Statistical Analysis Plan

Protocol Number and Title:	**SERES-013** ECOSPOR IV: An Open-Label Extension of Study SERES-012 and Open-Label Program for Evaluating SER-109 in Adult Subjects with Recurrent Clostridioides difficile Infection (RCDI)
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I confirm that I have reviewed this document and agree with the content.

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1. GLOSSARY OF ABBREVIATIONS

Abbreviation	Description
AE	Adverse Event
AESI	Adverse Event of Special Interest
ATC	Anatomical Therapeutic Chemical
CCNA	Cell Cytotoxicity Neutralization Assay
CDI	*Clostridioides difficile* infection
CDiff32	Clostridioides difficile Health-related Quality-of-Life Questionnaire
CI	Confidence Interval
CLIA	Clinical Laboratory Improvement Amendments
cm	Centimeter
CRF	Case Report Form
CTMS	Clinical Trial Management System
EAIR	Exposure-adjusted Incidence Rate
eCRF	Electronic Case Report Form
EIA	Enzyme Immunoassay
EQ-5D-5L	EuroQol 5 Dimension 5 Level
FDA	Food and Drug Administration
FMT	Fecal Microbiota Transplantation
HRQOL	Health-Related Quality of Life
IgG	Immunoglobulin
ITT	Intent-To-Treat
IXRS	Interactive Voice or Web Response System
kg	Kilogram
K-M	Kaplan-Meier
m	Meters
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mITT	Modified Intent-to-Treat
mmHg	Millimeters of Mercury
n	Number of observations
PCR	Polymerase Chain Reaction
PT	Preferred Term
RCDI	Recurrent *Clostridioides difficile* infection
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
[redacted]	[redacted]
SD	Standard Deviation
SOC	System Organ Class

Abbreviation	Description
TEAE	Treatment-Emergent Adverse Event
TLF	Table, Listing and Figure
UBM	Unformed Bowel Movement
VAS	Visual Analog Scale
WHO	World Health Organization

2. PURPOSE

The purpose of this statistical analysis plan (SAP) is to ensure that the data listings, summary tables and figures which will be produced, and the statistical methodologies that will be used, are complete and appropriate to allow valid conclusions regarding the study objectives.

This SAP is based on SERES-013 Protocol Amendment 8 dated 16 February 2021 and electronic case report form (eCRF) Version 6.0. dated 9 December 2021.

2.1. Timings of Analyses

Analysis will be performed after all subjects have completed the 24-week follow-up period, or otherwise terminated from the study.

2.2. Summary of Changes from Version 1.0

The table in Appendix A summarizes the changes made to the specified analysis in this version of the SAP (v2.0) compared to the original version (v1.0). These changes largely reflect changes amendments to the study protocol and additions to the previously specified statistical analysis.

3. STUDY OBJECTIVES

3.1. Study Objectives for Cohort 1

3.1.1. Primary Efficacy Objective

- To evaluate SER-109 in the reduction of *Clostridioides difficile* infection (CDI) recurrence rates and increase in sustained clinical response rate, determined by a toxin assay, up to 8 weeks after initiation of treatment

3.1.2. Secondary Efficacy Objectives

- To evaluate SER-109 in the reduction of CDI recurrence rates, determined using a polymerase chain reaction (PCR) algorithm (see Laboratory Manual) up to 8 weeks after initiation of treatment
- To evaluate the time to CDI recurrence, determined by a toxin assay, after initiation of a treatment regimen of SER-109
- To evaluate the time to CDI recurrence, determined using a PCR algorithm, after initiation of a treatment regimen of SER-109
- To evaluate the proportion of subjects experiencing CDI recurrence, determined by a toxin assay, up to 4, 12, and 24 weeks after initiation of a treatment regimen of SER-109
- To evaluate the proportion of subjects experiencing CDI recurrence, determined using a PCR algorithm, up to 4, 12, and 24 weeks after initiation of a treatment regimen of SER-109

3.1.3. Primary Safety Objective

- To evaluate the safety and tolerability of SER-109 in adult subjects with recurrent CDI

3.1.4. Exploratory Objectives

- To evaluate changes in the composition of the gut microbiome from Baseline up to 1, 2, 8, and 24 weeks after initiation of a treatment regimen of SER-109
- To evaluate changes in the fecal metabolome from Baseline up to 1, 2, and 8 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of mortality from all causes up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of all hospitalizations up to 8 and 24 weeks after initiation of a treatment regimen of SER-109

- To determine, for subjects who are hospitalized, the total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To assess health outcomes, including Health-Related Quality of Life (HRQOL), by using the EuroQol 5 Dimensions 5 Level (EQ-5D-5L) and the HRQOL survey for CDI (CDiff32) up to 24 and 8 weeks after initiation of a treatment regimen of SER-109, respectively

3.2. Study Objectives for Cohort 2

3.2.1. Primary Safety Objective

- To evaluate the safety and tolerability of SER-109 in adult subjects with recurrent CDI

3.2.2. Efficacy Objectives

- To evaluate SER-109 in the reduction of CDI recurrence rates and increase in sustained clinical response rates, determined by a toxin assay, up to 8 and 12 weeks after initiation of treatment

3.2.3. Exploratory Objectives

- To evaluate changes in the composition of the gut microbiome from Baseline to 1 week after initiation of a treatment regimen of SER-109
- To evaluate changes in the fecal metabolome from Baseline to 1 week after initiation of treatment
- To determine the incidence of mortality from all causes up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of all hospitalizations up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine, for subjects who are hospitalized, the total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To assess health outcomes using the EQ-5D-5L Visual Analog Scale at Screening and at Week 8
- To assess a Bowel Cleanse Patient Satisfaction Survey Measure at Week 8 on a bowel cleanse administered prior to SER-109

3.3. Brief Description

ECOSPOR IV is an open-label extension of Study SERES-012. This study is designed to evaluate the safety, tolerability, and efficacy of a treatment regimen, SER-109, in adult subjects 18 years of age or older with recurrent *Clostridioides difficile* infection (RCDI), who received a treatment regimen of SER-109 or placebo in Study SERES-012. In Cohort 2 of ECOSPOR IV, the primary study objective is to examine safety and tolerability in a cohort of subjects receiving SER-109 at the dose used in SERES-012.

This study will be conducted at approximately 140 study centers in North America. In Cohort 1, subjects who had a per-protocol recurrence of CDI within 8 weeks of receipt of a treatment regimen of SER 109 or placebo in Study SERES-012, and who have responded to 10 to 21 days of standard-of-care antibiotic treatment for CDI (i.e. vancomycin [125 mg QID] and/or fidaxomicin [200 mg BID]) will be eligible to enroll and receive a treatment regimen of SER-109 in Study SERES-013. A treatment regimen of SER-109 is administered orally as [redacted] in 4 capsules once daily for 3 consecutive days. Approximately 30 eligible subjects with RCDI disease from Study SERES-012 are expected to enroll in Cohort 1. Approximately 200 subjects will be enrolled through the Open-Label program in Cohort 2. For Cohort 2, subjects with one or more recurrences of CDI (including the current episode) who have responded to CDI antibiotic therapy defined as 10 to 42 days of treatment with vancomycin or 10 to 25 days with fidaxomicin [200 mg] will be potentially eligible to enroll in Study SERES-013 to receive a SER-109 treatment regimen.

The study duration is approximately 27 weeks, including a ~3-week Screening Period, an 8-week Efficacy Period, and a 16-week Follow-up Period with initiation of treatment on Day 1 after screening. Subjects treated with prolonged or tapering doses of antibiotics after prescreening confirmation of CDI will be screened during the 3-week screening period (Day -24 to Day -2).

In Cohort 1, favorable clinical outcome, or sustained clinical response, in this study will be determined by the absence of CDI recurrence up to 8 weeks after initiation of treatment of study drug, with CDI recurrence defined as ≥ 3 unformed stools per day over 2 consecutive days with a positive *C. difficile* test on a stool sample determined by a toxin assay performed by the central laboratory and a decision by the investigator (based on clinical assessment), that antibiotic treatment is needed. Data from the *C. difficile* toxin assay (either enzyme immunoassay [EIA] or cell cytotoxicity neutralization assay [CCNA]), performed at the central laboratory, will be used for the primary endpoint analysis. The central laboratory results will be communicated to the investigator and the decision to treat with antibiotics will be based upon the investigator's assessment.

In Cohort 2, favorable clinical outcome, or sustained clinical response, will be determined by the absence of CDI recurrence up to 8 or 12 weeks after initiation of treatment of study drug, with CDI recurrence defined in the same manner as for Cohort 1.

3.4. Subject Selection

3.4.1. Inclusion Criteria

To be eligible for enrollment, a subject must meet all the following criteria before undergoing any study related- procedures. Only the inclusion criteria from the latest study protocol are listed.

For Cohort 1:

1. Previously enrolled in Study SERES-012 and experienced a CDI recurrence within 8 weeks after receipt of a treatment regimen of SER-109 or placebo in Study SERES-012.
2. Signed informed consent prior to initiation of any study-specific procedure or treatment. The subject, or their legally authorized representative, must be willing to provide written informed consent and understand the potential risks and benefits from study enrollment and treatment.
3. The CDI recurrence in Study SERES-012 must have met the protocol definition of:
    a. ≥3 unformed stools per day for 2 consecutive days
    b. A positive *C. difficile* stool toxin assay
    c. The requirement of CDI standard of care antibiotic therapy (defined as 10 to 21 days of treatment with vancomycin [125 mg QID] or fidaxomicin [200 mg] mg BID).
    d. An adequate clinical response following standard of care antibiotic therapy, defined as (<3 unformed stools in 24 hours) for 2 or more consecutive days before initiation of study drug on Day 1.
4. If female, subject is non-lactating, and is either:
    a. Not of childbearing potential, defined as postmenopausal for at least 1 year or surgically sterile due to bilateral tubal ligation, bilateral oophorectomy, or hysterectomy.
    b. Of childbearing potential and is practicing at least 1 highly effective method of birth control including: the barrier method; oral or parenteral contraceptives; a vasectomized partner; or abstinence from sexual intercourse. The investigator will discuss with the subject the option of practicing more than 1 of the above methods for the duration of the study.
5. If male, and partner is of childbearing potential, subject agrees to practice at least 1 highly effective method of birth control for the duration of the study.

For Cohort 2:

1. Signed informed consent prior to initiation of any study-specific procedure or treatment. The subject, or their legally authorized representative, must be willing to provide written informed consent and understand the potential risks and benefits from study enrollment and treatment.
2. ≥ 2 episodes of CDI, inclusive of the current episode, with estimated total number of prior episodes.
3. The CDI recurrence must have met the protocol definition of:

a. ≥ 3 unformed stools per day for 2 consecutive days
b. A positive C. difficile stool toxin or PCR assay (either local or central laboratory).
c. The requirement of CDI antibiotic therapy (defined as 10 to 42 days of treatment with vancomycin or 10 to 25 days with fidaxomicin [200 mg]. It is acceptable if subject was started on metronidazole, switched to vancomycin or fidaxomicin and is treated for a minimum of 10 days of vancomycin or fidaxomicin with a total treatment duration of up to a maximum of 42 days for vancomycin or 25 days for fidaxomicin.
d. An adequate clinical response following antibiotic therapy, defined as <3 unformed stools in 24 hours) for 2 or more consecutive days before initiation of study drug on Day 1.
e. The requirement that the subject can be dosed with study drug within 4 days of antibiotic completion.

4. Male or female subject ≥ 18 years of age.
5. If female, subject is non-lactating, and is either:
    a. Not of childbearing potential, defined as post-menopausal for at least 1 year or surgically sterile due to bilateral tubal ligation, bilateral oophorectomy, or hysterectomy.
    b. Of childbearing potential and is practicing at least 1 highly effective method of birth control including: the barrier method; oral or parenteral contraceptives; a vasectomized partner; or abstinence from sexual intercourse. The investigator will discuss with the subject the option of practicing more than 1 of the above methods for the duration of the study.
6. If male, and partner is of childbearing potential, subject agrees to practice at least 1 highly effective method of birth control for the duration of the study.
7. If currently taking probiotics, must be willing to stop at time of consent, for the duration of the study.

3.4.2. Exclusion Criteria

A subject will not be enrolled if the subject meets any of the following criteria. Only the exclusion criteria from the latest study protocol are listed.

For Cohort 1:

1. Female subjects who are pregnant, breastfeeding, lactating, or planning to become pregnant during the study.
2. Known or suspected toxic megacolon and/or known small bowel ileus.
3. Admitted to or expected to be admitted to an intensive care unit for medical reasons (not just boarding). Note: nursing homes, rehabilitation, assisted living centers and acute care hospitals are acceptable.
4. Absolute neutrophil count of <500 cells/mm^3
5. Taking antibacterial therapy other than antibiotics for the most recent episode of CDI during the screening period (a single day- antibiotic prophylactic regimen is permitted), or projected to receive antibiotics during the 8-week period post-randomization.

6. Major gastrointestinal surgery (e.g., significant bowel resection or diversion) within 3 months before enrollment (this does not include appendectomy or cholecystectomy), or any history of total colectomy or bariatric surgery. (Bariatric surgery which does not disrupt the gastrointestinal lumen, i.e., restrictive procedures such as banding, are permitted).
7. History of active inflammatory bowel disease (ulcerative colitis, Crohn's disease, microscopic colitis) with diarrhea believed to be caused by active inflammatory bowel disease in the past 3 months.
8. Unable to stop loperamide, diphenoxylate/atropine, or cholestyramine prior to enrollment.
9. Unable to stop opiate treatment unless on a stable dose, including PRN dosing, as of the onset of diarrhea and no increase in dose planned for the duration of the study. Note: Short term opiate use is permitted (e.g., for a dental extraction).
10. Known positive stool cultures for other enteropathogens including, but not limited to, *Salmonella*, *Shigella*, and *Campylobacter* within the 30 days before enrollment.
11. Known stool studies positive for ova and/or parasites within the 30 days before enrollment.
12. Poor concurrent medical risks with clinically significant co-morbid disease such that, in the opinion of the investigator, the subject should not be enrolled.
13. Received a human monoclonal antibody against *C. difficile* toxin within 3 months before study entry.
14. Received an investigational drug or vaccine, or participated in any experimental procedure within 1 month (3 months for monoclonal antibodies) before study entry.
15. Any history of immunoglobulin (IgG) replacement therapy within the past 3 months.
16. Any history of fecal microbiota transplantation (FMT) within the past 3 months.
17. Known active intravenous drug or alcohol abuse or use of other drugs of abuse.
18. Concurrent intensive induction chemotherapy, radiotherapy, or biologic treatment for active malignancy (subjects on maintenance chemotherapy may only be enrolled after consultation with the study medical monitor).
19. Unable to comply with the protocol requirements, including the ability to take oral drugs; or any condition that, in the opinion of the investigator, might interfere with study objectives.
20. Life expectancy is 24 weeks or less.

For Cohort 2, all Cohort 1 exclusion criteria plus number 21, below, apply.

21. Previously enrolled in a Seres Therapeutics clinical study. An exception is made for subjects who screened in SERES-012 who did not receive SER-109 and did not previously roll-over to SERES-013.

3.5. Determination of Sample Size

Approximately 30 subjects are anticipated to roll-over to this study from SERES-012. The recurrence rates prior to 8 weeks after initiation of study drug assumed in SERES-012 are 16% in SER-109 and 36% in placebo. However, it is expected that only a fraction of subjects who recur in SERES-012 prior to 8 weeks will roll-over to this study, since a follow-up of 8 weeks from start of study drug in earlier protocol versions of SERES-012 (up to Amendment 5) was required before rolling over to SERES-013, regardless of when the CDI recurrence occurred in SERES-012. Additionally, approximately 200 subjects with RCDI may enroll in the Open-Label program (Cohort 2) into SERES-013. Approximately 230 subjects total will be assessed for safety and tolerability, and efficacy.

As of December 2021, enrollment for both Cohort 1 and Cohort 2 were completed. Twenty-nine subjects were enrolled in Cohort 1, and 234 subjects were enrolled in Cohort 2.

3.6. Method of Assigning Subjects to Study Treatment

This is an open-label study. All subjects who qualify for dosing will receive single daily doses of SER-109 ([redacted]) in 4 capsules administered over 3 consecutive days.

The interactive voice and web response system (IxRS) will assign appropriate bottles of SER-109 that will be available at the site for all subjects on their Day 1 study visit. Subjects who discontinue this study or who have previously received SER-109 in this study will not be permitted to re-enter. Similarly, SER-109 dispensed to a subject may not be re-used, even if the bottle(s) are returned unopened.

3.7. Maintaining the Randomization Codes and Breaking the Study Blind

Not applicable. This study is not randomized nor blinded.

3.8. Table of Assessments and Procedures

Please refer to the protocol (Amendment 8.0) for the Table of Assessments and Procedures for Cohort 1 and Cohort 2.

4. ENDPOINTS

4.1. Primary Efficacy Endpoint

For Cohort 1, the primary efficacy endpoint is recurrence of CDI and sustained clinical response as determined by a toxin assay up to 8 weeks after initiation of treatment. A recurrence is defined as (i) ≥ 3 unformed stools per day for 2 consecutive days and the requirement that subjects must continue to have diarrhea until antibiotic treatment is initiated, with (ii) a positive *C. difficile* test on a stool sample determined by a toxin assay, and (iii) assessment by the investigator that the clinical condition of the subject warrants antibiotic treatment. The requirement that subjects continue to have diarrhea until antibiotic treatment is initiated in (i) is met if the subject has ≥1 UBM each day during the period between having 2 consecutive days of ≥ 3 unformed stools and the start date of the CDI antibiotic treatment.

For Cohort 2, recurrence of CDI and sustained clinical response as determined by a toxin assay up to 8 and 12 weeks after initiation of treatment are the efficacy endpoints.

4.2. Secondary Efficacy Endpoints

For Cohort 1, secondary efficacy endpoints are the following:

- Recurrence of CDI as determined by PCR algorithm up to 8 weeks after initiation of treatment
- Time to recurrence of CDI from initiation of treatment as determined by a toxin assay
- Time to recurrence of CDI from initiation of treatment as determined by PCR algorithm
- Recurrence of CDI, as determined by a toxin assay, up to 4, 12 and 24 weeks after initiation of treatment
- Recurrence of CDI, as determined by a PCR algorithm, up to 4, 12 and 24 weeks after initiation of treatment

For Cohort 2, secondary efficacy endpoints are not being sought. As the information is collected for Cohort 2, the related data summaries will be presented for exploratory purpose.

4.3. Exploratory Efficacy Endpoints

For Cohort 1, exploratory endpoints are the following:

- Change in the composition of the gut microbiome from Baseline up to 1, 2, 8, and 24 weeks after initiation of treatment
- Change in the fecal metabolome from Baseline up to 1, 2, and 8 weeks after initiation of treatment
- Incidence of mortality from all causes up to 8 and 24 weeks after initiation of treatment
- Incidence of hospitalizations for RCDI up to 8 and 24 weeks after initiation of treatment

- Incidence of all hospitalizations up to 8 and 24 weeks after initiation of treatment
- Total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after treatment initiation (for subjects hospitalized)
- Changes from Baseline in HRQoL and health outcomes as assessed by the EQ-5D-5L up to Week 24, and assessed by the Cdiff32 HRQoL from up to Week 8, or at an ET or Recurrence visit prior to Week 8, after initiation of treatment

For Cohort 2, exploratory endpoints are the following:

- Change in the composition of the gut microbiome from Baseline up to 1 week after initiation of treatment
- Change in the fecal metabolome from Baseline up to 1 week after initiation of treatment
- Incidence of mortality from all causes up to 8 and 24 weeks after initiation of treatment
- Incidence of hospitalizations for RCDI up to 8 and 24 weeks after initiation of treatment
- Incidence of all hospitalizations up to 8 and 24 weeks after initiation of treatment
- Total length of stay (days) of hospitalization, including days in the intensive care unit, up to 24 weeks after treatment initiation (for subjects hospitalized)
- Change in the EQ-5D-5L Visual Analog Scale from Screening to Week 8
- Assess Bowel Cleanse Patient Satisfaction Survey Measure at Week 8

4.4. Safety Endpoints

Safety endpoints for both cohorts are the following:

- Incidence of adverse events (AEs)
- Laboratory evaluation results
- Vital sign measurements
- Physical examination findings

5. ANALYSIS POPULATIONS

5.1. Intent-to-Treat (ITT) Population

The ITT Population will consist of all enrolled subjects.

5.2. Modified Intent-to-Treat (mITT) Population

The mITT Population will be composed of all enrolled subjects who received any amount of SER-109, whose CDI was clinically controlled by antibiotic treatment before receiving SER-109, and who have at least 1 post-baseline evaluation.

For subjects in Cohort 1, subjects must have had RCDI diagnosis that occurred on the SERES-012 trial, as defined below:

Confirmation of the qualifying CDI episode requires a positive *C. difficile* test based on a toxin assay.

Requirements for the qualifying CDI episode to be clinically controlled by antibiotic treatment include:

- ≤2 unformed bowel movements (UBM) for at least 2 days prior to randomization
- Receipt of appropriate antibiotic, including adequate treatment duration, for the qualifying episode to roll over on to the SERES-013 Cohort 1 study

For subjects in Cohort 2, subjects with a RCDI diagnosis should have ≥2 CDI episodes prior to screening, inclusive of the current episode, as defined below: .

Confirmation of the qualifying CDI episode requires a positive *C. difficile* test based on a toxin or PCR assay. Earlier protocol versions (up to Amendment 8) required a positive *C. difficile* test based on a toxin assay only.

Requirements for the qualifying CDI episode to be clinically controlled by antibiotic treatment include:

- ≤2 unformed bowel movements (UBM) for at least 2 days prior to enrollment
- Receipt of appropriate antibiotic, including adequate treatment duration, for the qualifying episode

5.3. Safety Population

The Safety Population will consist of all enrolled subjects who received any amount of SER-109. All safety analyses will be conducted based on the Safety Population.

6. PROTOCOL DEVIATIONS

Protocol deviations are collected in [redacted], the Clinical Trial Management System (CTMS), used by [redacted], the contract research organization employed for this study. Protocol deviations will be assigned to a deviation sub-type within one of the following deviation types: inclusion/exclusion criteria, informed consent form issues, procedures/tests, laboratory, visit schedule, study drug, concomitant medication, and other. The protocol deviations will be further classified as key vs. non-key in [redacted] per [redacted] Protocol Deviation Criteria document and reviewed by the medical monitors on an on-going basis.

The deviations entered in [redacted] are then transferred into a cumulative protocol deviation listing organized by severity, subject, site, deviation sub-type, and type. This listing of [redacted] defined key and non-key protocol deviations is reviewed by a Seres team including the Medical monitor, Clinical operations, Data management, and statistical team to identify deviations as either Major or Minor.

Protocol deviations will be presented by severity category of Major vs. Minor, deviation type and deviation sub-type and summarized as follows: with number and percentages of subjects with at least one deviation in each deviation sub-type and type. Subjects with multiple deviation sub-types will only be counted once for a given deviation type within the major/minor deviation category and once for the protocol deviation sub-type within a deviation type The summaries will be presented for each cohort separately, and also for 2 cohorts combined. Deviations related to the COVID-19 pandemic will be included in protocol deviations listings.

A listing of all protocol deviations by subject, deviation sub-type and type will also be provided, indicating which are major/minor.

7. GENERAL ASPECTS FOR STATISTICAL ANALYSIS

7.1. General Methods

- All analyses and summaries will be produced using SAS® version 9.4 (or higher).
- Unless otherwise specified, summaries will be presented by the following groups:
  - For Cohort 1 subjects, their randomized treatment arm in SERES-012 (SER-109 and Placebo)
  - All subjects in Cohort 1
  - All subjects in Cohort 2
  - Cohort 1 and Cohort 2 combined
- Continuous variables will be summarized using the number of subjects with evaluable data, mean, standard deviation (SD), median, minimum and maximum. The same number of decimal places as in the raw data will be presented when reporting minimum and maximum, 1 more decimal place than in the raw data will be presented when reporting the mean and median, and 2 more decimal places than in the raw data will be presented when reporting the SD.

- Categorical variables will be summarized using the number of observations (n), frequency and percentage of subjects. All percentages will be presented as one-decimal point, unless otherwise specified. Percentages equal to 100 will be presented as 100% and percentages will not be presented for zero frequencies.
- Unless stated otherwise, the percentages will be based on the number of non-missing observations. The header will still contain the number of subjects in the treatment group. There will be a row for the number of non-missing observations in the table (at each time point, if required) for each variable being summarized.
- All relevant subject data will be included in listings. Subjects from Cohort 1 will be sorted by randomized treatment arm in SERES-012 (SER-109 and Placebo), Subject ID, and visit, as applicable, for all randomized subjects. Subjects from Cohort 2 will be sorted by Subject ID and visit, as applicable
- Unscheduled or repeat assessments will not be included in summary tables unless specified otherwise (e.g., unscheduled CDI assessments will be summarized), but will be included in the subject listings.
- All tables, listings and figures will include footers that identify the name of the program that created the item, together with the date and time on which it was created. Headers will include the total number of pages that the presentation contains and, for each page, the number of the page within the presentation.

7.2. Key Definitions

7.2.1. Study Day

Study Day 1 is defined as the first day of study drug administration in the SERES-013 study. Subsequent days are numbered consecutively (Day 2, Day 3, etc.). Before the day of study drug administration, study days are numbered sequentially with negative values (i.e., Day -1, Day -2, etc.). There is no Day 0.

7.2.2. Baseline Values

Baseline values will be taken as the last assessments on or before day of dosing with study drug in this study. In general, these will be taken from the pre-dose assessment on Day 1, unless otherwise specified.

7.3. Missing Data

Every effort will be made to collect all data required in this study, especially with regards to the primary endpoint. Contact with subjects is made weekly either by telephone or clinic visits up to Week 8, in which all the components of the CDI recurrence endpoint, specifically, (i) 2 or more consecutive days with ≥3 unformed stools, with the requirement that subjects continue to have diarrhea until antibiotic treatment is initiated, (ii) *Clostridioides difficile* test on a stool sample determined by a toxin assay and (iii) assessment by investigator that the condition warrants antibiotic treatment, are assessed.

Monitoring diarrheal symptoms differs between Cohort 1 and Cohort 2.

- Cohort 1 subjects will be instructed to complete a daily diarrhea log every day whether or not they experience diarrhea. At all scheduled telephone calls and study site visits, subjects will be queried regarding diarrheal symptoms, including the day, frequency, and quality of bowel movements described as diarrhea. Any subject suspected of having an episode of CDI per protocol definition will be asked to come in for an in-clinic visit, where possible, for a *C. difficile* stool toxin test and evaluation for recurrence of CDI.
- Cohort 2: at all scheduled telephone calls and clinic or home visits, subjects will be queried regarding diarrheal symptoms, including the day, frequency, and quality of bowel movements described as diarrhea. Any subject suspected of having an episode of CDI per protocol definition (≥ 3 unformed stools per day lasting ≥ 2 consecutive days) will be asked to arrange for an in-clinic or home visit for a *C. difficile* stool toxin test (to be sent to central laboratory) and evaluation for recurrence of CDI.

Daily diarrhea log is only used by subjects in Cohort 1. From the date of enrollment to the end of study (Week 24) assessment, subjects are given 24 hours to enter the number of UBMs from the previous day in the electronic diarrhea log, including recording when no UBMs are experienced on any given day. Subjects are instructed to do this daily until the end of the study. However, some missing data can be expected. Handling of missing data for components of the CDI recurrence endpoint is discussed below.

- For Cohort 1, if the number of UBM is missing on any day from the date of enrollment to the end of the study, then the missing UBM counts will be assumed to be ≥ 3.
- For Cohort 1, if a subject missed entry into the diarrhea log on any day, the site will call the subject to inquire how many UBMs they had on the day entry into the device was missed and remind them to enter their UBM count every day until the end of the study. Subject's response to how many UBMs they had on the day entry was missed will be entered in the EDC database, but not used to assess the primary endpoint, i.e. criteria (i) above will be evaluated based solely on the data entered in the diarrhea log by the subject.
- For Cohort 1, if entry into the device is missed for 1 day and the subject reports ≥3 UBMs for either of the adjacent days, the subject will be contacted by the site. If the subject reports ≥3 UBMs for the missed entry, the subject will be asked to return to the clinic for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI. If the subject reports having <3 UBMs on the missed day, then the site will complete the Suspected CDI Recurrence page in the eCRF, without requiring the subject to come to the site for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI.
- For Cohort 1, if entry into the device is missed for ≥2 consecutive days and the subject reports 2 consecutive days of ≥3 UBMs the next time the site is able to make contact, the subject will be asked to return to the clinic for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI. If entry into the device is missed for ≥2 consecutive days, but the subject reports not experiencing 2 consecutive days of ≥3 UBMs the entire time entry into the diarrhea log was missed at the next contact,

then the site will complete the Suspected CDI Recurrence page in the eCRF, without requiring the subject to come to the site for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI.

For the primary endpoint for both Cohort 1 and Cohort 2, subjects who are lost-to-follow-up, terminated from the study prematurely, or died without a CDI recurrence before 8 weeks after treatment (Day 58) will be defined as having CDI recurrence for the primary analysis. Reasons for withdrawal from the study will be recorded on the eCRF.

Data from the *C. difficile* toxin assay (either enzyme immunoassay [EIA] or cell cytotoxicity neutralization assay [CCNA]), performed at the central laboratory, will be used for the primary endpoint analysis. If the results of the *C. difficile* toxin assay from the central laboratory are missing, then the results of the *C. difficile* toxin test performed by a Clinical Laboratory Improvement Amendments (CLIA)-certified local laboratory using an Food and Drug Administration (FDA)-approved toxin test will be used, if available.

If any of the components of the CDI recurrence criteria is missing, and the non-missing components meet the CDI recurrence criteria, then CDI recurrence for the primary analysis will be imputed. However, if some of the components of the CDI recurrence criteria are missing, and at least 1 of the non-missing components does not meet the CDI recurrence criteria, then a CDI non-recurrence (ie, sustained clinical response) for the primary analysis will be imputed.

For recurrence of CDI by Weeks 4, 12, and 24, subjects will be considered as having had a recurrence using similar rules.

Missing data for the time to CDI recurrence analyses will be handled with censoring by the Kaplan-Meier method. Subjects who complete the study and do not experience a CDI recurrence by the end of the follow-up period will be censored on the date of last contact. Subjects who are lost to follow-up or who terminate the trial prematurely before experiencing a CDI recurrence will be censored on the date of last contact. Subjects who die before experiencing a CDI recurrence will be censored on their date of death. Subjects who were assessed to have a CDI recurrence due to missing or incomplete data for one or more of the 3 components of CDI recurrence will not be counted as an event but censored on their last date of contact. The last date of contact will be determined by either the last visit (clinic or phone) date in which the site made contact with the subject or the last entry date in the diarrhea log, whichever is later. A sensitivity analysis of the time to CDI recurrence will be conducted as detailed in Section 9.2.3.

Frequency (number and percentage) of subjects with an event (CDI recurrence) and censoring reasons will be presented. Thus censoring rules are summarized as follows: (1) study completion without CDI recurrence; (2) lost to follow-up, premature trial termination or death; (3) CDI recurrence due to missing or incomplete data for one or more of the 3 components and the non-missing components meet the CDI recurrence criteria. A sensitivity analysis will be performed where only subjects who complete the study will be censored with subjects in censoring rule 2 and 3 imputed as CDI recurrence.

7.4. Visit Windows

For the primary endpoint of CDI recurrence up to 8 weeks after the start of treatment, and CDI recurrence up to 4 weeks, 12 weeks, and 24 weeks after last treatment regimen received, CDI recurrences will be included in the analyses for the specified endpoints as follows:

Endpoint	Recurrences Included in Analysis
CDI recurrence up to 4 weeks after treatment	Up to Day 30
CDI recurrence up to 8 weeks after treatment	Up to Day 58
CDI recurrence up to 12 weeks after treatment	Up to Day 87
CDI recurrence up to 24 weeks after treatment	Up to Day 171

The number of subjects with hospitalization and the number of hospitalizations will also be summarized by timepoint (Week 4, 8 and 24) using the same cut-off days specified for the primary endpoint. Observed study visits will be used for other efficacy analyses, including responses to the questionnaire data.

For analyses of vital signs and laboratory data, data collected at an early termination visit will be presented separately.

A summary describing adherence to visit schedules will be provided for the ITT Population. The summary will include the count and percentage of subjects who have discontinued on or prior to the end of the previous clinic visit/phone contact window; and those who are still ongoing at the current visit/phone contact. Ongoing subjects are those who have not discontinued on or prior to the end of the previous clinic visit/phone contact window and are further classified into: (i) those with data collected within the window for the respective visit/phone contact, (ii) those with data collected outside the window for the respective visit/phone contact, (iii) those who experienced a CDI recurrence since the end of the previous clinic visit/phone contact window through the end of the current clinic visit/phone contact window, and (iv) those with missing data, which includes subjects who discontinued for an AE, withdrew consent, were lost to follow-up, had a protocol deviation, died, or other reason (excluding CDI recurrence) after the end of the previous clinic visit/phone contact window and on or prior to the end of the current clinic visit/phone contact window, and subjects that are ongoing but missed the clinic visit/phone contact. The percentages of subjects who have discontinued and are ongoing will be based on the ITT Population, while the percentages of subjects with data collected in and out of window, CDI recurrence and missing data at each visit/phone contact will be based on the number of ongoing subjects at the respective visit/phone contact.

Subject listings of telephone contacts, as well as of clinic visits and visit details will be provided.

7.5. Pooling of Centers

There is no planned pooling of centers.

7.6. Multiplicity Adjustments

No adjustments for multiple comparisons will be made.

8. DEMOGRAPHIC AND BASELINE CHARACTERISTICS

8.1. Subject Disposition and Withdrawals

Summary statistics will tabulate the number and percentage of subjects who are screened, screen failures, enrolled, who completed the study, and who prematurely discontinued the study together with reasons for discontinuation. The number and percentage of subjects included in each of the analysis populations will be presented. No statistical testing will be performed on these data. The number of subjects in the ITT Population for each group will be used as the denominator for percentages.

Subject listings of screen failure subjects, inclusion and exclusion criteria information and subject disposition data will be provided.

8.2. Demographic and Other Baseline Characteristics

Demographics (age, race, ethnicity, sex) and baseline characteristics (weight, height, body mass index (BMI)), number of previous CDI episodes, BI/NAP1/027 status when available, previous history of fecal microbiota transplantation (FMT), and additional subgroups defined in Section 8.4 that are not covered by the previous parameters will be summarized for the ITT, mITT and Safety Populations.

BMI will be calculated as:

- BMI (kg/m^2) = Weight(kg)/$[Height(m)]^2$

A subject listing of demographic data will be provided. A separate listing on the previous FMT history information will also be generated.

8.3. Medical History

A by-treatment summary table of the number and percentage of subjects with medical history by system organ class (SOC) and preferred term (PT) will be produced for subjects in the Safety Population. Medical history will be sorted by highest occurrence in the overall column in decreasing order of SOC and PT using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary, v20.0 (March 2017). For the summary tables, a subject may appear more than once if he has more than one medical history finding coded under different SOC terms or more than one medical history finding with a different PT under the same SOC term. However, the subject will be counted only once in the overall category.

A by-subject listing with coded SOC and PT along with verbatim term will also be provided.

8.4. Qualifying CDI episode Characteristics and Severity

Severity characteristics of the qualifying CDI episode will be summarized in the ITT population. All information collected on the qualifying CDI episode will be included in separate listings for the ITT Population.

The Bowel Prep data on the Screening Day will also be listed.

8.5. Prior and Concomitant Medication

Prior medications are defined as medications that started before the date of dosing. Any medication that started on the date of dosing will not be considered prior. Concomitant medications are defined as all medications (excluding study treatment) taken on or after the date of dosing. This also includes medications ongoing on the dosing date. Medications that started before the date of dosing and are ongoing after the date of dosing will be considered as both prior and concomitant.

Partial start dates in prior and concomitant medications will be imputed to the first day of the month (if missing day) or the first month of the year (if missing month). Partial end dates in prior and concomitant medications will be imputed to the last day of the month (if missing day) or the last month of the year (if missing month).

A subject listing of prior and concomitant medications use will be provided, coded by using the Anatomic Therapeutic Chemical (ATC) classification codes and preferred drug name according to the World Health Organization (WHO) Drug Dictionary Enhanced, (Sept. 1, 2016). Separate summary tables will be provided for prior and concomitant medications in the Safety Population, presenting the number and percentage of subjects, and will be sorted by descending frequency of ATC Level 2 and then PT in the overall column. For each subject, the medication will be counted only once within a given ATC level 2 and only once within a given preferred drug name level. A subject may appear more than once if he/she has more than one concomitant medication coded under different ATC categories; however, the subject will be counted only once in the overall category.

8.5.1. Antibiotic Medication

Summary tables for prior and concomitant antibiotic medication use will be provided separately in the Safety Population. A combined subject listing of prior and concomitant antibiotic use will also be generated.

9. EFFICACY

Efficacy is based on CDI recurrence. CDI recurrence will be determined based on the definition below:

- ≥ 3 unformed bowel movements per day over 2 consecutive days and the requirement that patients must continue to have diarrhea until antibiotic treatment is initiated. The requirement that subjects continue to have diarrhea until antibiotic treatment is initiated is met if the subject has ≥1 UBM each day during the period between having 2 consecutive days of ≥ 3 unformed stools and the start date of the CDI antibiotic treatment.
- Positive *Clostridioides difficile* test on a stool sample determined by a toxin assay from the central laboratory
- Assessment by the investigator (based on clinical assessment) that the patient's condition warrants antibiotic treatment.

The investigator will use the data collected from stool sample analysis (*C. difficile* stool test as described in protocol).

9.1. Primary Efficacy Endpoint and Analysis

9.1.1. Primary Analysis of the Primary Endpoint

For Cohort 1, the primary efficacy endpoint is the proportion of subjects who had a CDI recurrence through Week 8 in the ITT Population. For the primary analysis, subjects who are lost to follow up, terminate from the study prematurely, or die without a recorded recurrence of CDI before Week 8 will be defined as having a CDI recurrence. If any of the components of the CDI recurrence criteria is missing, and the non-missing components meet the CDI recurrence criteria, then a CDI recurrence for the primary analysis is imputed. However, if some of the components of the CDI recurrence criteria are missing, and at least 1 of the non-missing components does not meet the CDI recurrence criteria, then a CDI non-recurrence (ie, sustained clinical response) outcome for the primary analysis is imputed. Details regarding how missing data will be handled are provided in Section 7.3 .

The number and percentage of subjects in each group defined as having CDI non-recurrence (ie, sustained clinical response) and CDI recurrence outcomes will be reported with exact 95% confidence intervals (CIs) for each group. The CIs will be derived using the Clopper-Pearson exact method.

Subject listings of all suspected CDI recurrences on study including results for each CDI criteria, as well as subject listings of the severity assessment for these episodes will be provided. Subject listings of local and central laboratory *C. Difficile* test results will be generated. A subject listing of all subject recurrences, including qualifying and on-study recurrences, will also be provided.

For Cohort 2, the primary efficacy endpoint is the proportion of subjects who had a CDI recurrence through Week 8 and Week 12 in the ITT Population. The same analyses will be conducted for CDI recurrence through Week 8 and Week 12 for Cohort 2 subjects.

9.2. Analyses of Secondary Efficacy Endpoints

Secondary efficacy endpoints are only defined for Cohort 1 in the study protocol. The analysis methodology will be presented in this section. Same analysis outputs for Cohort 2 will also be generated for exploratory purposes.

9.2.1. Recurrence of CDI up to Week 8 as determined by a PCR Algorithm

The number and percentage of subjects with CDI recurrence determined by a PCR algorithm up to 8 weeks (Day 58) will be presented in the ITT Population. The number and percentage of subjects in each group defined as having CDI non-recurrence (ie, sustained clinical response) and CDI recurrence outcomes will be estimated and tested using the same methods as for the primary efficacy assessment at Week 8 (Day 58) in Section 9.1.1.

9.2.2. Time to Recurrence of CDI Determined by a Toxin Assay

Time to first recurrence of CDI determined by a toxin assay will be summarized for the ITT and the mITT Populations using the median and 25th and 75th percentiles from the Kaplan-Meier (K-M) analyses. The 2-sided 95% CIs for the median, calculated using the Greenwood formula,

will also be provided. Subjects who complete the study and do not experience a CDI recurrence by the end of the follow-up period will be censored on the date of last contact. Subjects who are lost to follow-up or who terminated the study prematurely before experiencing a CDI recurrence will be censored on the date of last contact. Subjects who die before having a CDI recurrence will be censored on the date of death. Subjects who were assessed to have a CDI recurrence due to missing or incomplete data for any of the components of CDI recurrence will not be counted as an event, but censored on their last date of contact. Subjects who were not dosed will have their time to recurrence measured from their enrollment date.

The plot of the K-M survival function estimates will be provided by treatment for the ITT population.

9.2.3. Sensitivity Analysis of Time to Recurrence of CDI Determined by a Toxin Assay

A sensitivity analysis of the time to first recurrence of CDI determined by a toxin assay endpoint will also be conducted using a different censoring rule for missing data in the ITT population. In this analysis, subjects who do not experience a CDI recurrence by the end of the study follow-up period will continue to be censored on the date of last contact. However, subjects who are lost to follow-up or who terminate the trial prematurely prior to experiencing a CDI recurrence will be counted as having a CDI recurrence on the date of last contact. Subjects who die prior to experiencing a CDI recurrence will be counted as having a CDI recurrence on their date of death. Subjects who were assessed to have a CDI recurrence due to missing or incomplete data for any of the components of CDI recurrence will be counted as having a CDI recurrence on the date of the earliest diarrhea, *C. difficile* stool test, assessment of investigator that the subject's condition warrants antibiotics or the date of last contact, whichever is the earliest, in the analysis.

Analyses will be conducted as described in Section 9.2.2 above.

Note that the preceding sensitivity analyses will only be conducted for CDI recurrences determined by a toxin assay.

9.2.4. Time to Recurrence of CDI Determined by a PCR Algorithm

The same analyses described in Section 9.2.2 for time to first CDI recurrence determined by a toxin assay will be conducted for the analysis of time to first CDI recurrence determined by a PCR algorithm. However, the corresponding Kaplan-Meier plots will not be generated.

9.2.5. Recurrence of CDI up to 4, 12, and 24 Weeks Post-Treatment Determined by a Toxin Assay

The number and percentage of subjects with recurrence of CDI determined by a toxin assay up to 4 (Day 30), 12 (Day 87), and 24 weeks after treatment (Day 171) will be presented in the ITT and mITT Populations.

The same analysis described in Section 9.1.1 will be conducted for CDI recurrences up to 4, 12 and 24 weeks post-treatment.

9.2.6. Recurrence of CDI up to 4, 12, and 24 Weeks Post-Treatment Determined by a PCR Algorithm

The same analyses described in Section 9.2.5 will be conducted for the recurrence of CDI determined by a PCR algorithm up to 4, 12, and 24 weeks post-treatment.

9.3. Exploratory Efficacy analyses

9.3.1. Microbiome Outcome Analysis

A separate Microbiome Statistical Analysis Plan will be provided by Seres Therapeutics.

9.3.2. Incidence of Hospitalizations

Hospitalizations are defined as resource use recorded in the healthcare resource utilization eCRF as "Veterans Affairs Hospital", "Hospital", "Hospice" "Long Term Acute Care" "Rehabilitation Center". The numbers and percentages of subjects who are hospitalized for RCDI through Weeks 4, 8, 12, and 24 will be summarized for the ITT Population. Subjects with more than one hospitalization within a time period will be counted only once. No statistical tests will be conducted.

A similar summary will also be produced for the incidence of all hospitalizations, regardless of reason.

The number of hospitalizations per subject for recurrent CDI and for any reason will be summarized with frequencies and percentages.

Descriptive statistics for the total length of stay (in days) of all hospitalizations through 24 weeks for recurrent CDI and for any reason will be provided. A hospital duration less than 24 hours is assigned 1 day. Hospital duration will be presented as a continuous variable and by categories:1-3; 4-7; 8-14; and >14 days. No adjustments for time on study will be made.

Subject listings of the health care utilization information will be provided. This analysis will be performed for both Cohort 1 and Cohort 2.

9.3.3. EQ-5D-5L Questionnaire

For Cohort 1 subjects:

The full EQ-5D-5L questionnaire was collected for Cohort 1 subjects only. . The questionnaire developed by the EuroQol Group, measures health outcomes in 5 dimensions, using 5 levels of responses indicating severity with higher responses indicating greater severity. A visual analog scale (VAS) is also included. The dimensions are: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. The EQ-5D-5L VAS records the respondent's overall self-rated health on a scale of 0 to 100.

A unique health state is obtained by combining the levels from each of the 5 dimensions into a 5-digit number – a maximum of 3125 possible health states is possible. For example, 11111 indicates no problems on any dimensions. 12345 indicates no problems with mobility, slight problems with washing or dressing, moderate problems with doing usual activities, severe pain or discomfort, and extreme anxiety or depression. Missing values for a dimension are coded as 9.

The EQ-5D-5L VAS is scored as 0 to 100, where 0 is the worst health you can imagine, and 100 is the best health you can imagine.

An index value for the EQ-5D-5L can be obtained by using the Crosswalk Index Value Calculator, downloadable from the EuroQol website.

Responses on each dimension will be summarized with frequencies and percentages at each visit, including the early termination visit. Shift tables from baseline to each visit, including the early termination visit, will also be constructed.

Summary statistics will be supplied for the Crosswalk Index Value and EQ-5D-5L VAS at each visit, including the early termination visit, and also for the changes from baseline.

Subject listings of the EQ-5D-5L data will be provided.

For Cohort 2 subjects:

For Cohort 2 subjects, only EQ-5D-5L VAS was collected for sites who enrolled subjects under protocol amendment Version 8.0. Summary statistics will be supplied for EQ-5D-5L VAS at baseline and Week 8 visit, and also for the changes from baseline. Subject listings for this measurement will be provided.

9.3.4. Cdiff32 Health-care Quality of Life (HRQOL) Questionnaire

The Cdiff32 HRQOL questionnaire was collected for Cohort 1 subjects only. It is a validated CDI-specific instrument (Garey et al, 2016) developed to assess HRQOL changes related to CDI with a focus on recurrent disease.

The questionnaire comprises 32 questions with 5 possible levels of response for each question. It measures health outcomes in 3 domains (physical, mental and social), and 5 sub-domains (general physical complaints, specific physical complaints, anxiety future, anxiety current, and relationship).

The original CRF responses are from 1 to 5 for each question. They will be transformed to scores from 0 to 100 for each question, with the worst response scored as 0 and incrementing by 25 points as the response becomes more positive. For example, for Question 1 ‘Have you had any difficulties and/or disruption carrying out your daily activities?’, the original CRF responses are Not at all = 1, A little bit = 2, Moderately = 3, Quite a bit = 4, and Extremely = 5, with the lower score being the best original response. This will be transformed as follows: Not at all = 100, A little bit = 75, Moderately = 50, Quite a bit = 25, and Extremely = 0. For all questions except for Question 19 and 32, they all have lower scores as the best original score and will be transformed using the same way. For Question 19 and 32, since lower original scores are the worst scores, they will be transformed in the other direction. For example, for Question 19 ‘Despite my C. diff infection I can live a normal life.’, the original CRF responses are Totally disagree = 1, Mostly disagree = 2, Don't know = 3, Mostly agree = 4, and Totally agree = 5, with the lower score being the worst original response. This will be transformed as follows: Totally disagree = 0, Mostly disagree = 25, Don't know = 50, Mostly agree = 75, and Totally agree = 100.

The overall score for each subject is derived using the average score of the subject’s responses to all 32 questions. Each domain and sub-domain score for each subject is similarly derived by taking the average of all of the subject’s responses to all questions within the domain and sub-

domain, respectively. The following items are included in the specified domains and sub-domains:

Domain	Sub-domain	Item Number
Physical	General physical complaints	1–4, 9-10
	Specific physical complaints	11-18
Mental	Anxiety future	5-8, 27
	Anxiety current	19-26, 28
Social	Relationship	29-32

Descriptive statistics of the overall score, as well as the domain and sub-domain scores will be presented for all study visits, including the early termination visit, at which they were collected for the ITT population. The change from baseline to each post-baseline visit, including the early termination visit, will also be summarized by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall.

All responses to the Cdiff32 HRQOL questionnaire will be listed.

9.3.5. Bowel Cleanse Questionnaire

The bowel cleanse questionnaire will be collected for Cohort 2 subjects only. Patient satisfaction with the bowel cleanse is collected in this questionnaire. The questionnaire has 2 domains.

- Domain 1 includes four items assessing the ease or difficulty of consuming the bowel-cleansing preparation, whether the patient is able to consume the entire preparation, the taste of the preparation, and the overall experience when using the preparation. Patients report on their ability to complete the entire preparation with a binary ‘yes’ or ‘no’ response. The three other questions in Domain 1 require patients to report satisfaction on a five-point scale. The three items of Domain 1 with a five-point scale are assigned scores from 0 to 4, with 0 representing the most favorable (“Very Easy” or “Excellent”) and 4 representing the least favorable (“Very Difficult” or “Bad”) answers; the dichotomous item are coded as 0 for yes and 1 for no. The raw score of each of the four items are then transformed to a range from 0 to 100 to standardize items. These values are then summed to generate a total satisfaction score between 0 and 400, where a lower score indicates higher satisfaction.
- Domain 2 included two additional ‘yes/no’ questions to assess participants’ willingness to accept or refuse the same bowel preparation for a future treatment of SER-109.

The following analyses will be conducted for this questionnaire in ITT population. The analyses in this Section follows the similar methodology as Hatoum et al. (2016).

- Reliability
  - Cronbach’s alpha will be used to measure the internal consistency of patients’ responses to the individual questions of Domain 1 of the satisfaction questionnaire. Values above 0.70 reflect acceptable reliability (internal consistency).

- Validity
  - The validity of the patient satisfaction instrument will be assessed by determining the 'ability to measure change' and the 'predictive validity' of test scores.
  - The ability to measure change will be assessed by examining the extent of floor and ceiling effects, as measured by the percentages of responses at either end of the response range using the raw item scores (an item is considered to have a ceiling effect if most responses fall on its highest value). The number of subjects and the percentage of responses at each level of the raw response to each question will be provided for this summary. All questions in Domain 1 and 2 will be included in this summary.
  - The predictive validity will be assessed by evaluating the relationship between the satisfaction scores (Domain 1) and the acceptability measure on the likelihood to use the same preparation in the future (Question 5 and 6 in Domain 2). Normality test based on Shapiro-Wilk W test will be used to test the Normality of the satisfaction scores. If the satisfaction scores are Normally distributed, T-test will be used to compare the satisfaction scores between the 2 groups of subjects who reported "Yes" or "No" for Questions 5 and 6. If satisfaction scores are not Normally distributed, Wilcoxon rank-sum test will be used for this comparison instead. Descriptive summaries for the satisfaction scores for these 2 groups of subjects will also be presented.
  - The relationship between transformed satisfaction scores (Domain 1) and EQ-5D-5L VAS will also be assessed. Correlation of total score of the four questions in Domain 1 as well as each individual question in Domain 1 with EQ-5D-5L VAS will be assessed. Correlation of individual questions in Domain 2 with EQ-5D-5L VAS will also be assessed.
  - The discriminant validity of the bowel cleanse satisfaction will be assessed by comparing Domain 1 total score with EQ-5D-5L VAS week 8 change from baseline. Mean and median bowel cleanse total score of Domain 1 will be calculated for patients that have no change, five-point improvement, and five-point decline on the EQ-5D-5L VAS. This will be repeated for ten-point change of EQ-5D-5L VAS.
  - The discriminant validity of willingness to use bowel cleanse in the future will be assessed by comparing Domain 2 individual questions with EQ-5D-5L VAS week 8 change from baseline. Percentages of patients with a "Yes" and a "No" answer to Domain 2 questions will be calculated for patients that have no change, five-point improvement, and five-point decline on the EQ-5D-5L VAS. This will be repeated for ten-point change of EQ-5D-5L VAS.

9.4. Subgroup Analyses

A summary table of the number and percentage of subjects in each group having CDI non-recurrence (ie, sustained clinical response) and CDI recurrence outcomes up to 8 weeks after treatment determined by a toxin assay (Day 58) will be reported with exact 95% confidence intervals (CIs), for the following baseline characteristics in the ITT Population:

- Age (<65 years old, ≥65 years old)
- Prior Antibiotic Regimen (Vancomyin, Fidaxomicin)
- Gender (Male, Female)
- Race (White, Black or African American, Asian, Other)
- Region (USA, Canada)
- SER-109 Donor Lot
- # of prior CDI episodes (not including qualifying episode) (1, 2, ≥3). All Cohort 1 subjects will be included in the category of ≥3 recurrences.
- Enrollment prior to Protocol Amendment 8 and post Protocol Amendment 8
- Qualifying episode defined by PCR alone vs toxin with/without PCR

Side by side forest plots of the proportion of subjects who had a CDI recurrence by Week 8 (Day 58) with the corresponding 95% CI for the different subgroups will be presented.

10. SAFETY

All safety analyses will be conducted in the Safety Population, unless specified otherwise.

10.1. Extent of Exposure and Treatment Compliance

Exposure and compliance will be assessed by the number of capsules taken on each of the 3 dosing days and overall, as well as the percentage of subjects who took each number in the Safety Populations. Subject listings of the study drug administration information will be provided.

10.2. Adverse Events

Adverse events will be coded using MedDRA v20.0 (March 2017). A listing of all AEs from the time of enrolment up to Week 8 (defined as up to Day 58) will be summarized; from Week 8 up to Week 24, only serious adverse events (SAEs) and adverse events of special interest (AESIs) will be collected and summarized.

An AESI (serious or non-serious) is one of scientific and medical concern specific to the product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor is appropriate. In this protocol, an invasive infection (e.g., bacteremia, abscess, meningitis) is designated as an AESI, and as such, will be reported and followed in the same manner as an SAE during the course of the study.

Only treatment-emergent adverse events (TEAE) will be collected and summarized in this study. A TEAE is any AE that newly appeared, increased in frequency, or worsened in severity following initiation of study drug.

The following TEAE summaries will be presented by the time interval of AE onset: At any time; Days 1- 58; Days 1-10; Days 11-14; Days 15-58, Days 59-and beyond (End of Study):

- An overall summary, including the number and percentage of
  - TEAEs
  - Subjects with At Least One TEAE
  - Subjects with No TEAEs
  - Study Drug Related or Possibly Related TEAEs
  - Subjects with Study Drug Related or Possibly Related TEAEs
  - Serious TEAEs
  - Subjects with Serious TEAEs
  - Treatment-Emergent AESIs
  - Subjects with Treatment-Emergent AESIs
  - Serious TEAEs Related or Possibly Related to Study Drug
  - Subjects with Serious TEAEs Related or Possibly Related to Study Drug
  - Treatment-emergent AESIs Related or Possibly Related to Study Drug
  - Subjects with Treatment-emergent AESIs Related or Possibly Related to Study Drug
  - Severe TEAEs
  - Subjects with Severe TEAEs
  - TEAEs Leading to Study Withdrawal
  - Subjects with TEAE Leading to Study Withdrawal
  - Related or Possibly Related TEAEs Leading to Study Withdrawal
  - Subjects with Related or Possibly Related TEAEs Leading to Study Withdrawal
  - Serious TEAEs Leading to Study Withdrawal
  - Subjects with Serious TEAEs Leading to Study Withdrawal
  - Serious and Related or Possibly Related TEAEs Leading to Study Withdrawal
  - Subjects with Serious and Related or Possibly Related TEAEs Leading to Study Withdrawal
  - Treatment-emergent AESIs Leading to Study Withdrawal
  - Subjects with Treatment-emergent AESIs Leading to Study Withdrawal

  - Related or Possibly Related Treatment-emergent AESIs Leading to Study Withdrawal
  - Subjects with Related or Possibly Related Treatment-emergent AESIs Leading to Study Withdrawal
  - TEAEs Leading to Death
  - Subjects with TEAEs Leading to Death
- TEAEs by System Organ Class (SOC) and Preferred Term (PT)
- TEAEs by Preferred Term (PT)
- Serious TEAEs by System Organ Class (SOC) and Preferred Term (PT)
- TEAEs Leading to Study Withdrawal by System Organ Class (SOC) and Preferred Term (PT)
- TEAEs by System Organ Class (SOC), Preferred Term (PT) and Maximum Severity
- TEAEs by System Organ Class (SOC), Preferred Term (PT) and Maximum Relationship to Study Drug
- TEAEs Occurring Before Antibiotic Use by System Organ Class (SOC), Preferred Term (PT) and Maximum Severity

The following subject listings will be provided:

- All TEAEs,
- Deaths,
- Serious TEAEs,
- AESIs, and
- TEAEs leading to study withdrawal.

For key TEAE tables, key subgroup summaries will also be performed. This includes the following tables:

- Overall TEAE summary
- TEAEs by SOC and PT
- Study Drug Related or Possibly Related TEAEs by SOC and PT

The key subgroups include the following:

- # of prior CDI episodes (not including qualifying episode) (1, 2,≥3). All Cohort 1 subjects will be included in the category of ≥3 recurrences.
- Enrollment prior to Protocol Amendment 8 and post Protocol Amendment 8
- Qualifying episode defined by PCR alone vs toxin and/or PCR
- Donor lot

For all TEAE tables summarized by SOC and PT, a subject contributes only once to the count for a given TEAE on the SOC level and on the PT level within SOC.

In the summary by maximum severity, subjects reporting AEs at different severities will be counted only once at the greatest severity reported within an AE level (SOC or PT). Severity categories will include mild, moderate, severe and missing.

In the summary by maximum relationship, subjects reporting AEs at different relationships will be counted only once at the strongest relationship reported within an AE level (SOC or PT). Relationship categories will include related, possibly related, unrelated and missing.

In all summary tables, TEAEs will be sorted in decreasing incidence, first by SOC and then by PT within the SOC, according to the incidence in the overall column. SOCs and PTs occurring at the 7same incidence will be sorted alphabetically, unless specified otherwise.

No statistical tests will be performed.

Additional analyses will determine the exposure-adjusted incidence rates (EAIR) per 100-person years of specific TEAEs occurring before subjects received antibiotics for recurrence of CDI, based on the number of days the subjects were followed up to Week 24/End of Study, including TEAEs for subjects who did not receive antibiotics for treatment of CDI before Week 24/End of Study. Incidence rates per 100-person years will be presented for the following:

1. subjects with at least one treatment-emergent SAE,
2. subjects with at least one treatment-emergent AESI, and
3. subjects with at least one TEAE leading to study withdrawal.
4. subjects with a TEAE of urinary tract infection

The EAIR per 100 person years will be calculated as (100*number of subjects with events)/total person years, where total person years equals the sum of the following: 1) [(earliest of the date of first antibiotic treatment before Week 24/End of Study or the date of the event of interest) – date of dose + 1]/365.25, summed across subjects who received antibiotics for treatment of CDI; and 2) [(earliest of the date of last contact up to Week 24/End of Study or the date of the event of interest) – date of dose + 1]/365.25, summed across subjects who did not receive antibiotics for treatment of CDI before Week 24/End of Study. A 95% CI obtained using the normal approximation to the Poisson distribution will be presented.

10.3. Laboratory Evaluations

All hematology, chemistry, blood screening, and pregnancy laboratory tests will be performed by a central laboratory. Descriptive statistics of the laboratory parameters will be presented for all study visits at which they were collected. The change from baseline to each post-baseline visit, including the early termination visit, and to the minimum and maximum post-baseline value will also be summarized.

Laboratory parameters will be defined as within or outside normal range limits and shift tables from baseline to each post-baseline visit will also be provided.

All laboratory evaluations will be included in the data listings.

10.4. Vital Signs

Vital signs data include measurements of weight (kg), height (cm), blood pressure (mmHg), respiratory rate (breaths/minute), body temperature (Celsius), and Body Mass Index (kg/m^2). Descriptive statistics of the vital signs will be presented for all study visits, including the early termination visit, at which they were collected. The change from baseline to each post-baseline visit, including the early termination visit and to the minimum and maximum post-baseline value, will also be summarized.

All vital signs data will be listed.

10.5. Physical Examination

A listing with physical examination findings will be provided.

10.6. Other Safety

For Cohort 1, all data in the Diarrheal Assessment Log will be listed.

Subject listings will also be generated for data on the central laboratory stool sample and samples for future biomedical research.

11. CHANGES FROM ANALYSIS PLANNED IN PROTOCOL

Analysis specified in this SAP supersedes those specified in the study protocol (SERES-013 Protocol Amendment 8 dated 16 February 2021). Substantive differences in the analysis specified in the SAP compared to those specified in the study protocol include:

- Sensitivity analyses specified for primary efficacy endpoint were removed
- Analysis of time to CDI recurrence where events were imputed rather than censored was added
- Secondary efficacy analysis was only specified for Cohort 1 in the protocol. These analyses will also be performed for Cohort 2

12. REFERENCE LIST

Garey K, et al. Clin Gastroenterol 2016; 50(8): 631–637. Development and Validation of a *Clostridium difficile* Health-related Quality-of-Life Questionnaire.

Hatoum H, et al. The Patient - Patient-Centered Outcomes Research 2016; 9(1), 27-34. Validation of a Patient Satisfaction Scale in Patients Undergoing Bowel Preparation Prior to Colonoscopy.

13. PROGRAMMING CONSIDERATIONS

Please review Section below and modify as per protocol requirements.

All tables, data listings, figures (TLFs), and statistical analyses will be generated using SAS for Windows, Release 9.4 or later (SAS Institute Inc., Cary, NC, USA). Computer-generated table, listing and figure output will follow the [redacted] templates and output specifications.

14. INDEX OF TABLES/LISTINGS/FIGURES

A separate document that contains the Table/Listing/Figure mock-ups will be developed. The index of Tables/Listings/Figures will be included in the document.

APPENDIX A. SUMMARY OF CHANGES

Table 1: Summary of Material Changes to SAP v2.0 compared to v1.0

Section Title **Section Number** (Refers to v2.0 numbering unless otherwise indicated)	**Description of Change**	**Rationale for Change**
Primary Efficacy Objective Section 3.1.1	Revised language for primary efficacy objective	Achieve consistency with latest protocol amendment
Version 1.0 Exploratory Objective Section 3.4	Removed 7th bullet related to DRG-adjusted hospital costs	Achieve consistency with latest protocol amendment
Study Objectives for Cohort 2.0 Section 3.2	Added section for study objectives specific to Cohort 2.0	Achieve consistency with latest protocol amendment
Brief Description Section 3.3	Revised and added text	Achieve consistency with latest protocol amendment
Subject Selection Section 3.4	Revised inclusion and exclusion criteria	Achieve consistency with latest protocol amendment
Determination of Sample Size Section 3.5.	Revised sample size consideration	Achieve consistency with latest protocol amendment. Reflects revised Cohort 1 rollover assumptions and added enrollment of Cohort 2 De Novo subjects.
Table of Assessments and Procedures Section 3.8.	Removed table in SAP and references source table in protocol	Minimize redundancy in documents and use protocol as source for this information
Endpoints Section 4.	Revised language with additions and deletions. Most substantive additions were endpoints relating to Cohort 2.	Reflect latest amendment of the protocol
Modified Intent-to-Treat Population Section 5.2.	Added detail on defining MITT population	Detail was needed to programmatically define MITT population
Protocol Deviations Section 6.	Added detail on analysis and presentation of protocol deviation data	Revisions reflect planned analysis.

Section Title **Section Number** (Refers to v2.0 numbering unless otherwise indicated)	**Description of Change**	**Rationale for Change**
General Methods Section 7.1.	Added detail on presentation groups for analysis tables Removed information on presentation of p-values Added information on presentation of subject data listings	Revisions reflect planned analysis.
Study Day Section 7.2.1.	Clarified definition of study to specify that relevant study for Day 1 is SERES-013	Revisions reflect planned analysis.
Baseline Values Section 7.2.2.	Revised definition of baseline to include assessments made on the same day initiation of study drug	Revisions reflect planned analysis.
Missing Data Section 7.3.	Clarified handling of missing data for CDI recurrence	Revisions reflect planned analysis.
Visit Windows Section 7.4.	Added the Week 4 time for hospitalization analysis	Revisions reflect planned analysis.
Time to Recurrence of CDI Determined by a Toxin Assay Section 9.2.2.	Added detail on how 95% confidence intervals for median is calculated (ie, Greenwood formula)	Revisions reflect planned analysis.
Incidence of Hospitalizations Section 9.3.2.	Added detail on analysis of hospitalization and duration of hospitalizations. Categories of healthcare resource use included to define hospitalizations was added. Treatment of hospitalization durations <24h and categories of hospital duration to be presented were specified.	Revisions reflect planned analysis.
EQ-5D-5L Questionnaire Section 9.3.3.	Added text specifying the analysis of data from Cohort 2 subjects.	Revisions reflect planned analysis of subjects enrolled under latest protocol amendment.
CDiff32 Health-care Quality of Life Questionnaire Section 9.3.4.	Added more detail on the derivation of CDiff32 scores for analysis from CRF entries.	Revisions reflect planned analysis.

Section Title **Section Number** (Refers to v2.0 numbering unless otherwise indicated)	**Description of Change**	**Rationale for Change**
Bowel Cleanse Questionnaire Section 9.3.5.	Added information of analysis of the Bowel Cleanse Questionnaire which was added in Protocol Amendment 8.0 of Cohort 2 De Novo subjects.	Revisions reflect planned analysis of assessment added to latest protocol amendment.
Subgroup Analysis Section 9.4.	Added subgroups to be evaluated for efficacy	
Adverse Events Section 10.2.	Specified time of onset intervals for presentation of adverse event summaries Specified which AE analyses will be conducted for subgroup analysis. Indicated that AEs with missing severity or relatedness information will be presented as missing. Added TEAEs of urinary tract infections for analysis of exposure-adjusted incidence rates.	Revisions reflect planned analysis.
Other Safety Section 10.6.	Removed information on data safety monitoring committee. Specified that data from Diarrheal Assessment Logs, central laboratory stool sample and samples for future biomedical research will be listed.	Revisions reflect planned analysis and revisions made to the protocol.