimbriae confer 90-100% protective efficacy against CF-homologous ETEC challenge, the critical next step is to demonstrate the passive protective efficacy of an anti-CS6 preparation in human subjects. This clinical investigation is designed to specifically identify 1) if the BSIgG products are safe and well tolerated and 2) if passive administration of bovine serum derived antibodies raised against CS6 and/or the CS6 expressing whole cell B7A ETEC protect against moderate-severe diarrhea following experimental infection with the CS6 expressing B7A ETEC challenge. With proof-of-principle that CS6 can serve as a protective antigen, a multivalent BIgG product could be conceived for broad coverage against ETEC expressing the most common CFs.

## 1.2 Rationale

Given the diversity of fimbrial types collectively expressed by ETEC disease isolates, one barrier to development of an affordable, effective BSIgG product for prevention of travelers' diarrhea due to ETEC is the potentially large number of antigens needed to produce a multivalent preparation that confers broad protection. Since Class 5 fimbrial CFs are collectively expressed by as many as two-thirds of ETEC diarrhea case isolates in some areas [92], class-wide coverage by a multivalent BSIgG product would be expected to have a clinically significant impact. If anti-adhesin-based BSIgG preparations are proven to be broadly protective, we postulate that a trivalent product containing BIgG with specificity for one representative adhesin from each of the three subclasses (i.e., anti-CfaE [5a], anti-CsbD [5b], and anti-CotD [CS2 fimbrial adhesin, 5c] would be such a product, conferring class-wide protective efficacy [49].

The purpose of this study is to determine if anti-CS6 BSIgG confers protection against oral challenge with B7A. Hyperimmune anti-CS6 BSIgG will be tested in parallel to hyperimmune anti-B7A whole cell killed BSIgG to demonstrate the homologous protective effects of anti-CF BSIgG while corroborating the importance of CS6 as a protective immunogen. We hypothesize that anti-CS6 BSIgG will confer protection against B7A mediated moderate to severe diarrhea upon challenge.

## 1.3 Previous Human Experience with BIgG Products

Concentrates of immunoglobulin from bovine milk or colostrum have been evaluated in several human clinical trials in hundreds of subjects as outlined in Table 2., and these products have been very well tolerated. The products have been investigated as both a prophylactic and treatment for infectious diseases caused by organisms like ETEC or EPEC [50, 51, 93-96] (Savarino, unpublished), Rotavirus [97-100], *Shigella* [53], *Cholera* [101], *Cryptosporidium parvum* [102-105], *Clostridium difficile* [106, 107] and *Helicobacter pylori* [108]. Depending on the target disease and population, the products have been tested for safety and efficacy in healthy adults, immuno-compromised adults and children, and healthy children or children hospitalized with diarrhea.

**Table 2. A summary of a Range of Clinical Trials Evaluating the Safety and Efficacy of BIgG Concentrates in Human Subjects[a]**

| Reference | Population and type of study | Cow vaccine | Daily dose, duration of treatment and controls used | # subjects | Results of safety and efficacy trials |
|---|---|---|---|---|---|
| BIgG anti-*E.coli* | | | | | |
| Otto et al (2011) | Healthy adults | 14 ETEC strains (including serogroup O78) | 400 mg BIgG taken thrice daily with bicarbonate buffer | 90 | 90% protective efficacy against diarrhea. No difference when formulated with/without buffer. Of note, 200 mg BIgG conferred 58% efficacy compared to placebo. |
| Tawfeek et al (2003) | Healthy infants at a Child Health Center, Iraq, prophylactic field study | EPEC (5 or 1 serotype) | 0.5g BIgG/ kg body weight (supplement milk formula), 7 days.<br>Control: milk formula only | 65 | Safe and well tolerated. Polyvalent BIgG reduced diarrhea, monovalent BIgG no effect |
| Casswall et al (2000) | Children, hospitalized with E. coli diarrhea, Bangladesh. Therapeutic study | ETEC (14 serotypes) or EPEC (15 serotypes) | 20g daily (5g x 4 doses), 4 days.<br>Control: BIgG from non-immunized cows | 32 | Safe and well tolerated. No therapeutic effect |
| Tacket et al (1999) | Healthy adults. Inpatient prophylaxis/challenge study | CFA/I, CS3, CS6 | 2.07g (0.69g x 3 doses), 5 days.<br>Control: BIgG from non-immunized cows | 10 | No report of side effects in publication. No efficacy. |
| Savarino et al (unpublished) | Healthy US soldiers deployed in Egypt | CFA/I | 1:1:1 enrollment to AEMI, 2.07g (0.69g x3 doses), uncoated granules or 50/50 mix of coated and uncoated granules or control. 10 days intake | 200 | No difference in protective efficacy between control and interventional groups |
| Freedman et al (1998) | Healthy adults. Inpatient prophylaxis/challenge study | CFA/I | 5.1g (1.7g x3 doses) or 1.3g (0.43g x 3 doses), 7 days.<br>Control: LactoFree infant formula | 15 | Safe and well tolerated. Protection from diarrhea. |
| Tacket et al (1988) | Healthy adults. Inpatient prophylaxis/challenge study. | ETEC (14 serotypes), CT, LT. | 10.65g (3.55g x3), for 7 days<br>Control: BIgG anti-rotavirus | 10 | Generally well tolerated[b0]. Protection from diarrhea. |
| Mietens et al (1979)[b] | Infants hospitalized with EPEC diarrhea, Germany. Therapeutic study. | EPEC (14 serotypes). | 1g/kg body weight (distributed over meals), 10 days.<br>Control: children with EPEC of a serotype not present in cow vaccine treated with BIgG | 60 | No report of side effects in publication. Better clearance of infection. |
| BIgG anti-Rotavirus | | | | | |
| Sarker et al (1998) | Infants hospitalized with rotavirus diarrhea, Bangladesh. Therapeutic study. | Rotavirus (4 serotypes). | 10g (in 4 doses), 4 days<br>Control: milk powder | 40 | Safe and well-tolerated. Reduced diarrhea. |
| Mitra et al (1995) | Infants hospitalized with rotavirus diarrhea, Bangladesh. Therapeutic study. | Rotavirus (4 serotypes). | 10g (in 3 doses), 3 days<br>Control: normal colostrum | 35 | Safe and well-tolerated. Reduced diarrhea duration and output. |

| | | | | | |
|---|---|---|---|---|---|
| Brunser et al (1992) | Children in Chile. Prophylactic field trial. | Rotavirus + *E. coli*. | 1g BIgG product (supplementing milk formula), 6 months. Control: milk formula alone | 117 | Safe and well-tolerated<br>No efficacy. |
| Davidson et al (1989) | Children hospitalized (not for diarrhea) in Australia. Prophylactic study. | Rotavirus (4 serotypes). | 50mL (concentration not reported), 10 days Control: infant formula | 55 | No report of side effects in publication. Prevented acquisition of symptomatic rotavirus infection. |
| Hilpert et al (1987) | Children hospitalized with rotavirus infection, Germany. Prophylactic study. | Rotavirus (4 serotypes), 3 products with varying titers. | 2g/kg/day for a winter season. Control: No treatment | 75 | No report of side effects in publication.<br>High-titer BIgG reduced excretion of virus and duration of diarrhea (latter non-significantly). Low-titer BIgG no efficacy. |
| Ebina et al (1985)[e] | Children hospitalized with rotavirus infection, Japan. Therapeutic study. | Rotavirus (1 serotype). | 20-50mL (concentration not reported, 3 days. Control: no BIgG treatment | 18 | Safe and well tolerated. No therapeutic effect. |
| Ebina et al (1985)[e] | Children in an orphanage in Japan. Prophylactic study (Rotavirus outbreak during the treatment phase) | Rotavirus (1 serotype). | 20mL (concentration not reported, approx 5 weeks. Control: market milk | 6 | Safe and well tolerated. Reduced acquisition of rotavirus infection from outbreak. |
| BIgG anti- *Cryptosporidium parvum* | | | | | |
| Okhuysen et al (1998) BB-IND-4122 | Healthy adults. Inpatient prophylaxis/challenge study. | *C. parvum*. | 30 g (10g x3 doses), 5 days. Control: nonfat powdered milk | 10 | Safe and well tolerated. No reduction in infection or diarrhea. |
| Greenberg & Cello (1996)[c] | AIDS patient with chronic diarrhea from *C. parvum*. Case report. | *C. parvum*. | 40g daily (10g x4 doses), 21 days | 23 | Generally safe and well tolerated. Reduction in diarrhea if product in powder form, not in capsule form. |
| Ungar et al (1990)[c] | AIDS patient with chronic diarrhea from *C. parvum*. Case report. | *C. parvum*. | Infusion by nasoduodenal tube, 20cm3/h for 60h, concentration not reported. | 1 | No report of side effects in publication. Reduced diarrhea. |
| Nord et al (1990)[c] | AIDS patients with diarrhea from *C. parvum*. Case report. | *C. parvum*. | 14g by infusion via nasogastric tube for 10 days. | 3 | No report of side effects in publication. Reduced stool output and oocyst excretion in 1/3 patients. 2/2 control patients (control colostrum) reduced stool output but not excretion. |
| Tzipori et al (1987)[c] | Immuno-deficient patients with diarrhea from *C. parvum*. Case report. | *C. parvum*. | 200-500mL by nasogastric tube for 10-21 days, concentration not reported | 3 | No report of side effects in publication. Resolved diarrhea in 3/3, resolved infection in 1/3. |
| BIgG anti-*Clostridium difficile* | | | | | |
| Warny et al (1999)[c] | Adults with end ileostomys, transit study. | *C. difficile* toxoid (A). | 5g on 4 separate occasions. | 6 | No adverse experiences. Not an efficacy study. |
| Kelly et al (1997)[c] | Healthy adults, transit study. | *C. difficile* toxoid (A and B). | 45g or 8g, one dose only. | 10 | No report of side effects in publication. Not an efficacy study. |
| BIgG anti-*Shigella flexneri* | | | | | |
| Tacket et al (1992) | Healthy adults. Inpatient prophylaxis/challenge study. | *Shigella flexneri*. | 30g (10g x3 doses), 7 days. Control: sodium bicarbonate alone | 10 | Safe and well-tolerated. Protection from diarrhea. |

# 2.0 OBJECTIVES

## 2.1 Primary Objectives

The primary objectives of this study are to assess the safety of serum-derived bovine immunoglobulins in healthy adult subjects when orally administered three times a day over 7 days and to estimate protective efficacy of those preparations against moderate-severe diarrhea upon challenge with B7A.

The primary endpoint for this study is moderate-severe diarrhea defined as follows post-inoculation:

- Moderate diarrhea: 4 to 5 loose/liquid stools or 401-800 g of loose/liquid stool in any 24-hour period
- Severe diarrhea: ≥ 6 loose/liquid stools or > 800 g of loose/liquid stool in any 24-hour period

## 2.2 Secondary Objectives

The secondary objectives include assessments of a variety of clinical endpoints, measuring mucosal and systemic immune responses and obtaining and archiving samples for future proteomics and/or systems biology efforts.

1. Measure mucosal and systemic immune responses to experimental infection
2. Obtain and archive samples for future proteomics, microbiome and/or systems biology efforts

A number of secondary endpoints will be determined in this study. Specific endpoints have been selected to support the primary outcome and are outlined below.

1. Maximum 24-hour stool output
2. Percent of subjects with severe diarrhea
3. Percent of subjects with diarrhea of any severity
4. Total weight of grade 3-5 stools passed per subject over 120-hour period
5. Number of grade 3-5 stools per subject
6. Percent of subjects with nausea, vomiting, anorexia, or abdominal pain/cramps rated as moderate to severe
7. Mean/Median time to onset of diarrhea
8. Number of subjects with moderate to severe ETEC illness
9. Number of cfu of the challenge strain per gram of stool 2 and 4 days after challenge
10. ETEC clinical severity score post-challenge

## 2.3 Exploratory Objectives

1. Exploratory immunology and systems biology analyses to include transcriptomics, proteomics, phosphoproteomics, cytokine secretion measurements, lymphocyte subpopulation characterizations, and antigen-specific memory B cell quantification.
2. Exploratory evaluation of the cognitive impact of acute diarrhea using psychomotor vigilance testing.
3. Evaluate the impact of both the B7A ETEC challenge and antibiotic exposure on short-term changes in host microbiota.
4. Explore the impact of the microbiome on disease susceptibility.
5. Evaluate the impact of the B7A ETEC challenge on short-term changes in intestinal inflammation/repair, epithelial barrier function, motility, and immune system modulation.

## 3.0 STUDY DESIGN

This is a Phase 1, randomized, double-blind, placebo-controlled study designed to investigate whether anti-CS6 BSIgG protects subjects against diarrhea upon challenge with a CS6-expressing ETEC strain B7A, compared to the protective efficacy of the positive control (anti-B7A BSIgG) and negative control (non-hyperimmune) BSIgG. The anti-B7A BSIgG will serve as a positive control and will not be directly compared to the anti-CS6 BSIgG. The study will also evaluate the safety and tolerability of these BSIgG products and describe the immune responses following challenge. The basic study design is depicted in Table 3.

**Table 3. Description of Study Groups**

| Product[a] | N | Dose (approximate) |
|---|---|---|
| Anti-CS6 BSIgG | 20 | 1.0g three times daily (tid) |
| Anti-B7A whole cell killed BSIgG | 20 | 1.0 g tid |
| Negative Control (Non-hyperimmune) BSIgG | 20 | 1.1 g protein total (equivalent) tid |

[a] All products will be given 3 times daily

Subjects (N=60) will be randomized into three groups receiving anti-CS6 BSIgG, anti-B7A whole cell killed BSIgG, or a placebo control (non hyperimmune BSIgG). Subjects will receive three doses a day of the test article 15 minutes (range 10 - 25 minutes) after each of their three daily meals (breakfast, lunch and dinner) for a period of 7 days (i.e., from study day -2 to study day 4). The study will be divided into two cohorts of approximately 30 subjects each. The target number of subjects to be challenged with ETEC is 60.

Unit doses of the test article (as detailed in Table 3) will be administered according to the relevant SSP. Doses of the test articles/placebo will be prepared by the research pharmacist, and will start on study day -2. On study day 0, after receipt of test article/placebo, subjects will be given 120mL of sodium bicarbonate buffer to neutralize their stomach acidity. About 1 minute later they will ingest approximately 1 x $10^{10}$ cfu of CS6 expressing B7A-ETEC strain diluted in 30 ml sodium bicarbonate buffer. Subjects will continue to receive three doses a day of the test articles/placebo until study day 4. Subjects meeting pre-determined criteria for early antibiotic administration will be treated with antibiotics and test article/placebo administration will be discontinued with initiation of treatment. Subjects who do not receive early antibiotic treatment will start antibiotic treatment on study day 5. Routine discharge is scheduled for day 8, when most subjects are expected to meet the discharge criteria of: they feel well (clinical symptoms resolved or resolving) and have taken at least two doses of antibiotic and have 2 consecutive stool culture negative for the challenge strain. Subjects may be discharged earlier than day 8 on a case-by-case basis if they meet discharge criteria. For subjects who do not meet the discharge criteria on day 8 will remain on the unit until discharge criteria has been met.

The duration of the active study period is approximately eleven months, encompassing up to 90 days of screening/enrollment, 4 weeks of the inpatient/outpatient phase when data and samples will be collected, 12 weeks for immunology assays, and 2 months for analysis and report. Additionally, subjects will be contacted 6 months after challenge to see if they are still well and to complete the functional bowel disorder survey.

## 4.0 STUDY POPULATION

Subjects will be recruited from the Baltimore-Washington and surrounding areas via advertisements and word of mouth and screened at the CIR. They will be healthy male and non-pregnant females, aged 18 to 50 inclusive. A sufficient number will be screened to provide 20 subjects in each of the three groups, with a

target of about 60 subjects enrolled. Up to 6 alternates per cohort will be recruited to replace anyone who does not report or is unable to participate at the time of planned admission. Two of these alternates may be admitted on Day-3 and stay overnight until the first dose of IP is given on Day -2 to replace subjects who become ineligible for continuation in the study on Day -2. Alternates will not be randomized unless they are replacing a study subject unable to participate in the study (prior to the first dose of test article/placebo). Alternates not replacing a subject will be discharged on Day-2 prior to IP receipt.

## 4.1 Subject Inclusion Criteria

Inclusion Criteria:

1. Male or female between 18 and 50 years of age, inclusive.
2. General good health, without significant medical illness, abnormal physical examination findings or clinical laboratory abnormalities as determined by principal investigator (PI) or PI in consultation with the research monitor and sponsor.
3. Demonstrate comprehension of the protocol procedures and knowledge of ETEC illness by passing a written examination (pass grade ≥ 70%)
4. Willing to participate after informed consent obtained.
5. Available for all planned follow-up visits.
6. Negative serum pregnancy test at screening and negative serum and/or urine pregnancy tests on the day of admittance to the inpatient phase for female subjects of childbearing potential. Females of childbearing potential must agree to use an efficacious hormonal or barrier method of birth control during the study. Abstinence is acceptable. Female subjects unable to bear children must have this documented (e.g., tubal ligation or hysterectomy).

## 4.2 Subject Exclusion Criteria

Exclusion Criteria:

General health criteria

1. Presence of a significant medical condition, (e.g. psychiatric conditions or gastrointestinal disease, such as peptic ulcer, symptoms or evidence of active gastritis or gastroesophageal reflux disease, inflammatory bowel disease, alcohol or illicit drug abuse/dependency), or other laboratory abnormalities which in the opinion of the investigator precludes participation in the study.
2. Immunosuppressive illness or IgA deficiency (serum IgA < 7 mg/dL or below the limit of detection of assay )
3. Evidence of confirmed infection with HIV, HBsAg, or HCV, with confirmatory assay.
4. Use of any investigational product within 30 days preceding the receipt of the investigational products, or planned use during the active study period
5. Significant abnormalities in screening lab hematology or serum chemistries, as determined by PI or PI in consultation with the research monitor and sponsor.
6. Lactation or breastfeeding.

Research-related exclusions applicable to challenge

7. History of microbiologically confirmed ETEC or cholera infection in last 3 years.
8. Occupation involving handling of ETEC or *Vibrio cholerae* currently, or in the past 3 years.
9. Travel to countries where ETEC or cholera infection is endemic (most of the developing world) within 3 years prior to dosing.

10. Symptoms consistent with Travelers' Diarrhea concurrent with travel to countries where ETEC infection is endemic (most of the developing world) within 3 years prior to dosing, OR planned travel to endemic countries during the length of the study.
11. Vaccination for or ingestion of ETEC, cholera, or E coli heat labile toxin within 3 years prior to dosing.
12. Any prior experimental infection with ETEC strain B7A.

Study-specific Exclusion Criteria (potential increased risk or complicating outcome ascertainment)

13. Abnormal stool pattern (fewer than 3 per week or more than 3 per day).
14. History of diarrhea in the 2 weeks prior to planned inpatient phase.
15. Regular use of laxatives, antacids, or other agents to lower stomach acidity (regular defined as at least weekly).
16. Use of antibiotics during the 7 days before receipt of any investigational product or proton pump inhibitors, $H_2$ blockers, or antacids within 48 hours of receipt of any investigational product.
17. Use of any medication known to affect the immune function (eg, systemic corticosteroids and others) within 30 days preceding the administration of challenge or planned use during the active study period.
18. Known allergy to fluoroquinolones.
19. Inability to tolerate 150 mL sodium bicarbonate buffer (based on requirement for frequent dosing).

# 5.0 STUDY PROCEDURES

## 5.1 Screening

The CIR may use a screening protocol approved by the Johns Hopkins School of Public Health (JHSPH) Institutional Review Board (IRB) in recruiting subjects for this study. The screening protocol is entitled "Screening of adult volunteers for eligibility to participate in clinical studies evaluating investigational vaccines, antimicrobial agents, or disease prevention measures or the pathogenesis of infectious agents" JHSPH IRB 200, JHSPH IRB H.22.04.02.19.A2. Subjects will be made aware that the screening process may take several visits to complete. Using this screening protocol, a medical history/exam and a series of clinical laboratory tests may be completed to rule out occult illness and pregnancy. These laboratory tests may include, but are not limited to complete blood count (CBC), serum chemistries, hepatitis B antigen, hepatitis C antibody, HIV-1 antibody, IgA levels, serum HCG (for females of childbearing potential), and urine toxicology (drug screening). (Confirmatory testing will be performed on subjects who test positive for hepatitis B, hepatitis C, or HIV-1 antigens.) Subjects who have ≤ 2 mild (grade 1) non-hematologic abnormalities may be included if the PI determines that their participation will not present undue risk to the subject. Subjects with > 2 mild abnormalities will not be included in the study. Subjects with clinical laboratory abnormalities of greater than mild severity will not participate in this clinical trial. The clinical toxicity grading scale that will be used as a guideline is based on the scale used by the Division of AIDS (DAIDS) for adverse events and the guidance from the US Food and Drug Administration (FDA) Center for Biologics Evaluation and Research. If any additional safety labs are performed, either scale may be utilized.

Potential subjects will be given a complete description of the study. To ensure comprehension of the study, all subjects will have to pass a written examination before inclusion in the study. Subjects who meet all inclusion criteria and none of the exclusion criteria, pass the comprehension test, and sign the study Informed Consent Document (ICD) may be eligible for the study.

Informed consent is an ongoing process which includes the informed consent document. Subjects will receive an oral presentation of the study. Each prospective subject will be given the written, IRB-approved informed consent, allowed ample time to read the consent, allowed to ask questions about the study, have his/her questions answered, and given time to decide if he/she would like to participate in the study. To document subjects' understanding of informed consent, immediately before the consent is signed, the person obtaining consent will administer a brief quiz or comprehension test. Incorrect answers will be discussed with subjects to reinforce the consent. A final acceptable test score is 70% or more answered correctly. Subjects who fail the comprehension test on the first attempt may retake the comprehension test on the same day, or they may come back on a separate visit to retake the test. Subjects failing after two attempts are not eligible for study enrollment. No coercion or influence is allowed in obtaining subjects' consent. Before subjects participate in the study, consent forms will be signed and dated by subjects as well as by the PI or designee. Subjects will receive copies of the signed consent prior to participation. As part of the consent process, subjects will also be asked to read and sign additional IRB approved forms including but not inclusive, a Medical Records/Lab Results Release, alternate information form, inpatient guidelines, with an opportunity to ask questions, if relevant.

Subjects will be asked to drink about 150 mL of sodium bicarbonate buffer to ensure tolerability for frequent dosing. Subjects will be asked to complete a Functional Bowel Disorder Survey (Rome III) to establish a baseline of general GI health for subsequent surveys (survey either taken by subjects or administered by study staff).

Additionally, samples for future ABO and RH blood typing may be collected following recent data suggesting correlation between ABO typing and susceptibility to moderate to severe diarrhea following challenge with ETEC strain H10407 (Fleckenstein, unpublished). RH typing has been previously reported to affect clinical outcomes in subjects infected with other enteric pathogens and as such similar associations will be assessed as part of this study [109-111].

## 5.2 Randomization

Subjects will be randomized in a 1:1:1 ratio to one of 3 treatment groups. An investigator at NMRC not involved in outcome assessment will prepare a randomization list, allocating volunteer identification numbers to the study groups using the PROC PLAN function of SAS v9.2 (Cary, NC). The randomization scheme will utilize block sizes of 6 in order to ensure comparable group sizes in the event that the targeted number of 60 subjects is not reached. NMRC staff will print the code, log and output of the SAS procedure, sign them, and store them under lock and key. A photocopy of the signed output will be provided to the research pharmacist prior to the first BSIgG administration day.

## 5.3 Group Assignment

Prior to the first dose of test article/placebo, subjects will be assigned a study number. Subjects will receive the test article/placebo in containers bearing their assigned identification numbers. This number will be linked to the randomization code list securely maintained throughout the clinical phase of the study by the designated NMRC staff and the JHU research pharmacist. Study identification numbers will identify all samples for laboratory analyses.

## 5.4 Blinding

Investigators and subjects will remain blinded as to group until completion of the clinical phase of the trial and validation of the clinical and immunological data. Each test article/placebo bottle is labeled with an open label as described in section 7.0. The research pharmacist will use the randomization list to prepare the IP. IP bottles will be sent to the research pharmacy prior to administration. All mixing and administration of the test

articles/placebo will be performed per formulation and product administration SSPs. Administration will occur in a separate room from where the doses are prepared.

Only in a medical emergency, when knowledge of the study treatment is essential for further management of subjects, will the randomization code be broken. In the event that this is necessary, the PI will provide the study identification number to the 24-hour pharmacy, who in turn will break the seal on that subject's envelope and provide the investigator with the broken code for that subject. The investigator will notify the Sponsor immediately and document the event on the appropriate Source Documents and electronic Case Report Forms (eCRF).

## 5.5 Clinical Evaluations

### 5.5.1 Monitoring During Inpatient Phase

Subjects will be monitored daily while inpatient for general, gastrointestinal, and systemic signs and symptoms, have medical conditions reviewed, and adverse effects noted. This will include examination by a study physician/nurse practitioner and solicitation of daily progress reports. Additionally, subjects will be examined for symptoms and signs of dehydration, including thirst, dizziness on standing, decreased skin turgor, and dryness of mucous membranes. Vital signs will be recorded three times daily, and more often when subjects are ill. If subjects develop moderate or severe diarrhea, postural blood pressure and pulse will be measured as necessary for clinical management according to the judgment of the physician/nurse practitioner.

Johns Hopkins Bayview Medical Center inpatient research facility is a self-contained unit, suited for conducting live-in studies.

Subjects will remain at the inpatient facility under clinical observation. Vital Signs will be assessed at least 3 times each day, once in the morning, in the afternoon and at bedtime. On challenge day, vital signs will be assessed 4 times, once prior to challenge, once about 30 minutes after challenge, and then 2 additional times this day. A clinician will conduct a daily medical interview and focused physical exam to assess health status, follow-up, monitor, and treat as indicated. All stools will be collected for weighing and grading. Following ETEC B7A challenge, up to 3 stool samples will be collected daily for culture as per SSP starting the day after challenge. If a subject is unable to provide a stool sample by 1300 hours, s/he will be asked to obtain a rectal swab. Swabs will be used starting the day after challenge.

Subjects will perform 5-minute psychomotor vigilance testing (PVT) at least three times a day during the inpatient phase. As an exploratory assessment, performance of the three PVTs per day will be predicated on the subject not undergoing other procedures or primary study related events. Missed PVTs will not be considered protocol deviations. Similarly, management of symptoms associated with ETEC or other illness will have priority over completion of PVTs.

Treatment for severe nausea or vomiting may be needed. Subjects who experience severe nausea or vomiting may be given ondansetron (Zofran) ODT or ondansetron IV.

#### 5.5.1.1 Rehydration Procedures

Subjects passing grade 3-5 stools post-challenge will be offered ORS or Gatorade to prevent dehydration, at the same volume as their stool output. For documentation purposes of concomitant medications, ORS will not be considered a concomitant medication while IV fluids will.

A subject may be administered IV fluids (clinician discretion) for the following reasons:

- Subject experiences abrupt onset of diarrhea defined by passage of an initial loose/liquid stool of > 300g or passage of > 400 g of loose/liquid stools over 2 hours.
- Subject becomes hypovolemic.
- It is determined necessary by the study physician, i.e., diarrhea with nausea/vomiting and unable to drink enough to keep up with output, or other reason.

Hypovolemia is a significant decrease in blood volume, characterized by:

- Orthostatic hypotension, confirmed systolic blood pressure (BP) < 90 mmHg and associated symptoms
- *Or* significant lightheadedness on standing with a confirmed postural change in BP or pulse. Postural vital signs will be measured lying and 2 minutes after standing. A significant change will be either of the following: decrease in systolic BP of > 20 mmHg, or diastolic BP of > 10 mmHg or increase in pulse of > 30 beats/min.

#### 5.5.1.2 Routine Discharge

Routine discharge is scheduled for study day 8. Two consecutive negative stool cultures for B7A are required before discharge (can be collected on the same study day). If the subject has not completed antibiotics, then the remaining doses of antibiotic will be given to the subject for self-administration. Vital signs will be collected.

#### 5.5.1.3 Early Discharge

Early discharge is permitted in cases where early antibiotic treatment has been initiated. The subject needs 2 consecutive stool cultures negative for B7A and to have taken two doses of antibiotic with resolved or resolving clinical symptoms before discharge. Remaining doses of antibiotic will be given to the subject for self-administration. Subjects discharged before study day 7 will return on day 7 and provide the requisite samples (stool, blood) as delineated in Table 1.

### 5.5.2 Monitoring During Outpatient Phase

On study day 28 (+/- 2 day), subjects will return to the clinic for a follow up visit as described in section 6.5. Some subjects may also be outpatients on day 7. In addition, subjects will also have a single phone follow-up on day 180 (+/- 1 month). Clinic visits during follow-up will include vital signs assessment, clinical checks, including concomitant medications and AEs, and sample collection for immunogenicity and exploratory outcome evaluation.

## 5.6 Concomitant Medications/Treatments

Only concomitant medications approved by the study physician will be used during the study. Subjects needing to take unapproved or excluded medication will not be eligible for enrollment in this study. As the subjects will stay in the inpatient facility after challenge until treatment, this should not be an issue. Subjects taking regular medication (i.e., birth control pills) prior to enrollment will be allowed to continue unless it is specifically excluded as part of the inclusion/exclusion criteria. Any medication ordered during the trial (i.e., Tylenol or ciprofloxacin or alternative antibiotics) will be documented in the subject's study chart and on the appropriate page of the eCRFs. Approved medications that were being taken prior to, as well as during the course of the trial will also be documented in this manner.

## 5.7 Laboratory Evaluations

### 5.7.1 Specimen Preparation, Handling and Shipping

Research microbiology, including the preparation of live inoculum and culturing of specimens, will be carried out in the laboratory of the CIR in the JHBSPH. Immunologic assays will be carried out at the Enteric Diseases Department at NMRC or the Core Lab of the JHBSPH. Samples collected under this protocol will be used to conduct protocol-related safety and immunogenicity evaluations. Samples for immunogenicity will be collected at the CIR and maintained at the CIR or core lab until transport to NMRC. Storage at NMRC of these biological samples will be handled according to appropriate procedures. Any study for the future use of these biological samples will have IRB approval. All subjects will consent for the future use of their specimens.

### 5.7.2 Clinical Laboratory Evaluations

Standard clinical laboratory tests for the purpose of inclusion and exclusion of potential subjects and for safety monitoring will be carried out at JHH, JH Bayview Medical Center, or Quest Diagnostics in Baltimore City. Microbiology tests will be done in the CIR bacteriology laboratory. Study related samples will be labeled according to the relevant SSP.

## 5.8 Outcome Measures

### 5.8.1 Clinical

The primary endpoint of this study is moderate to severe diarrhea according to the following definitions post-inoculation:

> Severe diarrhea: ≥ 6 grade 3-5 stools in 24 hours, or > 800 g of grade 3-5 stools in 24 hours and,
> Moderate diarrhea: 4-5 grade 3-5 stools in 24 hours or 401-800 g of loose/liquid stool in any 24-hour period

Stool will be graded based on a standard stool grading scale as follows:

Grade 1 = Fully formed (normal)
Grade 2 = Soft (normal)
Grade 3 = Thick liquid (diarrheal)
Grade 4 = Opaque watery (diarrheal)
Grade 5 = Rice-water (diarrheal)

Additional secondary endpoints have been selected as follows:

- Maximum 24-hour stool output
- Percent of subjects with severe diarrhea
- Percent of subjects with diarrhea of any severity
- Total weight of grade 3-5 stools passed per subject
- Number of grade 3-5 stools per subject
- Percent of subjects with nausea, vomiting, anorexia, or abdominal pain/cramps rated as moderate to severe
- Mean/median time to diarrhea onset
- Number of subjects with moderate to severe ‘ETEC illness’
- Number of cfu of the challenge strain per gram of stool 2 and 4 days after challenge

- ETEC systemic and diarrhea severity score post-challenge with B7A [112]

An exploratory assessment of the cognitive impact of ETEC challenge will be conducted with the use of PVT monitoring. The outcomes are exploratory in nature and will not be utilized as part of the regulatory, safety, immunogenicity, or efficacy evaluation of the study product. Subjects will use PVT device while inpatients, and PVT is a measure of a subject's ability to respond to a visual prompt by pushing a button. Three PVT 5-min tests per day will be performed by each subject up until discharge from the treatment facility or as outlined in the SSP. Comparisons will be made between symptom presence/severity and adjusted for other confounding variables.

### 5.8.2 Immunological

Blood will be collected per the Time and Events Schedule from subjects to assess for ETEC challenge antigen-specific serum IgA and IgG responses.

The serum will be processed at the CIR laboratory, transferred to the NMRC laboratory, and assayed for IgG and IgA antibody titers against LT using anti-ganglioside M1 (GM1)-enzyme-linked immunosorbent assay (ELISA), and against CS6 and LPS using methods previously described [113, 114]. For all antigens, pre- and post-dosing serum samples from the same individual will be tested side by side. The antibody titer ascribed to each sample will represent the geometric mean of duplicate determinations. Reciprocal endpoint titers <50 will be assigned a value of 25 for computations. Seroconversion is defined as ≥ four-fold increase in endpoint titer between pre- and post-challenge specimens AND a post-challenge reciprocal titer > 100. Exploratory immunological assays may include memory B cell evaluation, flow cytometric assays, and systems biological assays (transcriptomics, proteomics, phosphoproteomics, if funds are obtained) as outlined in the Time and Events Schedule.

Qualitative (responder rates) and quantitative assessments (log transformed values) will be made in addition to evaluation of the kinetics of the immune response. Median increases (fold-rises) of anti-ETEC (i.e., CS6, LT, and LPS) antibody concentrations and seroconversion rates will be calculated. Geometric mean titers will also be determined.

PBMCs will be assayed to determine antigen specific (CS6, LPS, and LT) ALS responses. ALS is a methodology that has been shown to be a replacement for enzyme-linked immunospot assay (ASC) methodology. PBMCs are incubated without stimulation and the supernatant is later assayed for antigen-specific IgG and IgA antibodies by ELISA. A positive ALS response will require a two-fold rise in antibody titers between pre and post challenge samples.

### 5.8.3 Microbiological

During the inpatient study [post-challenge day 0 to day 8 (or day of discharge)], stool samples (at least 1per subject per day post-inoculation) or rectal swab (if necessary) will be screened for the presence of B7A. Samples will be collected, processed and shipped as per the SSP, for qualitative cultures. Up to 10 *E. coli*-like colonies from MacConkey selective media will be subcultured onto CFA without bile salts agar and then screened for agglutination with challenge strain-specific antiserum using a slide agglutination technique.

Additional culture-independent methods may be used to quantitate B7A shedding.

### 5.8.4 Exploratory

Exploratory and expanded immunological assessments will be planned for this study. Among these, serum and PBMC samples may be collected for transcriptomic, cytokine, proteomic, and other systems biology

analyses to identify molecular signatures associated with ETEC infection. The cytokine analyses will encompass representation from multiple pathways including pro-and anti-inflammatory, and regulatory pathways.

Antigen specific memory B cell quantification may be performed with purified PBMCs to investigate the response generated following oral challenge. Briefly, following an *in vitro* stimulation/expansion to activate memory B cells, they are finally quantified as Ag-specific antibody-secreting cells.

Fecal and salivary IgA samples will be obtained to assess for mucosal IgA (including but not limited to total and anti-CS6, anti-LPS, and anti-LT) (Table 1). Subjects will be provided collection containers to collect all stools which will be processed per SSP.

Collection of a sublingual saliva sample will be performed utilizing synthetic oral swabs (Salimetrics Oral Swab; SOS). The subject will place a single swab in their mouth under the tongue, to collect saliva (only the lingual area—not from the parotid) for several (approximately 10) minutes. Subjects will be instructed not to eat or drink anything, including chewing gum, for 10 minutes prior to saliva sample collection. Subjects will be instructed to avoid drinking alcohol or using mouthwash for 24 hours and to avoid caffeinated beverages for 12 hours prior to collecting the sample. Saliva collection vials will be pre-loaded with 10uL of 100X HALT Protease Inhibitor Cocktail. Immunologic responders will be defined as subjects with a $\geq$ two-fold increase in reciprocal endpoint titer.

In addition, stool samples will be obtained to assess for exploratory endpoints to include microbiome characterization, culture-independent methods to quantitate B7A shedding, and PCR and transcriptomics (on the microbiome). This testing is subject to change as advances in research occur during the time that the stool is archived. These samples will be collected per SSP.

### 5.8.5 Outcome Adjudication Committee

In an effort to obtain an unbiased determination of the efficacy outcomes, an independent outcome adjudication committee, the members of which will be blinded as to the treatment regimens of the subjects, will evaluate challenge outcome data after completion of the inpatient phase of the study.

The committee will be comprised of at least 3 individuals, independent of the study sponsor and investigative team, who are experts on diarrheal illness case identification and pathogen diagnosis. The committee will also include a statistician/data analyst who will lead and coordinate the committee but will not have a voting role in deliberations.

The committee voting members will review all potential efficacy-related cases and endpoint data. Among the committee's responsibilities, they will (1) review and confirm all primary endpoint cases; (2) review all protocol-specified entry criteria, adherence, and compliance issues to ascertain classification in the per-protocol and other study populations; and (3) provide guidance regarding secondary and other endpoint classifications to include agreement on objective criteria for classification of endpoints. Specific duties and responsibilities will be outlined by charter prior to the start of the study.

## 6.0 STUDY SCHEDULE

The Time and Events Schedule (Table 1) details the study schedule. Subjects will receive unique, individual, study identification numbers either at screening or upon admission.

## 6.1 Screening (Day –90 to Day –5)

The following procedures will be completed at the JH200 and/or study-specific screening visit, between days -90 to -5, to determine and confirm study eligibility. An additional screening visit may be scheduled for any follow-up as needed, but is not required.

- Subject must fully understand the elements of the Informed Consent form, and sign and date the form prior to initiating protocol-specific procedures not covered in the JH200 screening protocol.
- Subject must take and pass (with ≥70% understanding) a comprehension test. Study staff will review any questions that the subject may have and the subject will be able to retake the comprehension test if they do not pass the first time.
- Assess inclusion and exclusion criteria.
- Record demographics and medical history, including gender, date of birth, race, height, weight, BMI, and any allergies.
- Complete physical examination including assessment of HEENT, heart, lungs, abdomen, skin, lymph nodes, neurological and musculoskeletal systems.
- Take vital signs (heart rate, blood pressure, respiratory rate, and oral temperature).
- Check health status.
- Blood draw for hematology (complete blood count with differential), serum chemistries (sodium, potassium, creatinine, glucose, SGPT/ALT), IgA level, blood typing.
- Urine for toxicology screen.
- Functional Bowel Disorder survey.
- Serum pregnancy test for women.

Additionally, approximately -30 days prior to admission to the inpatient unit (allowable range: Day -30 to Day -5), subjects will have a follow-up medical history and brief physical exam to ensure ongoing eligibility. An educational brief may also be provided to the subjects at this visit. The following procedures will be performed:

- Blood draw for serology (HIV, HCV and HbsAg)
- Blood for exploratory endpoints
- Confirm inclusion and exclusion criteria
- Check health status

If the initial screening visit is within the -30 day window, then all screening activities may be performed at the one visit.

Attempts will be made to inform subjects of their screening laboratory results either in person or over the telephone prior to admission on day –3. Subjects with clinically significant abnormalities (determined by PI) may be asked to have additional blood drawn. If the result(s) is confirmed, subjects may be referred to their primary care physician. A copy of the screening laboratory results may be provided to the subject at his/her request.

## 6.2 Inpatient Phase (Day –3 to Day 8)

### 6.2.1 Admission (Study Day –3)

The following procedures will occur on study day –3:

- Subjects will be admitted to the CIR
- Inclusion and exclusion criteria will be reviewed to confirm continued eligibility
- Vital signs (BP, HR and temperature) recorded
- Day -3 complete physical exam
- Medical history and concomitant medications since screening recorded
- Serum and/or urine pregnancy tests (for female subjects)
- Blood draw for hematology (complete blood count with differential), serum chemistries (sodium, potassium, creatinine, glucose, SGPT/ALT)
- Blood, stool and saliva samples for immunology (may be collected on D-3 or D-2)
- PVT demonstration

### 6.2.2 Study Days –2 to 4

On study day –2 subjects will be randomized as per section 5.2. Dosing will occur three times a day 15 minutes (range: 10 – 25 minutes) after each of three daily meals (breakfast, lunch and dinner) for a period of 7 days (day -2 to day 4). The time meals are completed will be recorded. Day 0, the day of ETEC challenge, is an exception to this order of events (see section 6.2.3). Subjects will receive test articles even if they do not eat. Vital signs (heart rate, blood pressure, and oral temperature) will be recorded at least three times daily. The study physician/nurse practitioner will monitor health status and adverse events by medical interview, and focused physical examinations. All stools will be collected, weighed and graded starting on Day -2 until discharge from the inpatient facility. Following ETEC challenge, one to three stool samples will be cultured daily per the relevant SSP. Rectal swabs may be used when stool specimens cannot be produced. Blood, saliva, and stool samples will be collected according to Table 1 and the relevant SSP.

### 6.2.3 ETEC Challenge (Day 0)

The dose and fasting time were determined as a part of NMRC.2015.0007.

On the day of ETEC challenge, subjects will be monitored as detailed above for days –2 to 4, with some modifications. Subjects will eat breakfast and then will receive test article/placebo in the morning approximately 90 minutes prior to challenge and will fast until the challenge. Approximately 1 minute prior to challenge, subjects will ingest 120 ml of bicarbonate buffer (buffer formulation: 13.35 gram of sodium bicarbonate in 1000 mL of sterile water for irrigation). For challenge, subjects will drink a solution of the challenge inoculum suspended in the remaining 30 mL of bicarbonate buffer at the appropriate inoculum doses. A second 90-minute fast will commence from the time of challenge in which subjects can only take the second dose of test article/placebo, and sips of water. Fifteen minutes (range: 10 – 25 minutes) after challenge, they will receive the second dose of test article/ placebo. See Table 4 for tabular listing of Day 0 schedule. Monitoring for post-challenge signs of adverse reactions will be conducted for at least 30 minutes, followed by taking vital signs. No Test article/placebo dosing is scheduled after lunch, but routine dosing will commence again at dinner. Table 1 and Table 4 outline these events. The meal times will be documented on study day 0.

**Table 4. Order of events on day of challenge**

| Event | Volume (approximate) |
|---|---|
| Breakfast | - |
| 1st daily dose of test article/placebo (range 10-25 min) | 150 ml |
| 90 minute fast | - |
| Bicarbonate buffer | 120 ml |
| 1 minute interval (up to 2 minutes) | - |

| | |
|---|---|
| Bicarbonate buffer + 1 x $10^{10}$ cfu B7A ETEC | 30 ml |
| Interval of 15 minutes (range 10-25 min) | - |
| 2nd daily dose of test article/placebo | 150 ml |
| Fast at least 90 minutes from challenge | - |
| Lunch | - |
| Dinner | - |
| 15 minutes after dinner complete (range 10-25 min) | - |
| 3rd daily dose of test article/placebo | 150mL |

## 6.3 Day 5-Discharge; Antibiotic Treatment

All subjects will be treated with ciprofloxacin (500 mg by mouth twice daily for three days). Alternate antibiotic treatment to which the strain is susceptible may also be considered as clinically appropriate. All antibiotic doses received prior to discharge will be directly observed by the investigator or designee.

All subjects are scheduled for routine antibiotic treatment on Day 5 (approximately 120 hours after challenge), per the protocol time and events schedule. Early antibiotic treatment after challenge may commence when any of the following criteria are identified and a study physician considers it to be warranted:

- Severe diarrhea (based on volume, 800 g in 24 hours)
- Stool output consistent with moderate diarrhea for 48 hours
- Mild or moderate diarrhea and 2 or more of the following symptoms: severe abdominal pain, severe abdominal cramps, severe nausea, severe headache, severe myalgias, any fever (≥ 38.0°C), or any vomiting
- A study physician determines that early treatment is warranted for any other reason

If, because of illness, a subject is unable to take oral antibiotics, intravenous antibiotics may be given (IV ciprofloxacin at an appropriate dose based on weight and clinical status). Subjects meeting discharge criteria may be released with the remaining antibiotic treatment to be taken at home. Subjects receiving early antibiotic therapy will **NOT** continue to receive test articles.

On study day 5, blood specimens (for hematology and serum chemistry) will be obtained from all subjects. Urine samples may be obtained on any of the study days at the discretion of the PI to assess surreptitious antibiotic intake or protocol restricted drug intake.

## 6.4 Inpatient Discharge

Discharge is routinely scheduled for day 8, when most subjects are expected to meet the discharge criteria of feeling well (with resolved or resolving symptoms of illness), having completed antibiotics, and having two consecutive stool cultures negative for ETEC. A clinical check including vital signs and a physician/nurse practitioner assessment is required before discharge. Blood, stool (except culture) and saliva specimens will be collected per Table 1 (from all subjects, inpatient and outpatient).

## 6.5 Outpatient Monitoring

Day 28 (± 2 days) is the scheduled follow-up safety visit. Subjects will be questioned about their health status and Adverse Events (AEs) that have occurred since discharge. Any reported AEs and/or concomitant medications will be recorded on the appropriate eCRF. Other procedures are vital signs, blood draw for serology and immunology.

Subjects will be told not to donate blood or blood products for one month following the completion of study participation and advised that the Red Cross will not allow blood donations for 1 year following participation in an investigational research study.

A phone-check will be conducted approximately six months (+/- 1 mo) after challenge to track the occurrence of any medically significant new chronic illnesses or serious health event, and completion of functional bowel disorder survey.

## 6.6 Early Termination

Subjects have the right to withdraw from the study at any time and for any reason without affecting the right to treatment by the investigator (for study-related conditions). The investigator also has the right to withdraw the subjects in the event of intercurrent illness, AEs, or for administrative/social reasons.

An excessive number of withdrawals can affect the scientific validity of the study, therefore unnecessary withdrawal should be avoided. Should withdrawals occur, efforts will be made to ensure subject safety and continued monitoring as thoroughly as possible. In case of subject withdrawal, for whatever reason, a final trial evaluation must be completed stating the reasons. Withdrawals due to non-attendance must be followed-up by the investigator to the extent possible to obtain the reason for non-attendance.

Subjects withdrawing from the study after receiving BSIgG (or placebo) will be asked to return on study day 5 for a brief physical exam and medical history, and blood draw for safety laboratory testing. Subjects withdrawing after receiving the CS6 expressing B7A-ETEC challenge will receive antibiotics for outpatient treatment and will be educated on the importance of complying with treatment. Attempts will be made to follow the subject for safety through study day 28.

# 7.0 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

## 7.1 Study Products

### 7.1.1 Antigens for Bovine Immunization

The antigens were used to vaccinate pre-selected cows for generation of the anti-CS6 and anti-B7A BSIgG products.

#### 7.1.1.1 CS6 Antigen

Recombinant CS6, given the lot designation 0840 was manufactured at the Walter Reed Army Institute of Research Pilot Bioproduction Facility under cGMP conditions in 26 Jan 2001 and released as a bulk material in 25 Jun 2001. Lot 0840 contains CS6 (derived from ETEC strain E8775), in a phosphate buffer, at a concentration of 2.56 mg/ml with an endotoxin content of 60 EU/ml. The manufacturing procedure for Lot 0840 involved the following steps: Plasmid DNA, containing the CS6 operon (cssA, cssB, cssC, and cssD) from a tox- E8775 ETEC strain was partially digested with HindIII and ligated to pUC19, which had also been digested with HindIII. The ligated plasmid was transformed into Escherichia coli (E. coli) DH5α. The CS6 genes were then subsequently subcloned into a pUC19 vector containing a kanamycin resistance marker in place of the gene for ampicillin resistance. The resulting vector containing the CS6 operon and kanamycin resistance marker was transformed into DH5α and then moved into the E. coli expression strain HB101. This clone was given the designation M346 and was used to generate cGMP master and production cell banks.

The bulk lot of purified recombinant CS6, Lot 0840, was analyzed using a panel of assays designed to assess protein content, identity, sterility, and purity. Protein content was quantified using the Lowry assay. A sterility test was conducted on Lot 0840 according to Code of Federal Regulations, Title 21, Section 610.12 (21 CFR 610.12). Identity was ascertained by SDS PAGE and Western blot analysis, and purity assessed by densitometric analysis of the CS6 protein band(s) after SDS PAGE separation. Endotoxin contamination was determined by the limulus amebocyte lysate (LAL) assay and immunogenicity of the CS6 protein was assessed through the immunization of New Zealand white female rabbits, demonstrating a greater than tenfold increase in serum IgG titers over pre-immunization serum titers.

#### 7.1.1.2 Whole-Cell Killed B7A ETEC

Inactivated whole-cell B7A ETEC was prepared by performing a phenol incubation of B7A. CS6 expression levels were ascertained using a CS6-specific inhibition ELISA. To produce the final vaccine lot, B7A was grown in CFA broth for 8-20 hours at 37°C in 5 L fermenters. Cells were harvested and inactivated with the optimal phenol concentration at 20°C with mild agitation for approximately 40 hours. The cells were washed and the OD600 adjusted to the desired final concentration. Vials containing a suitable amount of bacteria were prepared under aseptic conditions. The final whole-cell B7A vaccine batch was analyzed for sterility, CS6 content, pH, and visual appearance.

### 7.1.2 BSIgG Products

The IPs, anti-CS6 BSIgG, anti-whole cell killed B7A BSIgG, and nonhyperimmune BSIgG (placebo), were manufactured at SAB facilities. The anti-B7A BSIgG and anti-CS6 BSIgG are partially purified bovine polyclonal antibody products in development for use as a prophylactic for ETEC. These products were partially purified from bovine plasma collected from days 8, 11 and 14 post fourth (V4) through fifth (V5) vaccination with antigens/vaccine (CS6 and killed whole cell ETEC strain B7A) (see section 7.1.1). These products were manufactured into liquid form, with a total protein concentration of approximately >70 mg/mL formulated in phosphate buffered saline, and stored at -20°C ± 5ºC.

#### 7.1.2.1 Hyperimmune Plasma Collection

Plasma was pooled from three animals per vaccination group at approximately days 8, 11, and 14 post-vaccination under sterile conditions by using an automated plasmapheresis system (Baxter Healthcare, Autopheresis C Model 200). For this system, two catheters (BD T Catheter 14 gauge 3.5") were placed into the jugular of the donor. Whole blood was drawn through one catheter and was immediately stabilized with anticoagulant using an anticoagulant pump. The anticoagulant prevented the whole blood from clotting while the plasma is separated from the cells and platelets. The anticoagulant/whole blood was pumped through a spinning separation device which performed the plasma separation. The concentrated cells were returned to the donor using a cell pump through the second catheter. The plasma passed through the separation device and was collected into a bag (1-30L bioprocess film bags) on a scale. The total amount of plasma collected was up to 2.1% of the donor's body weight. Dedicated rooms were used to collect and store plasma.

#### 7.1.2.2 Negative Control Plasma (non-hyperimmune)

Bovine plasma was collected in bioprocess bags from four non-immunized cattle using plasmapheresis method developed at SAB. Up to 2.1% of body weight of non-immune plasma per animal was collected and three collections were performed every three to four weeks. Collected plasma bags were checked by QC and stored frozen until their release for manufacturing. At the time of release, 1.42 kg of negative control antibody was produced.

### 7.1.3 Packaging of Final Product

The liquid IP was packaged in multi-dose bottles. Quality control (QC) tests were performed for the final liquid product.

**Figure 2. Label for Anti-CS6 BSIgG Product**

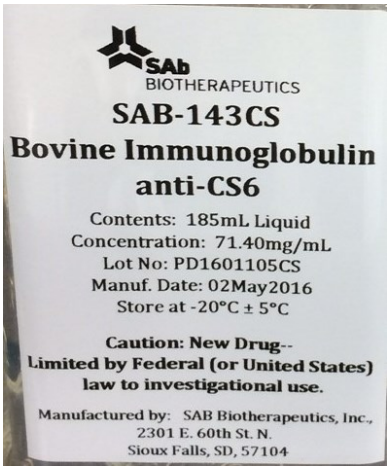



**Figure 3. Label for Anti-B7A BSIgG Product**

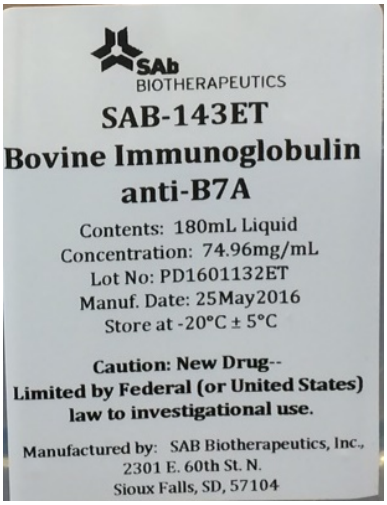
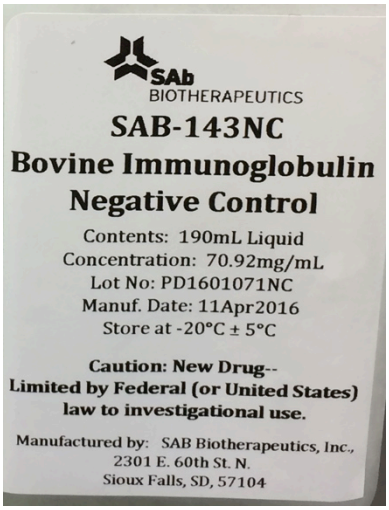



**Figure 4. Label for Negative Control Plasma Product**



### 7.1.4 Product Storage

BSIgG products will be stored at SAB in a locked freezer. Temperatures are monitored routinely per Standard Operating Procedures (SOP). SAB will have dedicated freezers for each product for storage prior to clinical evaluation.

### 7.1.5 Product Shipping

Prior to the commencement of the study, the IP will be transferred directly to the Research Pharmacy, CIR. Any use of the IP will be done under the supervision of the Research Pharmacy, and the Research Pharmacist will maintain IP accountability log which tracks the status of all IP received. Any bottles remaining at the end of the study will be returned to NMRC or destroyed per SSP.

### 7.1.6 Dose Preparation

Each dose of anti-CS6 BSIgG and anti-B7A whole cell killed BSIgG will be prepared per SSPs. A single unit-dose of both anti-CS6 BSIgG and anti-B7A whole cell BSIgG will contain approximately 1g of BIgG (pending the exact IgG content (>90% estimated)). The previous efficacy of 1g of Bovine IgG anti-CFA/I (AEMI Lot#43218) in preventing illness mediated by H10407 in the clinic [50]. As Bovine IgG anti- CS6 and anti-B7A are new products, the dose was fixed at 1g of IgG to normalize it with respect to Bovine IgG anti-CFA/I. More specifically, in these studies, the doses of bovine milk IgG product were protective against challenge with approximately 1 X $10^9$ cfu of ETEC strain H10407. The anti-CS6 and anti B7A whole cell ETEC bovine serum IgG to be used in this trial are very similar in physical characteristics and potency (based on ELISA) to the anti-CFA/I and anti-CfaE bovine milk IgG used in the recent JHU study. The non-hyperimmune BSIgG will be prepared per the formulation SSP. Section 6.2.2 describes how the test articles/placebo will be administered in relation to meals for Days -2 through 4. The test articles/placebo will be administered with bicarbonate buffer.

## 7.2 ETEC Challenge Strain

A strain dose-finding study was recently completed with CS6- expressing ETEC strain B7A under BB IND 16,517 (held by sponsor-investigator Dr. A.L. Bourgeois at CIR, JHBSPH). Briefly, in a cohort of 28 subjects, we assessed the optimal dose ($10^8$, $10^9$, and $10^{10}$ cfu) and fasting period (90 minutes and overnight) of the B7A challenge with B7A [identifying the optimal attack rate AR (seeking > 70.0%)]. The optimal dose and fasting time were determined from the outcome of NMRC.2015.0007. Subjects will be given approximately 1 x $10^{10}$ cfu of ETEC B7A with a 90 minute fast in this study.

### 7.2.1 Challenge Inoculum: The CS6-Expressing ETEC B7A

The IP to be used for challenge is ETEC strain B7A (O148:H28- $CS6^+$ $LT^+ST^+$). It was manufactured at the WRAIR Pilot Bioproduction Facility (PBF) in 1997. Each vial of the production cell bank contains approximately 9 x $10^8$ cfu of live ETEC B7A in Luria Broth (LB) with 15% glycerol as cryopreservative. There is approximately 1 ml of the bacterial suspension per vial. The lot number is 0481. Vials are stored at -80$\pm$10°C. Bacteria are not given directly from the vials to subjects; they are inoculated into media and grown overnight.

### 7.2.2 Packaging and Labeling

The B7A challenge strain is stored as 1 ml aliquots in 2 ml cryostorage tubes held at –80°C ± 10°C under controlled conditions at the Pilot Bioproduction Facility, WRAIR. The cryovials are labeled as shown below:

The label is as follows:

**Production Cell Bank for Enterotoxigenic *E. coli* CS6**
**Challenge Strain B7A**
**BPR No.: BPR-258-00** **Lot No.: 0481**
**Contents: 1.0mL**
**Cautions: For Manufacturing Use Only; Viable Organism.**
**Date of Mfg.: 09 Oct 97** **Storage: ≤-80 ± 10°C**
**Manufactured By: WRAIR, Washington, D.C. 20307**

### 7.2.3 Product Characterization

This Production Cell Bank (PCB) of B7A was used previously in a human challenge trial carried out by WRAIR investigators. Additionally, it was used in a challenge refinement investigation which optimized the dose and fasting period for its subsequent administration in this investigation (NMRC.2015.0007). Organisms prepared from the PCB will be used to challenge subjects participating in this trial. This strain is susceptible to ciprofloxacin and other common antibiotics.

### 7.2.4 Product Storage and Transfer

The B7A vials are stored at ≤-80°C ± 10°C. The challenge strain will be transferred on dry ice from the WRAIR PBF to the CIR Enterics Research Laboratory at JHBSPH, logged in and stored at -80°C ± 10°C in a locked and temperature-monitored freezer. Any use of these vials will be done under the supervision of the CIR Enterics Research Laboratory, JHSPH and tracked in an accountability log. Any vials remaining at the end of the study will be disposed of (via autoclaving) or returned to NMRC/WRAIR for use in non-clinical research studies.

### 7.2.5 Product Preparation

Fresh, plate grown organisms will be used for challenge inocula, a standard approach for ETEC challenge studies. Approximately 48 hours before challenge, a vial of the cGMP Master Cell Bank will be thawed and streaked onto CF antigen agar (CFA without bile salts [CFA] agar) and Mac agar (to document purity of the cGMP PCB and *E. coli* verification). After 22-24 hours of incubation at 35-37°C, 10 colonies will be used to prepare a suspension in sterile saline (0.9%). This suspension will be used to heavily inoculate approximately 6 CFA agar plates for incubation at 35 -37°C. CFA agar plates will be harvested in sterile saline after 18 - 20 hours and the resulting bacterial suspension further diluted in saline for optical density determination at 600 nm. The optical density of the suspension will be adjusted to the appropriate concentration of bacterial cells depending on study group. The number of cfu in the inoculum will be determined by titrating and plating on agar plates before and after administration to subjects. The final inoculum will be examined by Gram stain for purity and for CS6 expression by agglutination in anti-B7A anti-serum.

### 7.2.6 Product Administration

A sodium bicarbonate (USP-grade) solution of 2 g/150 ml water will be prepared. Each subject will drink 120 ml of this buffer one minute prior to ingesting the challenge inoculum, to neutralize gastric acidity. Within 2 minutes, the subjects will drink the challenge inoculum dissolved in the remaining 30 ml of buffer.

The bicarbonate buffer is prepared from USP grade Sodium bicarbonate by dissolving 13.35 gram of sodium bicarbonate in 1000 mL of sterile water for irrigation. When subjects ingest 150 mL of this buffer solution in conjunction with taking a dose of test article/placebo or receiving their ETEC challenge strain, they will receive a total dose of 2 grams of sodium bicarbonate

## 7.3 Accountability Procedures for the Investigational Products

The investigator must ensure that the IP supplies are stored as specified in the protocol and in a secured area, with access limited to authorized study personnel. The investigator has the following responsibility for the products: maintaining inventory; maintaining accurate records of the receipt of IP, including date received, randomization code, manufacture or expiration date, amount received and disposition; holding the amount of product needed; and adequate storage and dispensing of the vaccines. A record will be maintained that includes the dispensation date, amount of IP dispensed, initials and identification number. The IP must be administered only at the specified institution. Unused product will be shipped to:

Steven Poole, PhD
Naval Medical Research Center
503 Robert Grant Avenue, Silver Spring, MD 20910-7500

## 7.4 Assessment of Subject Compliance with Investigational Products

A member of the study team will witness the ingestion of the test article/placebo.

# 8.0 ASSESSMENT OF SAFETY

Safety monitoring will be conducted throughout the study; therefore, safety concerns will be identified by continuous review of the data by the PI, clinic staff, clinical monitor, research monitor, and the sponsor.

**Study Safety Management:** The research monitor and PI will review any safety concern. A data safety monitoring board (DSMB) is not required for this study.

**Research Monitor:** The research monitor will function as an independent safety advocate for subjects per AR 70-25 and Department of Defense (DoD) Instruction 3216.02. An independent research monitor is required to review all unanticipated problems involving risk to subjects or others, SAEs, and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum the research monitor should comment on the outcomes of the event or problem and, in the case of a SAE or death, comment on the relationship to participation in the study. The research monitor should also indicate whether he/she concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator or research monitor to be possibly or definitely related to participation and reports of events resulting in death should be promptly forwarded to the IRBs, ORP HRPO, and USAMRMC Division of Regulated Activities and Compliance.

The research monitor, in accordance with JHBSPH guidelines, will have the following responsibilities:

- Evaluate ongoing safety data and make recommendations in order to ensure subjects safety as required
- Be available for consultation by the clinical investigative team through the period of the clinical study in which there is an interaction with human subjects
- Be available to review all SAEs and other unanticipated problems involving risk to subjects
- Be available to discuss SAEs and significant safety issues
- Provide clinical advice, in accordance with the study protocol, on the clinical management of subjects. This advice may include, but is not limited to

  - Decisions on "borderline" laboratory values and eligibility for enrollment
  - Confirmation and discussion of treatment decisions for difficult clinical situations
- Must document all clinical decisions including date, time and signature
- Must communicate all decisions to the study PI and other study investigators, which must be stored with subject source documents

All safety reports (i.e., serious adverse events, deviations, unanticipated problems involving risk and subject deaths) will be submitted to the JHSPH IRB and NMRC IRB.

### 8.1 Vital Signs

Vital signs (temperature, blood pressure, heart rate) will be obtained throughout the inpatient period and at each study visit after discharge. Respiratory rates will be obtained on a case-by-case basis at the discretion of the study clinician. (See Table 5 for applicable AE coding.)

**Table 5. Reference Ranges and Adverse Event Coding for Vital Signs Parameters**

| Vital Signs | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| Heart rate | | | | |
| Tachycardia | 101–115 | 116-130 | >130 | ER visit or hospitalization for arrhythmia |
| Bradycardia | 50-54[a] | 45–49 | <45 | ER visit or hospitalization for arrhythmia |
| Fever (°C)<br>(°F) | 38.0–38.4<br>100.4–101.1 | 38.5–38.9<br>101.2–102.0 | >39.0<br>>102.0 | Life threatening hyperthermia |
| Blood Pressure | | | | |
| Hypertension (systolic, mm Hg) | 141–150 | 151 - 155 | >155 | ER visit/hospitalization for malignant hypertension |
| Hypertension (diastolic, mm Hg) | 91–95 | 96 – 100 | >100 | ER visit/hospitalization for malignant hypertension |
| Hypotension (systolic, mm Hg)[b] | 85–89 | 80 – 84 | <80 | ER visit/hospitalization for hypotensive shock |

[a] Grade 1 bradycardia will not be considered an abnormality for this study unless judged to be clinically significant by the PI or the PI in consultation with the Research Monitor and sponsor.

[b] If a subject has a baseline systolic BP in the 90's then a decrease in BP < 10 without associated clinical symptoms will not be considered an abnormality for this study unless judges to be clinically significant by the PI.

### 8.2 Physical Examination

A complete physical exam will be conducted during the screening visit and on Day -3 as part of the screening process; a targeted physical exam will be conducted prior to receipt of first IP, prior to challenge and daily during subject's inpatient stay. Subsequent focused clinical assessments will occur at each study visit with specific attention to the identification of local, systemic or other adverse reactions.

### 8.3 Laboratory Assessments

Venous blood samples will be collected for chemistry, hematology, and immunological parameters during the screening phase of this study and to provide a baseline sample. Hematology and chemistry analyses will be performed by commercial laboratory (Quest, Incorporated in Baltimore City or by Johns Hopkins Medical Institutions). Additional specimens may be collected to confirm and evaluate any abnormal values. Additional blood for chemistry and hematology are planned for collection following experimental infection per the time and events schedule. However, samples may be obtained as part of the clinical care of an individual subject. The clinical toxicity grading scale that will be used as a guideline is based on the

Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Subjects enrolled in Preventive Vaccine Clinical Trials and the DAIDS Table for Grading and Severity of Adult and Pediatric Adverse Events. Final grading determination will be made by the PI based on normal lab values for the specific lab and clinical symptoms. Abnormal laboratory values based on hematology, and clinical chemistry (SGPT/ALT, glucose, creatinine, and electrolytes) after test article dosing will be considered an AE and severity determination by the investigator will be based on clinical symptoms and using the attached grading scale as a guideline. In the event of a clinically significant abnormal laboratory value, the test will be repeated and followed up, if clinically relevant. Additional clinical laboratory evaluations may be performed at other times as required to follow up a serious or severe adverse event or as deemed necessary by the investigator. Slightly abnormal laboratory values that remain consistent from the time of screening throughout the study will not be recorded as AEs.

Serologic evidence of chronic HIV-1, HCV, and HBV infections will be obtained during the screening process. Evidence of current infection will make a subject ineligible. Additional testing will not be performed as part of this study beyond second tier confirmatory tests on those with preliminary positive tests on ELISA after HIV and/or HCV serology. Targeted drug screenings are planned for this study at screening and at the discretion of the study clinician.

A serum sample for pregnancy testing (female subjects) will be collected at the screening visit and on Day -3. A urine pregnancy test will be collected (female subjects) on Day -3 and at day 28. A positive pregnancy test prior to IP administration will result in disenrollment. Any subjects who become pregnant during the study will be removed from the study and followed until the end of their pregnancy. Procedures to be followed in the event a study participant becomes pregnant during the study period are outlined below.

**Table 6. Reference Ranges and Adverse Event Coding for Clinical Hematology Parameters**

| Test | Normal | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|---|
| Hemoglobin (g/dL) (for screening purposes only) | M: LLN = 11.0<br>F: LLN = 10.5 | | | | |
| Hemoglobin, low | | M: 10.0 to 10.9<br>F:9.5 to 10.4 | M: 9.0 to <10.0<br>F: 8.5 to <9.5 | M: 7.0 to <9.0<br>F:6.5 to <8.5 | M: <7.0<br>F: <6.5 |
| Eosinophils (cells/$mm^3$) | 15-500 | 551-1,500 | 1,501-5,000 | > 5,000 | Hypereosinophilic |
| Leukocytes (white blood cells) (cells/$mm^3$) | 2,500 to 10,800 | | | | |
| Leukopenia | | 2,000 to 2,499 | 1,500 to 1,999 | 1,000 to 1,499 | <1,000 |
| Leukocytosis | | 10,801-15,000 | 15,001-20,000 | 20,001-25,000 | > 25,000 |
| Lymphocytes, low (cells/$mm^3$) | >650 | 600 to <650 | 500 to <600 | 350 to <500 | <350 |
| Neutrophils, low (cells/$mm^3$) | >1,000 | 800 to <1,000 | 600to 799 | 400to 599 | <400 |
| Platelets decreased (cells/$mm^3$) | ≥125,000 | 100,000 to 124,999 | 50,000 to <100,000 | 25,000 to <50,000 | <25,000 |

**Table 7. Reference Ranges and Adverse Event Coding for Blood Chemistry Parameters**

| Test | Normal | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|---|
| BUN (elevation) | 7-25 | 26-28 | 29-31 | > 31 | Requires dialysis |
| Creatinine (elevation) | M: 0.7-1.4<br>F: 0.5-1.1 | 1.1 to 1.3 x ULN | > 1.3 to 1.8 x ULN OR Increase of > 0.3 mg/dL above baseline | > 1.8 to < 3.5 x ULN OR Increase of 1.5 to < 2.0 x above baseline | ≥ 3.5 x ULN OR Increase of ≥ 2.0 x above baseline |
| Glucose, Random (mg/dL) | 65 to115 | | | | |
| Hypoglycemia | | 55 to 64 | 40 to <55 | 30 to <40 | < 30 |
| Hyperglycemia | | 116 to 160 | >160 to 250 | >250 to 500 | > 500 |
| Potassium (mEq/L; *mmol/L*) | 3.4 to 5.6 | | | | |
| Hypokalemia | | 3.0 to < 3.4 | 2.5 to <3.0 | 2.0 to <2.5 | < 2.0 |
| Hyperkalemia | | >5.6 to <6.0 | 6.0 to <6.5 | 6.5 to <7.0 | ≥ 7.0 |
| SGPT/ALT (elevation) | M:9 to 46<br>F: 6 to 29 | 1.25 to <2.5 x ULN | 2.5 to <5.0 x ULN | 5.0 to <10.0 x ULN | ≥ 10.0 x ULN |
| Sodium (mEq/L; *mmol/L*) | 136 to145 | | | | |
| Hyponatremia | | 130 to <135 | 125 to <130 | 121 to <125 | ≤ 120 |
| Hypernatremia | | 146 to <150 | 150 to <154 | 154 <160 | ≥ 160 |

## 8.4 IND Safety Reporting

The following terms, as defined by 21 CFR 312.32, apply to IND safety reporting.

### 8.4.1 Adverse Event or Suspected Adverse Reaction

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. This includes an exacerbation or worsening of pre-existing conditions or events, intercurrent illnesses, injuries, or vaccine or drug interaction, or worsening of abnormal clinical laboratory values. Anticipated day-to-day fluctuations of pre-existing conditions that do not represent a clinically significant exacerbation need not be considered AEs. Discrete episodes or worsening of chronic conditions occurring during a study period will be reported as AEs to assess changes in frequency or severity. Stable, pre-existing conditions and/or elective procedures are not AEs.

AEs will be documented in terms of signs and symptoms observed by the investigator or designee or reported by the subjects at each study encounter, with a medical diagnosis stated. Pre-existing conditions or signs and/or symptoms (including any which are not recognized at study entry but are recognized during the study period) present in a subject prior to the start of the study will be recorded in the Medical History form within

the subject's eCRF. AEs occurring after informed consent is obtained, but prior to test article receipt, will be documented in the Medical History form within the subject's eCRF as instructed by the Study Monitor.

Hospitalization for elective surgery related to a pre-existing condition which did not increase in severity or frequency following initiation of the study, or for routine clinical procedures (including hospitalization for "social" reasons) that are not the result of an AE is not itself considered an AE, but must be recorded in the AE page of the eCRF. If hospitalization arises from a pre-existing condition, or was planned prior to the first test article dose, it will be recorded in the Medical History form of the eCRF. If planned after the first dose, it will only be recorded in the AE page of the eCRF. In both cases, it will be recorded as `Hospitalization (Not an AE)', and the relationship to test article receipts will be checked "No". Because hospitalization under these circumstances need not be considered an AE, it is therefore also not considered a SAE.

A "suspected" adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug. The investigator is responsible for documentation of AEs according to the detailed guidelines set out below. Subjects will be instructed to contact the investigator immediately should s/he manifest any signs or symptoms perceived as serious during the study period. Approximately six months after study completion, subjects will be contacted by phone to document any intervening medically significant new chronic illnesses or serious health events. These data will be documented in a telephone log and summarized in an annex to the final clinical study report.

All AEs will be recorded on the appropriate AE form of the subject's eCRF and recorded irrespective of severity or whether or not they are considered related to the test article or challenge inoculum. AEs will be tabulated separately for pre-and post-challenge data. The assessment of the safety of the BSIgG and control products will be primarily limited to the 2 days prior to receipt of the ETEC challenge. Following receipt of the challenge inoculum, gastrointestinal and systemic symptoms will likely attributable to B7A challenge strain unless temporally related to receipt of the BSIgG product/placebo or antibiotics. AEs occurring after receipt of the B7A challenge (day 0) will also be assessed as to their relationship with the challenge strain and the antibiotic (if treatment has started).

#### 8.4.1.1 Solicited and Anticipated Adverse Events

A solicited AE is a predetermined event, which may reflect safety concerns related to the IP. Previous clinical studies using much higher quantities of bovine colostrum products than planned for this study have been orally administered and well tolerated (e.g., 10 g/day anti-ETEC bovine milk IgG [93]; 30 g/day anti- *C. parvum* bovine milk IgG [103]; 30 g/day anti-*S. flexneri* bovine milk IgG [53].

This study includes a challenge with live CS6-expressing ETEC bacteria, and therefore all the symptoms of ETEC illness are expected. The most common effects of ETEC infection are moderate to severe diarrhea (which may lead to dehydration and the need for oral or intravenous rehydration), and abdominal cramping. Fever, nausea with or without vomiting, chills, loss of appetite, headache, muscle aches and bloating may also occur. The following ETEC-associated AEs will be solicited daily during the challenge phase:

1. Abdominal cramping
2. Abdominal pain
3. Anorexia (poor appetite)
4. Arthralgias
5. Bloating

6. Chills
7. Constipation
8. Excessive flatulence
9. Generalized myalgia
10. Headache
11. Lightheadedness
12. Malaise
13. Nausea
14. Urgency
15. Vomiting

The following will be documented via clinical assessments during the inpatient challenge phase:

1. Diarrhea (via stool logs)
2. Hypovolemia
3. Fever (oral temperature $\geq$ 100.4° F)

#### 8.4.1.2 Serious Adverse Event or Serious Suspected Adverse Reaction

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- Life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect (abortion, stillbirth and any malformation/disease must be reported as an SAE).

Although not considered SAEs, cancers will be reported in the same way as SAEs. Pertinent definitions include:

- Life threatening - An AE is life threatening if the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
- Disabling/incapacitating - An AE is incapacitating or disabling if it results in a substantial disruption of the subjects' ability to carry out normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as headache, nausea, vomiting, diarrhea, influenza, injection site reactions and accidental trauma (e.g. sprained ankle).
- Hospitalization: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for treatment that would not have been appropriate in the physician's office or outpatient setting. Hospitalization for either elective surgery related to a pre-existing condition which did not increase in severity or frequency following initiation of the study or for routine clinical procedures (including hospitalization for "social" reasons) that are not the result of an adverse event need not be considered as AEs and are therefore not SAEs.
- Routine Clinical Procedure: A procedure which takes place during the study and does not interfere with the test article administration or any of the ongoing protocol specific procedures.

Note: If anything untoward is reported during an elective procedure, that occurrence must be reported as an adverse event, either `serious' or non-serious according to the usual criteria. When in doubt as to whether 'hospitalization' occurred or was necessary, the AE will be considered serious. Important medical events that

may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

## 8.5 Serious Adverse Events

### 8.5.1 Unexpected Adverse Event or Unexpected Suspected Adverse Reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

### 8.5.2 Other Adverse Events

Other adverse events will be identified by the PI during the evaluation of safety data. Significant adverse events of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from the study, will be classified as other adverse events. For each, a narrative may be written and included in the clinical study report.

## 8.6 Relationship to Investigational Product (Assessment of Causality)

The investigator or designee must assign a relationship of each AE to the receipt of the IP. The investigator or designee will use clinical judgment in conjunction with the assessment of a plausible biologic mechanism, a temporal relationship between the onset of the event in relation to receipt of the IP, and identification of possible alternate etiologies including underlying disease, concurrent illness or concomitant medications. Every effort will be made to explain AEs and assess causal relationships, if any, to administration of the BSIgG test articles, B7A ETEC challenge, or antibiotic treatment. AEs occurring on study days –2 to 0 will be assessed as to their relationship with the BSIgG test articles. AEs occurring after receipt of the ETEC challenge (day 0) will be assessed as to their relationship with the BSIgG test articles, B7A challenge strain or the antibiotic, if applicable. The degree of certainty with which an AE can be attributed to these products (or alternative causes, e.g. natural history of the underlying diseases, concomitant therapy, etc.) will be determined by how well the event can be understood in terms of one or more of the following:

- Reaction of similar nature having previously been observed with BIgG products, ETEC challenge strains, or antibiotic administration
- Published literature accounts supporting causality
- Temporal relationship with administration

The following guidelines should be used by investigators to assess the relationship of an AE to study product administration. Only a physician or nurse practitioner can make this determination. The investigator will assess causality of all AEs as either 'related' or 'unrelated'. Non-serious and serious adverse events will be evaluated as two distinct types of events given their different medical nature. If an event meets the criteria to be determined 'serious' it will be examined by the investigator to the extent possible to determine ALL contributing factors applicable to the event. Other possible contributors include:

- Underlying disease
- Other medication
- Protocol required procedure
- Other cause (specify)

### 8.6.1 Causality

Causality of all AEs will be assessed by the investigator using the following criteria:

In the investigator's opinion, is there a reasonable possibility that the AE may have been caused by the product under consideration?

| | |
|---|---|
| **Definite** | The AE can only be explained by receipt of the product |
| **Probable** | AE occurs within a reasonable time after the administration of the product and cannot be reasonably explained by other factors (i.e., clinical condition, environmental / toxic factors or other treatments) |
| **Possible** | AE occurs within a reasonable time after the administration of the product but can also be reasonably explained by other factors (as mentioned above) |
| **Unrelated** | there is no suspicion that there is a relationship between the product and AE, there are other more likely causes, and administration of the product is not suspected to have contributed to the AE |

## 8.7 Recording of Adverse Events

### 8.7.1 Methods / Timing for Assessing, Recording and Analyzing Safety Endpoints

All AEs either observed by the investigator or one of his/her clinical collaborators or reported by subjects spontaneously or in response to a direct question will be evaluated by a study investigator. The nature of each event, date of onset, outcome, severity and relationship to test article, challenge, and/or antibiotic will be established. Details of any symptomatic/corrective treatment will be recorded on the appropriate page of the eCRF. Subjects will be asked non-leading questions initially when soliciting AEs, followed by more direct questions as necessary. AEs already documented in the eCRF, i.e., at a previous assessment, and designated as 'ongoing' will be reviewed at subsequent follow-up assessments. If resolved, documentation in the eCRF will be completed. If an AE changes significantly in frequency or intensity during a study period, a new record of the event will be started.

AEs, solicited AEs, and SAEs will be assessed at all study visits, documented in the source records, and recorded on the eCRFs using accepted medical terms and/or the diagnoses that accurately characterize the event. Solicited AE's will be recorded as individual events. Unsolicited AE may be recorded as a diagnosis. When a diagnosis is known, the AE term recorded on the eCRF will be the diagnosis rather than a constellation of symptoms. The investigator will assess all AEs for seriousness, relationship to IP, severity, and other possible etiologies. When an event has not resolved by the proscribed reporting period, it will be left open/without an end date on the AE eCRF and will be updated with end date or ongoing at visit.

The timeframe for the collection of AEs and SAEs begins at the time of IP administration through 28 days after receipt of the challenge strain. Additionally, subjects will be contacted by telephone approximately at 6 months after challenge to assess for any new onset SAEs or AEs of special interest mandated by the FDA.

### 8.7.2 Duration of Follow-up of Subjects after Adverse Events

Investigators are required to follow SAEs to resolution, even if this extends beyond the prescribed reporting period. Resolution is the return to baseline status or stabilization of the condition with the probability that it will become chronic. The SAE outcomes will be reported to the sponsor.
Investigators are not obligated to actively seek SAEs in former subjects; however, if a SAE, considered to be related to the IP is brought to the attention of the investigator *at any time* until closure of the study, the event will be reported.
Investigators should follow-up adverse events at least until the final study visit. This may include repeat safety laboratory analysis. Outcome should be assessed as:

- Resolved
- Resolved with sequelae
- Severity change (highest severity in a day will be recorded, if the severity on day 1 is mod, then mild and mod, it will be entered as moderate for the day only, then if on day 2 is mild, the moderate AE will stop and the AE will be reentered as mild)
- Ongoing at day 28
- Died
- Lost to follow up

All SAEs must be documented and followed until the event either resolves, subsides, stabilizes, disappears or is otherwise explained or the subject is lost to follow-up, but not longer than 6 months after the last receipt of test article. All follow-up activities have to be reported, if necessary on one or more consecutive SAE report forms, in a timely manner. All fields with additional or changed information must be completed and the report form will be forwarded to the study contact for reporting SAEs as soon as possible, but not more than 7 calendar days after receipt of the new information. Clinically significant laboratory abnormalities will be followed up until they have returned to normal or until stable. Reports relative to the subsequent course of an AE noted for any subject must be submitted to the Sponsor. The outcome of SAEs will be assessed in the same manner as all AEs.

### 8.7.3 Safety Assessment

All AEs will be assessed for severity by the investigator. Inherent in this assessment is the medical and clinical consideration of all information surrounding the event including any medical intervention required. Each event will be assigned one of the following categories: mild, moderate, severe, or life-threatening. The criteria below may be used for any symptom not included in the grading scale. Any grade 4 (life-threatening) AE must be reported as an SAE.

The eCRF for AEs will reflect only the highest severity for continuous days an event occurred.

| | | |
|---|---|---|
| **Mild** | Grade 1 | Does not interfere with routine activities; minimal level of discomfort |
| **Moderate** | Grade 2 | Interferes with routine activities; moderate level of discomfort |
| **Severe** | Grade 3 | Unable to perform routine activities; significant level of discomfort |

| | | |
|---|---|---|
| **Potentially life-threatening** | Grade 4 | Hospitalization or ER visit for potentially life-threatening event |

FDA guidelines for toxicity will be followed; however, if a subject is evaluated in an emergency room for nonlife threatening illness or symptoms (i.e., visits emergency department on weekend for mild problems because the physician's office is closed), the information from that visit will be reviewed and severity of the adverse event will be assessed according to the subject's clinical signs and symptoms.

As defined by the International Conference on Harmonization (ICH) guideline for Good Clinical Practice (GCP), the term "severe" is often used to describe intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself however, may be of relatively minor medical significance (such as severe headache). This is **not** the same as "serious", which is based on subject/event **outcome** or **action** criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

During the challenge phase of the study, ETEC disease-specific adverse events will be graded in accordance to the table below.

**Table 8. Challenge Phase ETEC Infection Anticipated Adverse Event / Endpoint Assessments**

| Adverse Event | Severity[a] | Parameter |
|---|---|---|
| Diarrhea, based on highest output of loose/liquid stools in any 24-hour period. (A diarrhea episode ends when there is a 48 hour window with no grade 3-5 stools.) | 1 | Mild: 2-3 loose /liquid stools totaling ≤400g |
| | 2 | Moderate: 4 to 5 loose/liquid stools or 401-800 g of loose/liquid stool |
| | 3 | Severe: 6 or more loose/liquid stools totaling >800g |
| | 4 | Life threatening |
| Body temperature (t) | 1 | ≥100.4ºF and ≤101.1ºF (38.0-38.4ºC) |
| | 2 | ≥101.1ºF and ≤102.0ºF (38.5-38.9ºC) |
| | 3 | >102.0ºF (39.0ºC) |
| | 4 | Life threatening hyperthermia |
| Vomiting | 1 | One episode within a 24-hour period |
| | 2 | Two episodes within a 24-hour period |
| | 3 | More than two episodes with a 24-hour period |
| | 4 | Life threatening consequence of emesis |
| Other solicited and non-solicited adverse events | 1 | Discomfort noted, but no disruption of normal daily activities; slightly bothersome; relieved with or without symptomatic treatment. |
| | 2 | Discomfort sufficient to reduce or affect normal daily activity to some degree; bothersome; interferes with activities, only partially relieved with symptomatic treatment. |
| | 3 | Discomfort sufficient to reduce or affect normal daily activity considerably; prevents regular activities; not relieved with symptomatic treatment. |
| | 4 | Life threatening |

[a]1=mild; 2=moderate; 3=severe; 4=life threatening.

## 8.8 Reporting Adverse Events

The PI will report all AEs to the sponsor and the local IRB in the appropriate safety, annual, and/or final reports. The NMRC staff in conjunction with the clinical site will draft annual and final clinical study reports and provide files to the sponsor for review and submission to the FDA.

### 8.8.1 Reporting Serious and Unexpected Adverse Events

All SAEs must be reported immediately by the investigator without filtration, whether or not regarded as possibly attributable to the test articles, placebo, or antibiotic. SAE reports will be provided to the Sponsor, medical monitor, JHSPH IRB, and NMRC IRB. The investigator must report SAEs within one calendar day of becoming aware of the event by telephone, fax or e-mail (if appropriate) to the study contact for reporting SAEs as described in the protocol. This initial notification will include minimal, but sufficient information to permit identification of the reporter, the subject, the test articles, AEs, and date of onset. The investigator will not wait for additional information to fully document the event before notifying. The first notification will be confirmed by an acknowledgement letter. The report is then to be followed by submission of a completed SAE Report Form provided by the sponsor as soon as possible but not more than 3 calendar days past the initial report, detailing relevant aspects of the AE in question. All investigator actions and event outcomes must also be reported immediately. SAE Report Forms are to be used for documentation of these various aspects regarding the event. Hospital records and autopsy reports will be obtained if applicable.

The research monitor is required to review all unanticipated problems involving risk to subjects or others, SAEs, and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the medical monitor will comment on the event outcomes, and in the case of a SAE or death, comment on the relationship to participation in the study. The medical monitor will indicate concurrence or nonconcurrence with the details of the report provided by the investigator. Reports for events determined by either the investigator or medical monitor to be possibly, probably, or definitely related to participation and reports of events resulting in death will be promptly forwarded to all germane IRBs (JHSPH IRB, NMRC IRB).

Unanticipated problems involving risk to subjects or others, SAEs related to participation in the study and all subject deaths will be promptly reported by phone (301-619-2165), by email, or by facsimile (301-619-7803) to the NMRC IRB. A complete written report will follow the initial notification.

#### 8.8.1.1 Reporting to the Sponsor

All SAEs and unexpected AEs must be reported promptly (within 72 hours) to the sponsor as per 21 CFR 312.64, whether or not the event is considered related to study product. Further, the investigator should comply with relevant study site SOPs on reporting SAEs.

The minimum information that the investigator will provide to the sponsor is specified in Table 10.

**Table 9. Study Contacts for Reporting Serious Adverse Events**

| | |
|---|---|
| **Sponsor** | A. Louis Bourgeois, Ph.D., M.P.H.<br>Center for Immunization Research<br>Department of International Health<br>Johns Hopkins Bloomberg School of Public Health<br>624 N. Broadway, HH, Rm 205<br>Baltimore, MD 20215 |
| **Institutional Review Board** | JHSPH IRB Office<br>615 N. Wolfe Street<br>Suite E1100<br>Baltimore, Maryland 21205<br>Phone: 410-955-3193<br>Toll-Free: 1-888-262-3242<br>Fax: 410-502-0584<br>Email: JHSPH.irboffice@jhu.edu |
| **Collaborating Institutional Review Board** | Naval Medical Research Center (NMRC) IRB<br>Research Services Directorate<br>Office of Research Administration<br>Code 025, Building 500, Rm 004<br>Silver Spring, MD<br>Telephone: 301-319-7276<br>Fax: 301-319-7277 |
| **Research Monitor** | Alexandra Singer, MD<br>Malaria Department<br>Naval Medical Research Center<br>503 Robert Grant Avenue<br>Silver Spring, MD 20910<br>Telephone: 301-295-0007<br>Fax: 301-295-8025<br>E-mail: Alexandra.l.singer.mil@mail.mil |

**Table 10. SAE Information to Be Reported to the Sponsor**

| **Notification Method** | **Information to be Provided** |
|---|---|
| Email or Telephone (within 72 hours) | IND number, sponsor study number, name of the IP, and investigator name and contact number |
| | Subject identification number |
| | SAE, onset date, date of IP administration, severity, relationship, and subject's current status |
| AND | |
| Email or Fax | Cover sheet or letter |
| | Adverse event case report form |
| | Serious adverse event report form |
| | Concomitant medication case report form or a list of concomitant medications |
| | Medical record progress notes including pertinent laboratory/diagnostic test results |
| NOTE: When submitting SAE reports via email, the subject line of each email notification will read as follows: **SAFETY REPORT – IND # _____, Study #_____, Subject# _____, Event term: _____** | |

In order to comply with regulations mandating sponsor notification of specified SAEs to the FDA within 7 calendar days, investigators must submit additional information as soon as it is available. The sponsor will report unexpected SAEs associated with the use of the challenge strain to the FDA as specified at 21 CFR 312.32 (c).

Investigators must follow all relevant regulatory requirements as well as specific policy at each institution regarding the timely reporting of SAEs to the local IRB and research monitor.

Reporting to the sponsor does not fulfill the investigator's duty to report all unanticipated problems involving risk to human subjects or others to the IRB. The PI will notify the local IRB and the research monitor.

#### 8.8.1.2 Reporting to the IRB

Unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and all subject deaths should be promptly reported by phone, email, or fax to the local JHSPH IRB and NMRC IRB. A written report will follow the initial notification.

Investigators are required to forward safety information provided by the sponsor's representative to the IRB. All SAEs will be reported to the JHSPH IRB according to IRB guidelines.

JHSPH IRB Guidelines: IRB Phone 410-955-3193; Fax 410-502-0584. Investigators are required to promptly report adverse events that fit the following criteria using the Problem/Event Report Form:

Event (including adverse event reports, injuries, side effects, breaches of confidentiality, or other problems) that occurs any time during or after the research study, which in the opinion of the principal investigator:

1. Involved harm to one or more participants or others, or placed one or more participants or others at increased risk of harm;
2. Is unexpected (an event is "unexpected" when it is not described with specificity in the protocol and informed consent document; or if described with specificity, it occurs beyond the expected frequency and/or severity identified); and
3. Is related to the research procedures (an event is "related to the research procedures" if in the opinion of the principal investigator, it was more likely than not to be caused by the research procedures.)

**Table 11. IRB Contact Information**

| IRB | Telephone | Fax | Address |
|---|---|---|---|
| NMRC | 301-319-7276 | 301-319-7277 | 500 Robert Grant Ave<br>Silver Spring, MD 20910 |
| JHSPH IRB | 410-955-3193;<br>1-888-262-3242 | 410-502-0584 | jhsph.irboffice@jhu.edu |

### 8.8.2 Immediately Reportable Events

#### 8.8.2.1 Pregnancy

Each pregnancy must be reported *immediately* **(within 72 hours of identification)** by email or fax to the sponsor and the IRB. The investigator must report any pregnancy on study subjects to the Research Monitor within 14 calendar days of learning of this occurrence.

Subjects who become pregnant after Day 0 through 3 months after the last study visit will be followed to term, and the following information will be gathered for outcome: date of delivery and health status of the

mother and child including the child's gender, height, and weight. Complications and/or abnormalities should be reported including any premature terminations. A pregnancy is reported as an AE or SAE only when there is suspicion that the IP may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy including a spontaneous abortion or an elective termination for medical rationale.

A pregnancy outcome other than abortion, stillbirth, and any malformation/disease as well as follow-up of the infant must be reported by the Investigator within 14 days of learning of its occurrence using local site procedures.

#### 8.8.2.2 AE-related Withdrawal of Consent

Any AE-related withdrawal of consent during the study must be reported *immediately* **(within 24 hours of identification)** by email or fax to the sponsor and the IRB.

#### 8.8.2.3 Pending Inspections/Issuance of Reports

The knowledge of any pending compliance inspection/visit by the FDA, Office for Human Research Protections (Department of Health and Human Services), or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters, or actions taken by any Regulatory Agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to IRB and the sponsor.

### 8.8.3 IND Reporting

#### 8.8.3.1 Annual Reports

The NMRC lead investigator will be responsible for the preparation of a detailed annual synopsis of clinical activity, including adverse events, for submission to the sponsor. Each annual report will summarize IND activity for 1 year beginning approximately 3 months before the IND FDA anniversary date. The sponsor will notify the NMRC lead investigator of the due date with sufficient time for the NMRC lead investigator to assemble the required information.

#### 8.8.3.2 Final Clinical Study Report

A final study report will be prepared in accordance with "Guidance for Industry: Submission of Abbreviated Reports and Synopses in Support of Marketing Applications" and ICH E3 Guideline "Structure and Content of Clinical Study Reports" and provided to the sponsor for review and approval. The sponsor representative will use this report to prepare the final clinical study report for submission to the FDA. The investigative team will report all AEs to the sponsor and the local IRB in the appropriate safety, annual, and/or final reports.

## 8.9 Safety Criteria for Stopping Doses

The PI, along with the research monitor, may determine if certain events warrant discontinuation of challenges and/or IP administration for all subjects in a cohort. If any of the additional following events occur, administration of the IP will be discontinued for all subjects in that cohort, and the PI and the research monitor will undertake a thorough review of the events:

- The occurrence of one or more serious adverse events (SAEs) determined to be related to the IP.
- One serious or unexpected AE evaluated by the PI, research monitor and sponsor determined to be an

unacceptable risk to the health and safety of other subjects.
- Systemic allergic reaction, including but not limited to generalized urticaria, generalized petechiae, or erythema multiforme, occurring in two or more subjects in a group. Bronchospasm or anaphylaxis occurring in any subject.

Based on prior experience with ETEC challenge studies, it is expected that some subjects will have severe AEs (such as severe diarrhea).

AEs which will prompt stopping the BSIgG test article administrations for an individual subject include:

- SAEs unrelated to the test articles (event will be discussed with the medical monitor so as to determine if the event precludes further participation and vaccination.)
- The investigator deems that stopping test article administration is in the best interest of the subject

Additional reasons for subject withdrawal include:

- The subject does not wish to continue with the study
- The subject is lost to follow-up

Further challenge, in accordance with the protocol, may be resumed with the concurrence of the research monitor, sponsor, PI, and the FDA.

## 8.10 Treatment of Adverse Events

Treatment of an AE is the responsibility of the investigator according to the best treatment currently available. The applied measures will be recorded in the eCRF of the subject.

## 8.11 Study Termination Criteria

The PI, research monitor, NMRC IRB, JHSPH IRB, Sponsor, or FDA may stop or suspend the use of this product at any time.

## 8.12 Six Month Follow-up Safety Surveillance

Data will begin to be entered into the study database beginning on or after the inpatient period for a cohort, verified, and subsequently locked. However, approximately 6 months after study completion, subjects will be contacted by phone to track the occurrence of any medically significant new chronic illnesses or serious health events and functional bowel disorder survey. If a subject cannot be contacted after three attempts, a registered letter will be mailed asking them to contact a study investigator. These data will be documented in a telephone log and summarized in an annex to the final clinical study report.

# 9.0 CLINICAL MONITORING

Monitoring will be conducted according to an approved monitoring plan. Local monitoring will commence prior to beginning, at initiation, during the study, and at closeout.

The study monitor shall be available for consultation with the investigator. The study monitor or other authorized representatives of the Sponsor may inspect all documents and records maintained by the investigator, including, but not limited to, medical records (office, clinic or hospital) and pharmacy records for the subject in this study. The clinical study site will permit access to such records. The investigator will obtain, as part of informed consent, permission for authorized representatives of the Sponsor, or regulatory authorities, to review, in confidence, any records identifying individuals in this clinical study.

The investigator will notify the Sponsor within 24 hours following contact by a regulatory agency. The investigator and study coordinator will be available to respond to reasonable requests and audit queries made by authorized representatives of regulatory agencies. The investigator will provide the Sponsor with copies of all correspondence that may affect the review of the current study or his/her qualification as an investigator in clinical studies conducted by the Sponsor. The Sponsor will provide any needed assistance in responding to regulatory audits or correspondence. The investigator will permit independent auditors (employees of the Sponsor or an external company designated by the Sponsor) to verify source data validation of the regularly monitored clinical trial. The auditors will compare the entries in the eCRFs with the source data, and evaluate the study site for its adherence to the clinical study protocol and GCP guidelines and applicable regulatory requirements.

The study team and data management group will arrange visits prior to beginning, at initiation, during the study, and at closeout by the study monitor or designee.

# 10.0 STATISTICAL CONSIDERATIONS

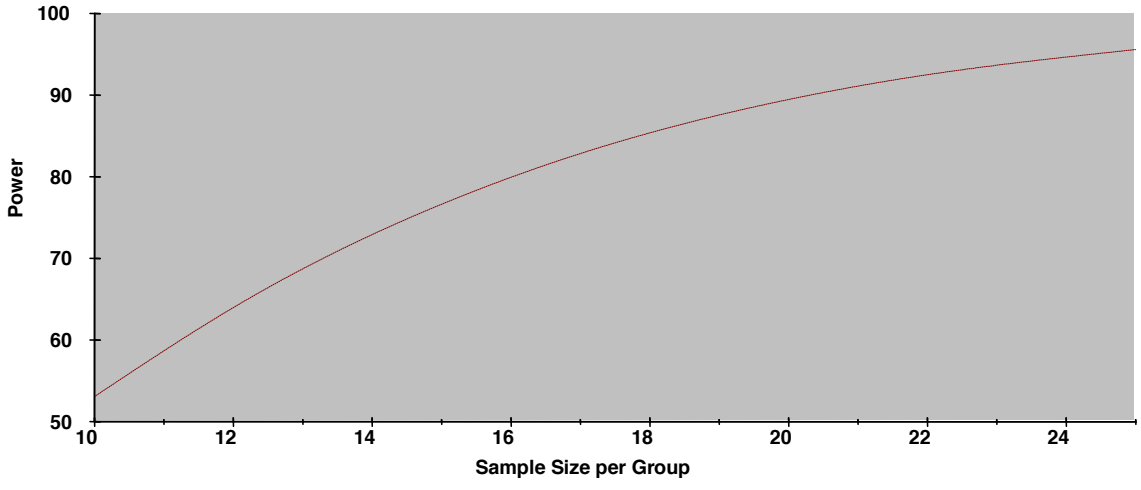
## 10.1 Introduction

Safety, efficacy, clinical outcomes, and immunogenicity data will be entered into the eCRFs using standard software for data management. Data will be edited with standard strategies for range and consistency checks. AEs for all subjects, regardless of the number of doses they have received, will be included in the safety analysis. The null hypotheses for this study are that the diarrhea rates will be the same in groups receiving the placebo and the 1) anti-CS6 BSIgG, 2) anti-B7A whole cell BSIgG.

## 10.2 Sample Size Considerations

The hypothesis being considered is that (1) anti-CS6 BSIgG confers ≥ 60% protective efficacy against diarrhea upon challenge with B7A (in comparison to the placebo group); and (2) anti-B7A whole cell killed BSIgG confers ≥ 60% protective efficacy against diarrhea upon challenge with B7A (in comparison to the placebo group). An attack rate of 87.5% of diarrheal illness has been found with B7A at an inoculum of 1 X $10^{10}$ cfu. For the current study, assuming a one-sided alpha = 0.05 and an attack rate of 80% in the placebo group, the power to detect a preliminary efficacy of 60% in the immunoprophylaxis groups is 90% (Figure 3) when groups each contain 20 subjects.

**Figure 5. Power Curve for Sample Size Calculation**



## 10.3 Analysis

### 10.3.1 Safety

Assessment of BSIgG product safety is limited to the two days prior to receipt of the ETEC challenge dose (day –2 and –1). Unless AEs are temporally related to receipt of BSIgG products, most will likely be attributed to the ETEC inoculum. During each day of the inpatient period, subjects will be monitored for loose stools (not meeting the diarrhea definition), diarrhea, nausea, vomiting, abdominal cramps, fever, headache, abdominal tenderness, abdominal distention and an abnormal abdominal exam. Additionally, subjects will have vital signs taken at least 3 times per day (in cases of moderate to severe diarrhea postural BP and pulse will be taken as necessary for clinical management according to the judgement of the physician/nurse practitioner).

The sample size is designed to indicate trends but not to show statistically significant differences between groups. All subjects who receive BSIgG products or placebo, irrespective of number of doses or receipt of the ETEC challenge will be included in the safety analyses. AEs will be summarized and compared between study groups for the periods prior to and after ETEC challenge. Summaries of the number and proportion of subjects who report a given coded term will be reported. Safety data, including AEs, vital signs, and laboratory tests will be listed by study subject.

### 10.3.2 Protective Efficacy of anti-CS6 and anti-B7A Whole Cell Killed BSIgG Products

The primary endpoint for determination of efficacy is moderate to severe diarrhea occurring during previously defined post-challenge period. However, subjects will be monitored for additional GI and non-GI complaints daily. Side effects, coded as 'possible, probable or definite relationship', as defined in the protocol will be listed (group, time of onset, duration, and severity). During each day of the inpatient period, subjects will be monitored for loose stools (Grade 3-5 not meeting the diarrhea definition), diarrhea, nausea, vomiting, abdominal pain or cramps, fever, headache, abdominal tenderness, abdominal distention or otherwise abnormal abdominal exam, along with solicited symptoms noted above. Vital signs will be taken 3 times a day or more, particularly if the subject meets the study definition for severe diarrhea. All AEs will be summarized and compared between dose groups. Safety data, including AEs, stool information, specified vital signs, and laboratory tests, will be listed by study subject. The planned statistical evaluation is based on the proportion of subjects meeting prospectively defined clinical, microbiological and immunological endpoints. The attack rate will be calculated for all study groups, using the standard definition of: (# with endpoint / # receiving inoculum) x 100%. Summary tables will also be created to detail quantitative and temporal features of the illness such as diarrhea stool frequency and volume, maximum temperature observed, and time to illness and infection. Continuous variables will be analyzed using nonparametric statistics. In addition, tables will be prepared to list each commonly observed adverse event, the number of subjects who experienced an event at least once, and the rate of subjects with adverse event(s). Adverse events will be divided into defined severity grades (mild, moderate, severe and life-threatening).

Data will be analyzed to determine the incidence of diarrheal illness as outlined above among subjects in the placebo vs. each of the passive BSIgG prophylactic groups (with no alpha adjustment for multiple comparisons'). Each rate and PE estimate will be calculated with 95% confidence intervals. Preliminary vaccine PE (%) will be calculated by the formula below:

$$PE\% = \frac{\text{diarrhea incidence (placebo)} - \text{diarrhea incidence (prophylactic)}}{\text{diarrhea incidence (placebo)}} \times 100$$

Additional efficacy analyses will evaluate protection from colonization, moderate and severe diarrhea, moderate and severe abdominal cramps, moderate and severe nausea, as well as evaluating time to diarrhea onset, diarrhea duration (controlling for early antibiotic treatment), need for intravenous re-hydration and early antibiotic treatment.

Initial efficacy analyses will be based on an intent to treat and will include all subjects who receive each of the BSIgG products/placebo and the B7A challenge. A secondary, per protocol analysis will limit the number of subjects evaluated. Subjects who miss more than one dose of BSIgG (or placebo) in the 24 hours prior to receipt of the challenge inoculum will be excluded from this secondary analysis. Subjects who miss more than one dose of BSIgG in the 72 hours following receipt of the challenge inoculum and who do not meet the primary outcome before missing their second dose will also be excluded. A similar analysis will be performed for the secondary outcomes of moderate and severe diarrhea. Analysis of subjects who miss doses not included in this time period will only be descriptive in nature.

### 10.3.3 Immunogenicity

Immunologic outcomes following ETEC challenge will be reported and compared between the three study groups. Analysis will include both ordinal (responder rates) and continuous (geometric mean titers) outcomes. Immunological outcomes will also be summarized in a tabular format and graphed to demonstrate kinetics of response. Qualitative (responder rates) and quantitative assessments (log transformed values) will be analyzed. Median increases (fold rises) of antibody concentrations and seroconversion rates may be calculated along with their 95% confidence intervals. Geometric mean titers may also be determined and presented with their 95% confidence intervals. All statistical tests will be interpreted in a two-tailed fashion using an *alpha* = 0.05 with no alpha adjustments for multiple comparisons.

## 11.0 DATA MANAGEMENT

The investigator will maintain complete and accurate documentation for the study, including records of medical treatments external to the research received during the study, records detailing the progress of the study for subjects, laboratory reports, source documents, signed informed consent forms, drug disposition records, correspondence with the IRB, the study monitor and the sponsor, AE reports, and information regarding subject discontinuation and completion of the study. All required data will be clearly and accurately recorded by authorized study personnel in the eCRFs. Only designated study site personnel will record or change data in an eCRF. The investigator will be responsible for procuring data and for quality of data recorded in the eCRFs. Complete source documentation (study visits, laboratory reports, etc) is kept for each subject in his/her individual study chart. All laboratory specimens, reports, study data collection, and administrative forms will be identified by coded number only to maintain participant confidentiality. eCRFs using coded identifiers will be used to record data for subjects enrolled in the study. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. All information regarding study subjects is kept in password-protected computer files or in locked file cabinets that can be accessed only by authorized study personnel. Samples are identified by coded subject number only. Chart information and information from study records is not released without written permission of the volunteer.

The source documents will be retained at the site. All study related documents will be kept in locked cabinets in locked rooms with limited access. Information in the electronic database is password-protected and access is available only to authorized research team members. Additionally, each authorized research team member is assigned a level of security clearance (also password-protected) with mandatory password changes every 90 days) for the purpose of limiting access to certain areas or functions of the database. Any information printed from this database is stored in locked files until its use is complete and then shredded.

For this study, an electronic data capture (EDC) database system will be used for the collection of the study data. The EDC database system will be designed based on the protocol requirements, the approved eCRF layouts and specifications, and in accordance with 21 CFR Part 11. The eCRF layouts and specifications define and identify the applicable source data that will be collected and captured into the EDC database system. The applicable source data will be electronically transcribed by the site designee into the eCRF in the EDC database system. The investigator is ultimately responsible for the accuracy of the data transcribed on the eCRF. Data monitoring and management will be performed in the EDC database system by the study monitor and the designated Data Management group.

A detailed data management plan will be written and approved by the study team, the PI, and the data management group.

## 12.0 RECORD AND SPECIMEN ARCHIVAL

All records pertaining to this protocol will be stored in a locked filing cabinet at JHU or at an offsite, locked storage facility per regulations for a minimum of 5 years. Access to these records will be limited to researchers in the Enteric Disease Department at NMRC and the JHU CIR as well as those responsible for regulatory monitoring of data to include representatives of the DoD and JHU. A copy of study records will be made available to the Sponsor. The investigator will obtain permission from the sponsor in writing before destroying any study records and the sponsor will notify the investigator in writing when records can be destroyed. Relevant IRBs will be notified in writing prior to destruction of any research records. Specimens will be stored indefinitely in the JHU or the ETEC laboratory at NMRC.

## 13.0 OBLIGATIONS AND ROLES OF THE SPONSOR, INVESTIGATOR AND STUDY PERSONNEL

This study will be conducted using GCP and in accordance with all federal regulations regarding the protection of human participants in research including The Nuremberg Code, The Belmont Report, US 21 CFR Part 50 – Protection of Human Subjects, 32 CFR 219 (The Common Rule) and all regulations pertinent to the Department of Defense.

The investigators agree to conduct the research in strict accordance with this protocol, the ICH Guideline for GCP (CPMP/ICH/135/95), as well as in conformity with any federal, provincial or local regulations regarding the conduct of clinical studies. The sponsor and investigator must comply with all applicable regulations. In addition, the investigator must follow local and institutional requirements including, but not limited to, IP, clinical research, informed consent and IRB regulations. The Sponsor will provide notification to the investigator of protocol and amendment approvals by regulatory authorities when applicable. Except where the investigator's signature is specifically required, it is understood that the term "investigator" as used in this protocol and on source documents refers to the investigator or appropriate study personnel that the investigator designates to perform a certain duty. The investigator is ultimately responsible for the conduct of all aspects of the study. Sub-investigators or other appropriate study personnel are eligible to sign for the investigator on designated source documents.

## 14.0 QUALITY CONTROL AND ASSURANCE

### 14.1 QA/QC monitoring

During the study, the investigator will maintain complete and accurate documentation for the study, including records detailing the progress of the study for each subject, laboratory reports, eCRFs, signed informed consent forms for each study subject, drug disposition records, correspondence with the IRB, the study monitor and the sponsor, adverse event reports and information regarding subject discontinuation and

completion of the study. All required study data will be clearly and accurately recorded by authorized study personnel in the eCRFs. Only designated study site personnel shall record or change data in an eCRF. During the study, the investigator will be responsible for the procurement of data and for quality of data recorded in the eCRFs. The study monitor will ensure accuracy of the eCRFs.

## 14.2 Protocol Deviation Management

All amendments to the protocol, consent form and/or questionnaires, including a change of PI, will be submitted to the JHSPH IRB and NMRC IRB for review and approval prior to implementation. Other-than-minimal-risk changes and all unanticipated major problems involving human subjects or others will be reported promptly to the IRBs, and no such changes will be made to the research without IRB approval unless necessary to eliminate apparent immediate hazards to human subjects. Minor minimal-risk deviations necessitated during the course of the trial will be made on site as needed, and documented for subsequent review within a reasonable time period. Deviations from the protocol that potentially impact on subject safety will be promptly reported to the Research Monitor, IRBs, and the Sponsor. Other deviations will be reported at the time of continuing review.

# 15.0 HUMAN SUBJECTS PROTECTIONS CONSIDERATIONS

## 15.1 Risks / Benefit

### 15.1.1 Risks

The BSIgG products are expected to be safe with possible mild to moderate discomfort likely related to consumption of the sodium bicarbonate buffer, such as bad taste, bloating, nausea, gas, etc. As with any investigational drug or biologic, there is a possibility of severe allergic reaction.

Naturally acquired illness caused by ETEC ranges from mild-to-severe watery diarrhea. Nausea, vomiting, abdominal cramping, headache, abdominal gurgling or gas, anorexia, fever, muscle and/or joint aches, and malaise, may occur. For most adults the illness is not life threatening but often leads to mild to moderate dehydration and significant inconvenience associated with loss of sleep and activity. Study facilities will have personnel and resources capable to manage diarrheal illness and potential complications. Side effects to the antibiotic (ciprofloxacin) used to treat the ETEC infection are possible.

Therapeutic antibiotics for use in this study are licensed approved medications that have been used extensively and shown to be very safe with only rare side effects. The most commonly reported side effects for ciprofloxacin are gastrointestinal symptoms (nausea, vomiting, and diarrhea) in as many as 5 persons in 100. Other reported symptoms in less than 1 person in a 100 include rash, dizziness, and headache. Rarely, allergic reactions to these medications have been observed. Ciprofloxacin is not recommended for use in pregnancy due to concerns of joint damage to the unborn child (based on studies in young animals). Pregnancy is exclusionary for study participation and is documented through testing prior to study interventions and provided discussion on methods to prevent pregnancy during study. Fluoroquinolones, including ciprofloxacin, are associated with an increased risk of tendonitis and tendon rupture in all ages. The risk of developing fluoroquinolone-associated tendonitis and tendon rupture is further increased in older patients usually over 60 years of age, in patients taking corticosteroid drugs, and in patients with kidney heart or lung transplants, all of whom are excluded from this study. *Clostridium difficile* associated diarrhea (CDAD/pseudomembranous colitis) has been reported with use of nearly all antibacterial agents.

Good nursing practices are performed during blood draws, which minimizes the risk to the subject. Hand-washing and sanitary disposal of feces (including pretreatment with bleach) are the main elements of personal hygiene and will minimize the spread by person-to-person infection; hand washing will be emphasized to the

subjects and subjects will be instructed not to share food or beverages. Subjects and staff will be trained in proper techniques of hand washing. Subjects will be instructed as to the importance of completing the 3-day course of antibiotics and this instruction will be documented. Risk of secondary transmission is highly unlikely due to antibiotic treatment and because subjects are required to submit two confirmed, consecutive negative stool samples prior to discharge.

There is a minimal risk of pain, hematoma or infection at the site of venipuncture. The maximum amount of blood drawn from a subject in total, and daily, will fall within applicable regulations.

There may be physical, psychological and social risks if subjects test positive for hepatitis B, hepatitis C and/or HIV. Subjects testing positive will be counseled and referred for treatment.

Recent studies also suggest an increased risk of post-infectious irritable bowel syndrome (PI-IBS) following bacterial enteritis, and infection with ETEC has been found to be associated with these sequelae [35, 115-117]. PI-IBS, a functional bowel disorder characterized by unexplained abdominal discomfort or pain associated with changes in normal bowel patterns, has been described in a recent systematic review to occur 6-7 times more frequently after an acute enteric infection compared to similar matched controls without such a history [118].

Medical records associated with this protocol are subject to provisions of the Privacy Act of 1974, 5 U.S.C., Section 552A, and AR 340-21. All data and medical information obtained about subjects will be considered privileged and held in confidence. Subjects will not be identified by name in any published report/presentation of the results. Complete confidentiality cannot be promised to subjects who are military personnel, because appropriate medical command authorities may require reporting information bearing on the health of their personnel. Representatives of the Sponsor, NMRC IRB, JHSPH IRB, or FDA may inspect the records of this research as part of their responsibility to oversee research and ensure protection of subjects. Study results and data may be published in scientific/medical journals; the identity of individual subjects will not be disclosed.

### 15.1.2 Risk Mitigation Strategies

Subjects will be questioned and examined daily for evidence of infection and diarrhea complications. Vital signs will be recorded at least three times per day. Based on prior studies, infected subjects tend to develop illness with incubation periods of approximately 1-3 days. Therapeutic benefit seems to be optimal if treatment is given within the first three days of symptom onset. The risk of diarrhea complications will be minimized by a conservative approach to timing of antibiotic administration well within an interval that has been shown to be efficacious as well as daily clinical monitoring. Stool output will be closely monitored. The plan will be to treat all subjects no later than day 5 post-dosing.

Aggressive fluid management will be undertaken to ensure the most common complication, dehydration, does not occur. The procedures to institute early oral and/or intravenous rehydration therapy are detailed above. In addition to rehydration therapy, prospectively defined criteria and procedures to institute early antibiotic therapy are also fully described above. In order to ensure clinical resolution and limit the potential for secondary spread upon discharge, predefined discharge criteria have been established. Subjects will be discharged from the inpatient phase of the study when clinical symptoms are resolved or resolving AND two consecutive stool cultures are negative for ETEC.

Systemic or severe gastrointestinal complications rarely occur with ETEC infection. The following clinical findings necessitate immediate consideration and management of complicated enteritis:

- Physical examination compatible with an acute abdomen

- Severe GI bleeding (any evidence of GI blood loss other than hemoccult positivity only, with evidence of hemodynamic instability, decrease in hemoglobin, hypovolemia)
- Sepsis (high fever: temp. >102ºF (39ºC), rigors, hemodynamic instability).

Any of these findings require prompt clinical management and discussion with the independent Research Monitor.

The ETEC strain has the potential for risk to both the environment and to the research personnel; however, the risk to the environment in regards to potential transmission outside of the CIR facility is low. There is a minimal risk of acquiring ETEC infection associated with subject inoculum administration, patient care activities on the ward, or processing ETEC-infected stool. The risk to the environment will be reduced by ensuring that all human waste products from inpatients are disinfected with bleach prior to disposal, ensuring all subjects comply with discharge criteria (two consecutive negative stool cultures for ETEC), emphasizing importance of handwashing for subjects and staff, ensuring proper disposal/cleaning of linen, and cohorting subjects in the CIR while shedding ETEC. Additionally, subjects will not be discharged until they are no longer shedding the challenge strain as per procedures outlined in the protocol.

Subjects with prior history of abnormal bowel patterns who might be at higher risk of post-infectious sequelae are excluded. Predefined criteria to assure early treatment as appropriate also may further reduce risk of post-infectious sequelae and is likely to reduce the risk associated with PI-IBS given the positive association between diarrheal illness duration and PI-IBS risk [119] .

There is no risk associated with collecting stool samples; however slight discomfort is possible when using rectal swabs. A breach of confidentiality in which private health information is made public is possible. There may be physical, psychological and social risks if subjects test positive for hepatitis B, hepatitis C and/or HIV. Subjects testing positive will be counseled and referred for treatment. Medical records associated with this protocol are subject to provisions of the Privacy Act of 1974, 5 U.S.C., Section 552A, and AR 340-21. All data and medical information obtained about subjects will be considered privileged and held in confidence. Subjects will not be identified by name in any published report/presentation of the results. The sponsor and the FDA may inspect the records of this research as part of their responsibility to oversee research and ensure protection of subjects. Study results and data may be published in scientific/medical journals; the identity of individual subjects will not be disclosed.

### 15.1.3 Benefits

There is no benefit that can be guaranteed to subjects for participating in this research study. However, there is potential societal benefit of the development of a product to prevent ETEC.

## 15.2 Subject Compensation

Compensation for participation will occur as detailed below. Compensation will be provided only for completed study procedures designated for compensatory payment. If a Subject is eligible to participate in the investigational protocol after screening, and s/he completes all study visits, procedures and follows all the rules s/he will receive the following compensation:

If enrolled in the study, the Subject will be compensated for participation time and travel in this trial as follows:

- $80 total for screening (only if enrolled in the study or presents as an alternate)
- $2,400 for the inpatient period (as long as all study requirements are met)

- $80 for outpatient study visit: Days 28
- $60 for the follow up telephone contact: Day 180
- $400 bonus upon completion of inpatient phase and outpatient visits

The payment schedule is:

- $2,480 at the completion of the inpatient period (approx. Day 8)
- $480 on Day 28
- $60 after completion of the telephone contact follow up, Day 180

Maximum compensation is $3,020 for participation.

If a subject is not eligible for discharge on day 8 because of illness or not having 2 consecutive negative stool culture results s/he will receive $200 per additional inpatient day. Subjects will not be paid for missed outpatient visits, and may forfeit some or all of their bonus as a result of missed visits or non-compliance.

For active duty military subjects, compensation for this study depends on duty status. By regulation, active duty personnel and federal employees can be compensated only for visits in which blood draws occur, and then only $50 per visit, unless the visits occur during off-duty hours or when they are on leave. If the volunteer is off-duty or on leave, he or she will be paid the same as non-military/non-federal personnel. The total amount of compensation may vary depending on the number of visits completed.

- $50 total for screening (only if enrolled in the study or presents as an alternate)
- $250 for the inpatient period (as long as all study requirements are met)
- $50 for outpatient study visit: Day 28
- $0 for the follow up telephone contact: Day 180
- $0 bonus upon completion of inpatient phase and outpatient visits

Maximum compensation is $350 for participation of an active duty service member.

## 15.3 Research-Related Injury

All study-related medical care will be provided to subjects without cost. Should a subject be injured as a direct result of participating in this research project, s/he will be provided medical care by the staff at the Walter Reed National Military Medical Center (or other military-affiliated medical center), at no cost to the subjects, for that injury. The subjects will not receive any injury compensation, only medical care. The subjects will not be compensated for care if s/he chooses to seek care from his/her own physician.

If a subject is injured because of participation in this research and is a DoD healthcare beneficiary (e.g., active duty in the military, military spouse or dependent), the subject is entitled to medical care for that injury within the DoD healthcare system, as long as the subject remains a DoD healthcare beneficiary. This care includes, but is not limited to, free medical care at Army hospitals or clinics.

If a subject is injured because of participation in this research and is not a DoD healthcare beneficiary, the subject is entitled to free medical care for that injury at a DoD hospital or clinic. It cannot be determined in advance which DoD hospital or clinic will provide care. If the subject receives care for research-related injuries outside of a DoD hospital or clinic, the subject or the subject's insurance will be responsible for medical expenses.

During the challenge phase, subjects who require medical treatment beyond what can be provided safely at the CIR will be transferred to the Johns Hopkins Hospital or Johns Hopkins Bayview Medical Center for care. If a subject is injured during the study, the study doctor will help the subjects find medical care. Medical care at Johns Hopkins is open to all subjects as it is to all sick or injured people. Neither Johns Hopkins Bloomberg School of Public Health nor the John Hopkins Hospitals have any plan to provide compensation to the subjects if they experience injury or other bad effects which are not the fault of the study doctors. Subjects will only be treated for injuries that are directly caused by the research study. In the event this occurs, the sponsor agrees to reimburse the Hospital for all reasonable expenses incurred by the Hospital in providing medical treatment and/or hospitalization reasonably necessary to address any injury to a Subject that, in the reasonable judgment of Hospital and Sponsor, occurs directly as a result of the administration of the IMPs or performance of study procedures in accordance with the Protocol, but only to the extent such expenses are not:

- the result of a foreseeable side effect as indicated in the Protocol
- reimbursed by (or submitted for reimbursement to) the Subject's insurance or any governmental program or other third-party payer providing medical or hospital coverage; provided, however, that this provision shall not obligate Hospital to submit such costs to the prospective Subject's insurance or any governmental program or other third-party payer coverage
- attributable to a failure of Hospital, or any of the Investigator Personnel, including PI, to adhere to the terms of the Protocol, Sponsor's written instructions or Applicable Law
- attributable to the negligence, recklessness or willful misconduct or omission of Hospital or any of its Investigator Personnel, including PI
- attributable to a pre-existing abnormal medical condition or underlying disease of the Subject or treatment that would have been provided to the Subject in the ordinary course notwithstanding participation in the study, or
- attributable to the failure of the Subject to follow the reasonable instructions of Investigator Personnel or Subject's physician.

Transportation to and from military hospitals or clinics will not be provided. No reimbursement is available if the subject incurs medical expenses to treat research-related injuries from outside or private providers. No compensation is available for research-related injuries. The subject is not waiving any legal rights. The subject should contact the PI if the subject believes he or she has sustained a research-related injury. The subject should contact the PI for any questions.

Requests for other benefits, such as compensation for lost time from work, are processed independently of this protocol. Military members retain the right to pursue military disability benefits, and Federal civilian employees retain the right to pursue relief through established workers compensation processes, but neither military disability benefits nor workers compensation benefits are guaranteed. The right of other parties to seek redress against the United States Government is limited to that set forth by existing agency regulations and the Federal Tort Claims Act. The subject should understand that this does not constitute a waiver or release of legal rights. This issue is addressed in the informed consent and will be discussed with the subject by the investigator or designee before the subject signs the informed consent to participate in the study.

## 15.4 Compensation for Investigators

There is no financial compensation for investigators in this study. All investigators will be required to complete a form for the disclosure of significant financial interest.

## 15.5 Fair and Equitable Selection of Subjects

Subjects will not be discriminated against on the basis of race, sex, or religion. Due to the early stage of development of this IP, we have excluded individuals under 18 and women who are pregnant or nursing and we have excluded individuals who are over the age of 50 due to the frequency of exclusionary medical conditions. Any individual who is unable to consent due to any reason will not be included in this study.

## 15.6 Informed Consent

The informed consent process and document(s) will be reviewed and approved by the NMRC IRB and the JHSPH IRB prior to initiation of the study. The consent document(s) will contain a full explanation of the possible risks, advantages, and alternate treatment options, and availability of treatment in the case of injury, in accordance with 21 CFR 50. Subjects will receive an oral presentation of the study in language (i.e., using lay terms as appropriate) they can understand. Subjects will be given the written, IRB-approved informed consent, allowed ample time to read the consent, allowed to ask questions about the study, have the questions answered, and given time to decide if he/she would like to participate in the study. To document subjects' understanding of informed consent, immediately before the consent is signed, the person obtaining consent will administer a brief quiz or comprehension test. A subject must achieve ≥70% correct to be eligible for inclusion in the study. Incorrect answers will be discussed with subjects to reinforce the consent. Subjects who fail the comprehension test on the first attempt will be given one additional opportunity, either on the same day or another day, to take the test after reviewing the quiz, re-reading the consent, and listening to the study brief again. A final acceptable test score is ≥70% answered correctly. Subjects failing the comprehension test on the second attempt are not eligible for study enrollment. No coercion or influence is allowed in obtaining subjects' consent. Before subjects participate in the study, consent forms will be signed and dated by subjects as well as by the PI or designee. Subjects will receive copies of the signed consent prior to participation. As part of the consent process, subjects will also be asked to read and sign a Medical Records/Lab Results Release, with an opportunity to ask questions, if relevant. Subjects will also be asked to sign a separate information form for HIV-1 testing. The consent document indicates that by signature, the subject, or where appropriate, legal guardian, permits access to relevant medical records by the sponsor's representative and by representatives of the FDA. The sponsor's representative will submit a copy of the initial IRB- and sponsor's representative-approved consent form to the FDA and will maintain copies of revised consent documents that have been reviewed and approved by the IRB/ethics committee.

A written informed consent document, in compliance with 21 CFR Part 50, 32 CFR Part 219, the Belmont Principles will be signed by the subject before any study-related procedures are initiated for that subject. This consent document must be retained by the investigator as part of the study records. The investigators or their designees will present the protocol in lay terms to individual subjects. Questions on the purpose of the protocol, protocol procedures, and risks to the subjects will then be solicited. Any question that cannot be answered will be referred to the PI. The subject will be allowed to take the consent document home to consider and discuss it with others and return to the CIR at a later time to sign it. The subject should understand that the study product is investigational and is not licensed by the FDA for commercial use, but is permitted to be used in this clinical research. Informed consent includes the principle that it is critical the subject be informed about the principal potential risks and benefits. This information will allow the subject to make a personal risk versus benefit decision and understand the following:

- Participation is entirely voluntary.
- Subjects may withdraw from participation at any time.
- Refusal to participate involves no penalty.
- The individual is free to ask any questions that will allow him/her to understand the nature of the protocol.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US law.

All non-exempt research involving human subjects shall, at a minimum, meet the requirement of 32 CFR 219.116(a)(6) in the Code of Federal Regulations.

### 15.7 Recruitment

Newspaper ads and study fliers posted on the JHU campus and community bulletin boards will be used to recruit prospective subjects. Additionally, subjects in previous studies that have expressed interest in participating in future trials will be contacted about the proposed study. All study-related advertisements will be reviewed and approved by the JHSPH IRB, NMRC IRB and HRPO-ORP, if applicable. Subjects responding to the advertisements by a phone call to the center will be screened for eligibility based on a standard screening questionnaire administered by the CIR recruiter. Some elements of the inclusion/exclusion criteria will be discussed with the subject at that time and a preliminary determination will be made regarding the individual's eligibility for study participation. Active duty military members will not specifically be recruited for this study.

## 16. PRIVACY AND CONFIDENTIALITY

### 16.1. Storage of Data and Samples

All original records involving this protocol will be stored securely at CIR or a locked, offsite storage facility for at least 5 years. Copies of databases will be stored securely at NMRC (and made available to the Sponsor). All samples will be stored under appropriate conditions in laboratories in the Enteric Disease Department at NMRC and/or the JHU laboratories.

### 16.2. Provisions Protecting Privacy and Confidentiality

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties, other than those cited below, is prohibited. Subject confidentiality will be further ensured by utilizing subject identification code numbers and subject initials. Neither NMRC nor the JHSPH are HIPAA-covered entities.

Confidentiality agreements may be developed with other clinical trials groups (e.g. at the University of Maryland Vaccine Research Center or Walter Reed Clinical Trials Center), and the investigative team may check verbally with these sites to see if subjects have participated in studies that would preclude their participation in this study. No written list will be exchanged with these sites.

### 16.3. Safeguards for Vulnerable Subjects

This study will not include individuals less than 18, incarcerated or unable to meet the requirements to sign the informed consent form. Military personnel will not be specifically recruited for this study. All active duty military subjects will need to have written permission from their superior to participate in this study.

## 17. PROTOCOL REVIEW PROCESS

The protocol will undergo scientific and ethical review at the two primary collaborating institutions: CIR and NMRC. In addition to these reviews, the JHU Biosafety Committee and Pharmacy and Therapeutics Committee will review the protocol. The protocol will also require FDA review as part of the IND application. The IND sponsor will be Dr. Louis Bourgeois. Continuing review will be undertaken in accordance with existing regulations.

The investigator may deviate from the protocol without prior approval when the change is necessary to eliminate an apparent immediate hazard to the subject. In that event, the investigator will notify the sponsor promptly by phone, will notify the CIR (JHSPH IRB) and NMRC IRB, and will confirm notification to the sponsor in writing within 5 working days after the change is implemented. All protocol deviations, including minor deviations not impacting subject safety, will be noted in the continuing review reports, the annual report to the Sponsor, and in the final study report. Any modification to the protocol, consent form and/or questionnaires, including changing the PI, must be submitted to both IRBs for review and approval prior to implementation of the modification. Any deviation to the protocol that may have an effect on the safety or rights of the subject or the integrity of the study must be reported to the NMRC ORA, JHSPH IRB and USAMRMC HRPO-ORP, if applicable, as soon as the deviation is identified.

## 18. PUBLICATION POLICY

All data collected during this study will be used to support this IND. All publications and presentations are governed by the standards and norms detailed in NAVMEDRSCHCENINST 5721.1. All authors will submit the proposed publication/presentation at least 30 days prior to the submission date. Prior to submission, the directorate will conduct a substantive scientific and professional review. The document is routed to the Office of Research Administration (ORA) for review and routing for Command review and approval, ultimately by the NMRC Public Affairs Officer. Once it is cleared at NMRC, it will be forwarded to BUMED through NMSC, if appropriate. Prior to publication, an author must have a completed Publication Clearance Request Submission Form with signatures from all approving and reviewing authorities.

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