Immunologic Responses to a Live Attenuated Oral Cholera Vaccine

Protocol Number: Cholera 1

Sponsored by:

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Principal Investigator:

Nadine Rouphael, MD

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2

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Statement of Compliance

The trial will be carried out in accordance with Good Clinical Practices (GCP) as required by the following:

- United States Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 54, 21 CFR Part 56, and 21 CFR Part 312);
- International Conference on Harmonization (ICH) E6; 62 Federal Register 25691 (1997);

Compliance with these standards provides public assurance that the rights, safety and wellbeing of study subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Investigator:

Signed: _____ Date: _____

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Protocol Summary

Title: Immunologic Responses to a Live Attenuated Oral Cholera Vaccine

Population: 10 subjects, 18 to 49 years old, who are in good health and meet the eligibility criteria

Clinical Site: The Hope Clinic of the Emory Vaccine Center, Emory University

Study Duration: Approximately 15 months

Subject Duration: Approximately 12 months

Description of Agent: One dose delivered orally of the FDA-approved live attenuated cholera vaccine

Objectives:

Primary:

- To evaluate the antibody response to the cholera vaccine in healthy subjects.

Secondary:

- To evaluate additional markers of the adaptive immune response including plasmablasts, activated B cells, memory B cells, T cell responses in healthy subjects receiving cholera vaccine.
- To produce monoclonal antibodies against cholera.
- To evaluate the safety and reactogenicity in healthy subjects receiving cholera vaccine.

Endpoints:

Primary:

- Level of antibody titers at days 1 (prevaccination) and 29.

Secondary

- Levels of plasmablasts, activated B cells, memory B cells, and T cell responses at days 1, 8, 11, 15, and/or 29.
- Correlations between antibody titers and the levels of plasmablasts, activated B cells, memory B cells, T cell responses corresponding time points.
- Production of monoclonal antibodies against cholera.

- Solicited and unsolicited adverse events in healthy subjects receiving cholera vaccine at day 8 and day 29 respectively.
- Serious adverse events in healthy subjects receiving cholera vaccine for the duration of the study.

KEY ROLES

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1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Background Information

Cholera is a life-threatening diarrheal illness caused by the ingestion of toxin-producing bacteria *Vibrio cholerae.*[1] Annually, there are approximately 1.3 to 4.0 million cases of cholera worldwide, resulting in 21,000 to 143,000 deaths.[2] The bacteria is spread through fecal contamination of water or food. Up to 50 countries with limited access to clean water are more susceptible to outbreaks.[2] In the United States most cases are linked to travel to endemic countries; however, there are 10-15 cases acquired locally each year resulting from undercooked seafood.[1]

Cholera is highly contagious and shed in the feces of all carriers. The spectrum of illness can vary from asymptomatic colonization to severe acute watery diarrheal illness that can result in death by dehydration in a previously healthy individual within hours.[2] Two serogroups of *Vibrio cholerae,* O1 and O139, are known to cause illness. In survivors, infection with cholera leads to protective immunity; which starts to wane after 5 years and returns to baseline at 10 years.[3] Recently Dr. Wrammert's Laboratory characterized the human response to cholera at the single-plasmablast, monoclonal antibody level.[3] This study identified novel antigenic targets and uncovered both the basis of cross-reactivity between different *V. cholerae* serotypes and the impact of prior enterotoxigenic *Escherichia coli* exposure on the response to cholera.

Vaxchora, (PaxVax, San Diego, CA), is a live attenuated cholera vaccine provides immunity against *V. cholerae* serogroup O1 and has been approved by FDA since June 2016. Vaxchora is not effective in protecting against serogroup O139, the other serogroup able to cause disease in humans. Since October, 2016, this vaccine has been recommended by the ACIP for certain travelers 18 through 64 years of age going to cholera-affected areas.[4] Efficacy has not yet been evaluated in individuals previously exposed to cholera, through illness or vaccination, or in populations living in countries where cholera is endemic.[4] Outside the United States, there are two whole cell killed cholera vaccines available (Dukoral and Shanchol). While these have shown modest efficacy, the longevity of protection is very poor.

Here we attempt to better understand the immune responses to the live-attenuated cholera vaccine (Vaxchora) in healthy naïve subjects and compare these responses to those observed in cholera infected patients. We will also generate monoclonal antibodies that in addition to increasing our understanding of the biology of the vaccine induced immune responses, may potentially be used for cholera therapy, which is currently limited

to antibiotic use and oral/IV rehydration therapy, or for diagnostic purposes.

1.2 Rationale

The immune responses to cholera vary between natural infection and vaccination in terms of magnitude, quality and longevity. Here we attempt to better understand the immune responses to cholera vaccine in healthy subjects who are naïve to cholera vaccination or infection.

1.3 Potential Risks and Benefits

The study utilizes an FDA-approved live cholera vaccine, Vaxchora. See package insert for full risks and benefits of the vaccine.[4]

1.3.1 Potential Risks

The potential risks to subjects are those associated with oral administration of the live cholera vaccine, possible reactions to the vaccine and having blood drawn.

The potential risks of receiving the live cholera vaccine include but are not limited to: tiredness (30%), headache (28%), abdominal pain (18%), nausea/vomiting (17%), lack of appetite (16%), diarrhea (4%), and fever (0.6%).[4] If any participant develops severe watery diarrhea we will clinically assess the patient and facilitate access to treatment

Clinical trials found no related serious adverse reactions to the Vaxchora vaccine. Rarely, a subject may experience an allergic reaction, which produces rash, hives, or difficulty breathing. Vaccine products have been shown to be shed in stool for at least one week following administration, posing a potential risk.

Blood sample collection involves transient discomfort and may cause fainting, which is managed by having the subject lie down prior. The blood draw site may bruise, and this can be ameliorated by holding pressure to this site following the blood draw. The sites of blood draw are potential sites of infection, but this risk is made very unlikely by the use of sterile technique.

1.3.2 Known Potential Benefits

While individuals are generally not at risk for acquiring cholera in the United States, the vaccine can offer protection to subjects if traveling to endemic countries**.** The benefit for the study is predominantly scientific, allowing a better understanding of the immune

response to this particular vaccine and possibly production of monoclonal antibodies. However, individuals who receive this vaccine are likely to receive some level of protection from future V. cholerae exposures.

2 OBJECTIVES

2.1 Study Objectives

Primary:

- To evaluate the antibody response to the cholera vaccine in healthy subjects.

Secondary:

- To evaluate additional markers of the adaptive immune response including plasmablasts, activated B cells, memory B cells, and T cell responses in healthy subjects receiving cholera vaccine.
- To produce monoclonal antibodies against cholera.
- To evaluate the safety and reactogenicity in healthy subjects receiving cholera vaccine.

2.2 Study Outcome Measures

Primary:

- Level of antibody titers at days 1 (pre-vaccination) and 29.

Secondary

- Levels of plasmablasts, activated B cells, memory B cells, and T cell responses at days 1, 8, 11, 15, and/or 29.
- Correlations between antibody titers and the levels of plasmablasts, activated B cells, memory B cells, T cell responses corresponding time points.
- Production of monoclonal antibodies against cholera.
- Solicited and unsolicited adverse events in healthy subjects receiving cholera vaccine at day 8 and day 29 respectively.
- Serious adverse events in healthy subjects receiving cholera vaccine for the duration of the study

3 STUDY DESIGN

This study will be a prospective pilot study, with 10 subjects total, who are between ages 18 and 49 years of age, in good health and meet all eligibility criteria. Each subject will make a total of 7 visits to the Hope Clinic. Subjects will be recruited from the Atlanta metro area.

4 STUDY POPULATION

4.1 Selection of the Study Population

The target sample size will be 10 subjects who will receive the Vaxchora live cholera vaccine. With the exception of the inclusion/exclusion criteria, there will be no intentional recruitment of particular ethnic or racial groups. Subjects will be screened for eligibility according to the inclusion/exclusion criteria by history. Informed consent will be obtained for study participation.

4.2 Inclusion/Exclusion Criteria

Subjects eligible to participate shall meet all of the following **inclusion criteria:**

1. Capable of informed consent and provision of written informed consent before any study procedures.
2. Capable of attending all study visits according to the study schedule.
3. Males or females between the ages of 18 and 49 years of age (inclusive).
4. Are in good health, as determined by medical history and targeted physical exam related to this history.
5. Female subjects of childbearing* age must have a negative urine pregnancy test before study vaccination, and must use two forms of contraception** to avoid pregnancy within one month of Vaxchora administration.

** Not sterilized via tubal ligation, bilateral oophorectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at least 90 days after the procedure, and still menstruating or <1 year of the last menses if menopausal.*
***Includes, but is not limited to, non-male sexual relationships, abstinence from sexual intercourse with a male partner, monogamous relationship with vasectomized partner who has been vasectomized for 180 days or more prior to the subject receiving the first study vaccination, barrier methods such as condoms or diaphragms with spermicide or foam, effective intrauterine devices, NuvaRing®, and licensed hormonal methods such as implants, injectables, or oral contraceptives ("the pill).*

Subjects eligible to participate shall not meet any of the following **exclusion criteria**:

1. Have an acute illness within 72 hours before vaccination.
2. Have any acute or chronic medical condition that, in the opinion of the principal investigator, would make vaccination unsafe or interfere with the evaluation of immune response to study vaccination.

3. Have a suppressed immune system as a result of illness, immunosuppressive medication, chemotherapy, or radiation therapy within 3 years prior to study vaccination.
4. Have taken oral or parenteral corticosteroids of any dose within 30 days before study vaccination.
5. Reside with individuals under the age of 2 or with an immunocompromised individuals.
6. Have a known history of autoimmune disease.
7. Have a history of Guillain-Barre Syndrome.
8. Have plans to receive any vaccine from 28 days prior to study vaccination until Day 29.
9. Has previously received a cholera vaccine or have a known history of cholera infection.
10. Have donated blood or blood products within 56 days before study vaccination, plan to donate blood at any time during the 56-day duration of subject study participation, or plan to donate blood within 56 days after the last blood draw.
11. Have known hypersensitivity or allergy to any component of the vaccine or history of anaphylaxis with a vaccine or vaccine component.
12. Have allergy to tetracycline and/or ciprofloxacin.
13. Are pregnant or breastfeeding or plan to within one month of vaccination.
14. Travelled to a cholera endemic area and had traveler's diarrhea in the previous 5 years.
15. Have abnormal stool pattern (fewer than 3 stools/ week or greater than 2 stools/ day) or regular use of laxatives in the last 6 months.
16. Have current or recent antibiotic use in the past 14 days.
17. Are healthcare workers who have direct contact with patients who are immunocompromised, have unstable medical conditions, or are under the age of 2.
18. Are childcare workers who have direct contact with children who are 2 years or younger.
19. Are workers in the food industry.
20. Have received any vaccine within the previous 21 days.

5 STUDY PROCEDURES/EVALUATIONS

5.1 Study Procedures

Medical history will be obtained by interview of study subjects on Day 1 (Visit 1) prior to the study vaccination. Subjects will be asked about a known history of significant medical disorders, cancer, immunodeficiency, allergies, psychiatric illness, substance use, and autoimmune diseases.

Medications history will include a review of all current medications and any medications taken in the last 30 days before study vaccination. Medications included in this history will include prescription medication, over-the-counter medication, vitamins, supplements, and prohibited treatments listed in the above Inclusion/Exclusion Criteria section, such as antibiotics within the last 14 days and vaccines.

On Day 1 (Visit 1) and before the study vaccination, a targeted physical examination may be performed by the investigator, who is licensed to make medical diagnoses. At visits 2-7, a targeted physical examination may be performed based on interim health history.

Vital signs (oral temperature, pulse rate, and blood pressure) will be measured at all study visits.

Height and weight will be measured on Day 1 (Visit 1) before study vaccination

Subjects will be observed in the clinic for at least 20 minutes after study vaccination on Day 1 (Visit 1).

5.1.1 Laboratory Evaluations/Assays

Urine pregnancy tests will be performed by the site laboratory on the same day as and prior to study vaccination (Day 1). Results must be negative and known prior to study vaccination.

5.1.2 Special Assays or Procedures

The immunologic assays will be performed at Dr. Wrammert's Laboratory on the Emory University main campus.

5.1.3 Specimen Collection, Preparation, Handling and Analyses

Blood will be collected at each of the seven study visits as per the Study Schedule in Appendix A.

6 STUDY SCHEDULE

6.1 Screening/Enrollment/Baseline, Visit 01, Day 01

Study subjects will be given a description of the study and have an opportunity to have their questions and concerns addressed by the study principal investigator or designee.

Subjects will be given the informed consent form to read and discuss with study staff, and if they wish to enroll will sign the document before any study vaccination, procedures, or lab draws are performed.

A medical history and targeted physical exam, if necessary, as outlined in the Study Procedures section will be conducted in order to assess for compliance with study inclusion and exclusion criteria.

A list of any medications taken by subjects in the past 30 days and those to be taken for 28 days after vaccinations will be documented on the appropriate study form.

A urine pregnancy test will be conducted for female subjects, and a negative result will be required and be documented on the appropriate study form prior to any study interventions.

Vital signs, including pulse rate, blood pressure, oral temperature, and height and weight will be measured and documented on the appropriate study form.

Blood will be collected for baseline study labs as outlined in the Study Procedures section.

A single dose of the FDA-approved oral live cholera vaccine (Vaxchora) will be administered. Eating and drinking should be avoided for one hour before and after vaccine administration. Subjects will be asked to remain at the clinic for 20 minutes to monitor for any reactions or adverse events. Any reactions or adverse events will be documented on the appropriate study form. Subject will be instructed to use adequate hand hygiene to avoid fecal-oral transmission of the vaccine to others.

Subjects will be informed to contact the study clinic should they experience any concerning reactions to the vaccine, and they will be asked to visit the clinic for evaluation should the principal investigator or designee deem the reaction in need of evaluation.

6.2 Follow-up and Final Visits

6.2.1 Visit 02 Day 08, Visit 03 Day 11, Visit 04 Day 15 and Visit 05 Day 29

Interim medical history and targeted physical exam, if necessary, will be conducted.

Medications taken during the interim from the previous visit and any adverse or serious adverse events will be documented on the appropriate study form.

Vital signs, including pulse rate, blood pressure, and oral temperature will be measured and documented on the appropriate study form.

Blood will be collected for immunogenicity analysis.

A review of the memory aid will be done at Visit 02.

6.2.2. Visit 06 Day 90 and Visit 07 Day 365

Interim medical history and targeted physical exam, if necessary, will be conducted.

Any serious adverse event will be documented on the appropriate study form.

Vital signs, including pulse rate, blood pressure, and oral temperature will be measured and documented on the appropriate study form.

Blood will be collected for immunogenicity analysis

6.3 Early Termination Visit (if needed)

The following will be performed at any early termination visit, if necessary, for subjects who withdraw or who are withdrawn from the study:

Interim medical history and targeted physical exam, if necessary, will be conducted.

A review of the memory aid will be conducted (if visit occurs within 7 days of vaccination).

Medications taken during the interim from the previous visit will be documented on the appropriate study form (if prior to D29).

Vital signs, including pulse rate, blood pressure, and oral temperature will be measured and documented on the appropriate study form.

Any adverse (prior to D29) or serious adverse events will be documented on the appropriate study form.

Blood will be collected for immunogenicity analysis.

7 STATISTICAL CONSIDERATIONS

The goal of this study is to study the immune responses to the live attenuated cholera vaccine Vaxchora in healthy, cholera infection/vaccination naïve subjects. This is a pilot study designed to demonstrate the potential utility of a subsequent larger scale study.

Since this is an exploratory study, our aim is to study a convenient sample of 10 subjects receiving the cholera vaccine.

The biostatistician will perform descriptive statistics to describe the immune responses to cholera vaccine.

8 QUALITY CONTROL AND QUALITY ASSURANCE

The study will undergo internal quality control and quality assurance per the Hope Clinic standard operating procedures.

9 ETHICS/PROTECTION OF HUMAN SUBJECTS

9.1 Ethical Standard

The site principal investigator will ensure that this trial is conducted in full conformity with the principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 25691 (1997), if applicable.

9.2 Institutional Review Board

Prior to enrollment of subjects into this trial, the approved protocol and informed consent form will be reviewed and approved by the Emory IRB. The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this study.

9.3 Informed Consent Process

The site principal investigator and designee will choose subjects in accordance with the eligibility criteria detailed in Section 4. Before any study procedures are performed, subjects must sign an informed consent form that complies with the requirements of 21 CFR Part 50 and 45 CFR 46 and the local IRB.

Informed consent is a process that is initiated prior to an individual agreeing to participate in a study and continuing throughout the individual's study participation. Before any study procedures are performed, subjects will receive a comprehensive explanation of the proposed study procedures and study interventions/products, including the nature and risks of the trial, alternate therapies, any known adverse effects, and the other elements that are part of obtaining proper informed consent. Subjects will also receive a detailed explanation of the proposed use and disclosure of their protected health information, including specifically their serum samples. Subjects will be allowed sufficient time to consider participation in the study, after having the nature and risks of the trial explained to them, and have the opportunity to discuss the trial with their family, friends or legally authorized representative or think about it prior to agreeing to participate.

Informed consent forms describing in detail the study interventions/products, study procedures, risks and possible benefits are given to subjects. The informed consent form must not include any exculpatory statements. Informed consent forms will be IRB-approved and subjects will be asked to read and review the appropriate document. Upon reviewing the appropriate document, the site principal investigator (or designee) will explain the research study to subjects and answer any questions that may arise. Subjects must sign the informed consent form, and written documentation of the informed consent process is required prior to starting any study procedures/interventions being done specifically for the trial, including administering study product.

Study personnel may employ IRB-approved recruitment efforts prior to obtaining the subjects consent; however, before any study procedures are performed to determine protocol eligibility an informed consent form must be signed. Subjects will be given a copy of all informed consent forms that they sign.

By signing the informed consent form, subjects agree to complete all evaluations required by the trial, unless the subject withdraws voluntarily, or is withdrawn or terminated from the trial for any reason.

The rights and welfare of subjects will be protected by emphasizing to subjects that the quality of their medical care will not be adversely affected if they decline to participate in or withdraw from this trial.

9.4 Exclusion of Women, Minorities, and Children (Special Populations)

This trial will be inclusive of all adults who meet the Subject Inclusion/Exclusion Criteria, regardless of religion, sex, or ethnic background.

9.5 Subject Confidentiality

Subjects will have code numbers and will not be identified by name. Subject confidentiality is strictly held in trust by principal investigator and the Hope Clinic personnel directly involved in the study. This confidentiality is extended to cover testing of biological samples, in addition to the clinical information relating to participating subjects. The study protocol, documentation, data, and all other information generated will be held in strict confidence.

9.6 Future Use of Stored Specimens

Subjects will be asked for permission to keep any remaining samples for possible use in future research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria. The samples will not be sold or used directly for production of any commercial product. Human genetic tests will be performed on samples. Each sample will be labeled with a unique tracking number to protect subject's confidentiality. There are no benefits to subjects in the collection, storage and subsequent research use of specimens. Reports about future research done with subject's samples will NOT be kept in their health records.

Subjects may be given the option to decide if they want their samples to be used for future research or have their samples destroyed at the end of the study. The subject's decision can be changed at any time prior to the end of the study by notifying the study doctors or nurses in writing. However, if the subject originally consents to future use and subsequently changes his/her decision, any data from a previously collected sample may still be used for this research.

10 LITERATURE REFERENCES

1. Epocrates an Athena Health Company [Internet]. Cholera. https://online.epocrates.com/diseases/451/Cholera [accessed 28 March 2017].

2. World Health Organization [Internet]. Cholera. Geneva: WHO; 2015. Available from: http://www.who.int/Cholera/en/ [accessed 28 March 2017].

3. Kauffman RC, Bhuiyan TR, Nakajima R, Mayo-Smith LM, Rashu R, Hoq MR, Chowdhury F, Khan AI, Rahman A, Bhaumik SK, Harris L, O'Neal JT, Trost JF, Alam NH, Jasinskas A, Dotsey E, Kelly M, Charles RC, Xu P, Kováč P, Calderwood SB, Ryan ET, Felgner PL, Qadri F, Wrammert J, Harris JB. Single-Cell Analysis of the Plasmablast Response to Vibrio cholerae Demonstrates Expansion of Cross-Reactive Memory B Cells. MBio. 2016 Dec 20;7(6).

4. Vaxchora [package insert]. PaxVax, Inc., Redwood City, CA; 2016.

5. U.S. Food and Drug Administration Guidance for Industry:Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.

SUPPLEMENTS/APPENDICES

Appendix A: Study Schedule

Study Visit Number	V01	V02	V03	V04	V05	V06	V07
Study Day +/- Window (days)	**D1**	**D8+1**	**D11+1**	**D15 +/-2**	**D29 +/-3**	**D90 +/-14**	**D365 +/-14**
Obtain Informed Consent[#]	X*						
Review Eligibility Criteria	X*						
Medical History[+]	X*	X	X	X	X	X	X
Concomitant Medications	X*[%]	X	X	X	X		
Vital Signs (Oral Temperature, Pulse Rate, and BP)	X*[&$]	X[$]	X[$]	X[$]	X[$]	X[$]	X[$]
Height and Weight	X*						
Targeted Physical Examination	X*	(X)	(X)	(X)	(X)	(X)	(x)
Urine Pregnancy Test	X*[@]						
Study Vaccination	X						
20-minute Evaluation Period After Study Vaccination	X						
Venous Blood Collection for Study Assays	X	X	X	X	X	X	X
Memory Aid review		X					
Adverse events	X	X	X	X	X		
Serious adverse events	X	X	X	X	X	X	X

[#]Prior to study procedures

*Prior to study vaccination.

[%]All current medications and medications taken within 30 days prior to day 1 of the study.

[&]Vital signs assessed on Day 1 (Visit 1) before the study vaccination will be considered as baseline.

[$]Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

() Targeted physical examination if indicated based on review of complete or interim medical history.

[@]Must be performed on all female subjects of childbearing potential within 24 hours prior to each study vaccination and results must be negative and known prior to each study vaccination.

[+]Complete medical history by interview of subjects to be obtained on Day 0 (Visit 01) prior to the first study vaccination and interim medical history by interview of subjects to be obtained at follow-up visits after study vaccination.

Appendix B: Adverse Events Grading

Vital Signs*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever** (°C) (°F)	 38.0- 38.4 100.4- 101.1	 35.8- 38.9 101.2- 102.0	 39.0- 40.0 102/1- 104.0	 >40.0 >104.0
Tachycardia (beats per minute)	101-115	116-130	>130	ER visit or hospitalization for arrhythmia
Bradycardia*** (beats per minute)	50-54	45-49	<45	ER visit or hospitalization for arrhythmia
Hypertension-systolic (mm Hg)	141- 150	151- 155	>155	ER visit or hospitalization for malignant hypertension
Hypertension-diastolic (mm Hg)	91- 95	96- 100	>100	ER visit or hospitalization for malignant hypertension
Hypotension-systolic (mm Hg)	85-89	80-84	<80	ER visit or hospitalization for hypotensive shock

* Should be at rest for all vital sign measurements

** Oral temperature; no recent hot or cold beverages or smoking

*** When resting heart rate is between 60-100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic (General)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Nausea/vomiting	No interference with activity or 1-2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2-3 loose stools or <400 gms/24 hours	4-5 loose stools or 400-800 gms/24 hours	6 or more watery stools or >800 grms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever> 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Abdominal pain	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Lack of appetite	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization