Rebiotix Inc.

Protocol 2015-01

An Open-label Efficacy and Safety Assessment of Rebiotix RBX2660 (microbiota suspension) for the Treatment of Recurrent *Clostridium difficile* Infection

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An Open-label Efficacy and Safety Assessment of Rebiotix RBX2660 (microbiota suspension) for the Treatment of Recurrent *Clostridium difficile* Infection

[redacted]

Sponsor: [redacted] Rebiotix Inc
2660 Patton Road
Roseville, Minn. 55113 USA
[redacted]

Study Product: RBX2660 (microbiota suspension)

Protocol #: 2015-01
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List of Abbreviations and Acronyms

AE – adverse event
AIDS – Acquired Immunodeficiency Syndrome
ALT – alanine aminotransferase
AST – aspartate aminotransferase
BUN – blood urea nitrogen
CBC – complete blood count
CDI – *Clostridium difficile* infection
CFR – Code of Federal Regulations
CLIA – Clinical Laboratory Improvement Amendments
DCF – data clarification form
eCRF – electronic case report form
FDA – Food and Drug Administration
FT – fecal transplant
GCPs – Good Clinical Practices
HIV – human immunodeficiency virus
IBD – inflammatory bowel disease
IBS – irritable bowel syndrome
ICF – informed consent form
ICH – International Council on Harmonisation
ICU – intensive care unit
IgG – Immunoglobulin
IRB – Institutional Review Board
IVIG – intravenous immunoglobulin
mL - milliliter
NA – not applicable
PAL – Product Accountability Log
PCR – polymerase chain reaction
REB – Research Ethics Board
SAE – serious adverse event
SAP – Statistical Analysis Plan
SAR – suspected adverse reaction
SOC – system organ class
SOP – standard operating procedure
USP – United States Pharmacopeia
VRE – vancomycin-resistant *enterococci*

Synopsis

Title	An Open-label Efficacy and Safety Assessment of Rebiotix RBX2660 (microbiota suspension) for the Treatment of Recurrent *Clostridium difficile* Infection
Investigational Product and Indication for Use	RBX2660 (microbiota suspension). RBX2660 is being studied for the treatment of adult subjects as an adjunct to antibiotics for the treatment of recurrent *Clostridium difficile* infection (CDI)
Control Product	Not applicable
Study Purpose	The purpose of this study is to evaluate the efficacy and safety of RBX2660 for the treatment of recurrent CDI in subjects who have had either a) at least two recurrences after a primary episode (primary episode + ≥ two recurrences, i.e., at least three episodes) and have completed at least two rounds of standard-of-care oral antibiotic therapy or b) have had at least two episodes of severe CDI resulting in hospitalization.
[redacted]	[redacted]
Objectives	Primary Objective To compare the efficacy of one treatment with RBX2660 versus antibiotic-treated historical controls. (One treatment consists of 2 enemas of RBX2660 7 ± 2 days apart.) Secondary Objectives 1. To evaluate the safety of RBX2660. 2. To evaluate the effect of RBX2660 on pre-existing conditions other than recurrent CDI. 3. To evaluate quality of life as measured by the SF-36 Health Survey. 4. To evaluate the efficacy of *C. difficile* anti-infection therapies administered to confirmed treatment failures.

Study Design	This is a prospective, multicenter, open-label study assessing RBX2660 as an adjunct to antibiotics for the treatment of recurrent CDI. Up to approximately 150 subjects will be enrolled in this study. Efficacy of RBX2660, measured by the recurrence-free rate of CDI diarrhea without the need for retreatment with *C. difficile* anti-infective therapy or fecal transplant through 56 days after completion of study treatment with RBX2660, will be evaluated by comparing the recurrence-free rate observed in the study population to the recurrence-free rate identified from antibiotic-treated historical controls. Subjects who meet all inclusion and exclusion criteria, including having a positive *C. difficile* test within 60 days prior to enrollment and experiencing either a) at least two recurrences after a primary episode and have completed at least two rounds of standard-of-care oral antibiotic therapy or b) have had at least two episodes of severe CDI resulting in hospitalization prior to enrollment, will receive treatment with RBX2660. One completed study treatment consists of two RBX2660 enemas administered 7 ± 2 days apart (no antibiotics between doses); the second enema may be administered sooner if CDI diarrhea reoccurs in less than 7 days. Subjects will already be taking or will start a course of antibiotics to control recurrent CDI diarrhea at the time of enrollment, [redacted] An in-office follow-up visit will occur at 8 weeks after completing study treatment for the assessment of AEs, efficacy, collection of the Subject Diary and blood tests. Telephone assessments for adverse events including serious adverse events (SAEs) and the new onset of chronic diseases and CDI symptoms will occur at weeks 1, 2, 3, and 4 and at 3, 6, and 12 months after completing study treatment. Assessment of SAEs and the new onset of chronic diseases will occur via phone call at 24 months after completing study treatment. Subjects will keep a detailed Subject Diary to allow for the assessment of solicited adverse events from the date of enrollment to 1 week after completing study treatment. Treatment failures (as determined by the site investigator at the time of CDI recurrence) are eligible to receive another therapy deemed most appropriate by their study investigator; see "Management of Treatment Failures," below.
Study Dosing	A study treatment consists of two enemas (doses) of RBX2660 administered 7 ± 2 days apart (the second enema may be administered sooner if CDI diarrhea reoccurs in less than 7 days).

Definition of Treatment Success	Treatment success is defined as: the absence of CDI diarrhea without the need for retreatment with *C. difficile* anti-infective therapy or fecal transplant through 56 days after completion of study treatment.
Definition of Treatment Failure (CDI Recurrence)	Treatment failure (CDI recurrence) is defined as: 1) the presence of CDI diarrhea, with or without other CDI symptoms, < 56 days after completion of study treatment; 2) a positive stool test for *C. difficile*; 3) need for retreatment for *C. difficile* infection; and 4) no other cause for CDI diarrhea has been determined. [redacted]
Determination of Success/Failure	The site investigator makes the determination of success based on: the absence of CDI diarrhea without the need for retreatment with *C. difficile* anti-infective therapy or FT through 56 days after completion of study treatment. The investigator makes the determination of failure based on 1) the subject's reporting the presence of CDI diarrhea, with or without other CDI symptoms, < 56 days after completion of study treatment; 2) a positive stool test for *C. difficile*; 3) need for retreatment for *C. difficile* infection; and 4) no other cause for CDI diarrhea symptoms is determined.
Medical Monitor Adjudication	The Medical Monitor will adjudicate SAEs and other adverse events of interest to provide an objective, qualified judgment of the events.
Sample Size	[redacted] The study will enroll up to approximately 150 subjects to allow for loss-to-follow and additional safety numbers if needed. [redacted]
[redacted]	[redacted]

Blinding	This is an open-label study; no blinding will be employed.
Study Population	The target population is adults (≥ 18 years old) with recurrent CDI who have had either a) at least two recurrences after a primary episode [redacted] and have completed at least two rounds [redacted] of severe CDI resulting in hospitalization.
Inclusion Criteria for Treatment Subjects	1. ≥ 18 years old. 2. Medical record documentation of recurrent CDI including a positive *C. difficile* test within 60 days prior to enrollment and either: a) at least two recurrences after a primary episode and has completed at least two rounds of standard-of-care oral antibiotic therapy or b) has had at least two episodes of severe CDI resulting in hospitalization. 3. Already taking or will start a course of antibiotics to control recurrent CDI symptoms at the time of enrollment. [redacted] 4. Willing and able to have an enema(s). 5. Willing and able to complete the stool and serum testing required for the study. 6. [redacted] 11. Willing to complete the required Subject Diary. 12. Willing and able to meet all study requirements, including completing the assessment visit and phone calls.
Exclusion Criteria for Treatment Subjects	1. A known history of continued CDI diarrhea despite being on a course of antibiotics prescribed for CDI treatment. 2. Requires continuous antibiotic therapy for a condition other than CDI. 3. Previous successful (resolution of CDI diarrhea) fecal transplant for recurrent CDI < 6 months prior to study enrollment. 4. Previous unsuccessful (recurrent CDI diarrhea was unresolved) fecal transplant.

	5. Previous treatment with RBX2660. 6. Diagnosis of inflammatory bowel disease (IBD), e.g., ulcerative colitis, Crohn's disease, or microscopic colitis. 7. Diagnosis of irritable bowel syndrome (IBS) as determined by Rome III criteria. 8. History of chronic diarrhea. 9. History of celiac disease. 10. Disease symptoms caused by a confirmed intestinal pathogen other than *C. difficile*. 11. Colostomy. 12. Intraabdominal surgery within the last 60 days. [redacted] 18. Life expectancy of < 12 months. 19. Compromised immune system [redacted]
Study Sites	Up to 40 US and Canadian sites will participate in this study.
Study Sponsor	Rebiotix Inc 2660 Patton Road

	Roseville MN 55113 USA www.rebiotix.com [redacted]
[redacted]	[redacted]

1.0 Introduction

1.1 Background

RBX2660 (microbiota suspension) is an intestinal microbial suspension prepared from human stool obtained from carefully and thoroughly screened healthy human donors. It is being studied for the treatment of recurrent *Clostridium difficile* infection (CDI). RBX2660 is prepared from a standardized amount of stool mixed with saline [redacted]

Rebiotix conducted its first clinical study of RBX2660 in an open-label, non-controlled Phase 2 study demonstrating the safety of the product and is currently conducting a prospective, multicenter, randomized, double-blinded, placebo-controlled, 3-arm Phase 2B study designed to demonstrate the efficacy and safety of RBX2660 for the treatment of recurrent CDI. The purpose of this current study is to gather additional efficacy and safety data on the use of RBX2660 for recurrent CDI in subjects who have had either a) at least two recurrences after a primary episode [redacted] and have completed at least two rounds of standard-of-care oral antibiotic therapy or b) have had at least two episodes of severe CDI resulting in hospitalization.

This study will be conducted in compliance with this protocol and each site's governing Institutional Review Board/Research Ethics Board; in accordance to relevant regulations in 21 CFR Part 11, 50, 54, 56 and 312; and the ICH E6 Good Clinical Practice: Consolidated Guidance.

1.2 Investigational Agent

[redacted] One study treatment consists of two doses of RBX2660 administered 7 ± 2 days apart.

1.4 Preclinical Data

[redacted]

[redacted]

1.5 Phase 2 Clinical Data

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

1.6 Treatment Rationale and Risk/Benefit

This study is assessing RBX2660 as an adjunct to antibiotics for the treatment of recurrent CDI. RBX2660 (microbiota suspension) was developed after careful, controlled component and process testing performed by Rebiotix. [redacted]

2.0 Study Objectives and Endpoints

2.1 Primary Objective

To compare the efficacy of one treatment with RBX2660 versus antibiotic-treated historical controls. (One treatment consists of 2 enemas of RBX2660 7 ± 2 days apart.)

2.2 Secondary Objectives

1. To evaluate the safety of RBX2660.
2. To evaluate the effect of RBX2660 on pre-existing conditions other than recurrent CDI.
3. To evaluate quality of life as measured by the SF-36 Health Survey.

4. To evaluate the efficacy of *C. difficile* anti-infection therapies administered to confirmed treatment failures.

2.3 Primary Endpoint

Efficacy, measured by the recurrence-free rate of CDI diarrhea without the need for retreatment with *C. difficile* anti-infective therapy or fecal transplant through 56 days after completion of study treatment with RBX2660, will be evaluated by comparing the recurrence-free rate observed in the study population to the recurrence-free rate identified from antibiotic-treated historical controls.

2.4 Secondary Endpoints

1. The frequencies and severity grades of solicited AEs from the first day of assigned study treatment through seven days following the last enema of assigned study treatment will be collected.
2. Adverse events, including serious adverse events and the onset of new chronic diseases, will be collected at in-office visits at 1, 4 and 8 weeks and at 3-, 6-, and 12-month calls; they will be categorized by frequency, severity, seriousness, and causality.
3. Serious adverse events and new onset of chronic diseases will be collected at a telephone call at 24 months and will be categorized by frequency, severity, seriousness, and causality.
4. Frequencies of major complications of CDI including death, septic shock, toxic megacolon, colonic perforation, emergency colectomy, or ICU admission will be collected through the 24-month telephone assessment.
5. The effect of RBX2660 on pre-existing conditions other than recurrent CDI will be assessed by comparing the change in the condition throughout the course of the study.
6. SF-36 scores recorded at the Week 8 visit will be compared to scores recorded at the screening visit.
7. Efficacy of *C. difficile* anti-infection therapies administered to confirmed treatment failures will be compared to each other for the rates of success at resolving CDI diarrhea for at least 56 days after completion of the anti-infection therapy.

3.0 Study Design

3.1 Study Purpose

The purpose of this study is to evaluate the efficacy and safety of RBX2660 for the treatment of recurrent CDI in subjects who have had either a) at least two recurrences after a primary episode (i.e., at least three episodes) and have completed at least two rounds of standard-of-care oral antibiotic therapy or b) have had at least two episodes of severe CDI resulting in hospitalization.

3.2 Study Design

This is a prospective, multicenter, open-label study assessing RBX2660 as an adjunct to antibiotics for the treatment of recurrent CDI. Up to approximately 150 subjects will be enrolled in this study. Efficacy of RBX2660, measured by the recurrence-free rate of CDI diarrhea without the need for retreatment with *C. difficile* anti-infective therapy or fecal transplant through 56 days after completion of study treatment with RBX2660, will be evaluated by comparing the recurrence-free rate observed in the study population to the recurrence-free rate identified from antibiotic-treated historical controls. Subjects who meet all inclusion and exclusion criteria, including having a positive *C. difficile* test within 60 days prior to enrollment and experiencing either a) at least two recurrences after a primary episode and have completed at least two rounds of standard-of-care oral antibiotic therapy or b) have had at least two episodes of severe CDI resulting in hospitalization prior to enrollment, will receive treatment with RBX2660. One study treatment consists of two RBX2660 enemas administered 7 ± 2 days apart (no antibiotics between doses); the second enema may be administered sooner if CDI diarrhea reoccurs in less than 7 days. Subjects will already be taking or will start a course of antibiotics to control recurrent CDI diarrhea at the time of enrollment, [redacted] An in-office follow-up visit will occur at 8 weeks after completing study treatment for the assessment of AEs, efficacy, collection of the Subject Diary, and blood tests. Telephone assessments for adverse events including SAEs and the new onset of chronic diseases and CDI symptoms will occur at weeks 1, 2, 3, and 4 and at 3, 6, and 12 months after completing study treatment. Assessment of serious adverse events and the new onset of chronic diseases will occur via phone call at 24 months after completing study treatment. Subjects will

keep a detailed Subject Diary to allow for assessment of solicited adverse events (see Section 7.1.3) from the date of enrollment to 1 week after completing study treatment. Treatment failures (as determined by the site investigator at the time of CDI recurrence) are eligible to receive another therapy deemed most appropriate by their study investigator; see "Management of Treatment Failures," below.

[redacted]

3.4 Definition of CDI Diarrhea

Clostridium difficile diarrhea is defined as the passage of ≥ 3 watery stools in ≤ 24 hours for at least two consecutive days.

3.5 Definition of Treatment Success

Treatment success is defined as: the absence of CDI diarrhea without the need for retreatment with *C. difficile* anti-infective therapy or FT through 56 days after completion of study treatment.

3.6 Definition of Treatment Failure

Treatment failure (CDI recurrence) is defined as: 1) the presence of CDI diarrhea (see Section 3.4), with or without other CDI symptoms, < 56 days after completion of study treatment; 2) a positive stool test for *C. difficile*; 3) need for retreatment for *C. difficile* infection; and 4) no other cause for CDI symptoms has been determined. [redacted]

3.7 Management of Treatment Failures

Treatment failures (as determined by the site investigator at the time of CDI recurrence) are to be treated with the therapy(ies) deemed most appropriate by their study investigator. All CDIs, including reoccurrences and new episodes, are to be captured in detail on the CDI History and On-study Episodes eCRF.

3.8 Determination of Treatment Success/Failure

The site investigator makes the determination of success based on: the absence of CDI diarrhea without the need for retreatment with *C. difficile* anti-infective therapy or FT < 56 days after completion of study treatment. S/he makes the determination of failure based on the four criteria for treatment failure listed in Section 3.6, above.

3.9 Blinding

This is an open-label study; no blinding will be employed.

4.0 Treatment Subject Selection and Withdrawal

4.1 Inclusion Criteria

All responses must be "yes" to include a subject in the study:

1. ≥ 18 years old.
2. Medical record documentation of recurrent CDI including a positive *C. difficile* test within 60 days prior to enrollment and either: a) at least two recurrences after a primary episode and has completed at least two rounds of standard-of-care oral antibiotic therapy or b) has had at least two episodes of severe CDI resulting in hospitalization.
3. Already taking or will start a course of antibiotics to control recurrent CDI symptoms at the time of enrollment. [redacted]
4. Willing and able to have an enema(s).
5. Willing and able to complete the stool and serum testing required for the study.
6. [redacted]

10. [redacted]
11. Willing to complete the required Subject Diary.
12. Willing and able to meet all study requirements, including completing the assessment visit and phone calls.

4.2 Exclusion Criteria

All responses must be "no" to include a subject in the study:

1. A known history of continued CDI diarrhea despite being on a course of antibiotics prescribed for CDI treatment.
2. Requires continuous antibiotic therapy for a condition other than CDI.
3. Previous successful (resolution of CDI diarrhea) fecal transplant for recurrent CDI < 6 months prior to study enrollment.
4. Previous unsuccessful (recurrent CDI diarrhea was unresolved) fecal transplant.
5. Previous treatment with RBX2660.
6. Diagnosis of inflammatory bowel disease (IBD), e.g., ulcerative colitis, Crohn's disease, or microscopic colitis.
7. Diagnosis of irritable bowel syndrome (IBS) as determined by Rome III criteria.
8. History of chronic diarrhea.
9. History of celiac disease.
10. Disease symptoms caused by a confirmed intestinal pathogen other than *C. difficile*.
11. Colostomy.
12. Intraabdominal surgery within the last 60 days.

[redacted]

18. Life expectancy of < 12 months.
19. Compromised immune system [redacted]

[redacted]

4.3 Subject Recruitment and Screening

Subjects are recruited by qualified and trained site personnel when they are identified as experiencing either a) at least two recurrences of CDI after a primary episode (i.e., at least three episodes) and have completed at least two rounds of standard-of-care oral antibiotic therapy or b) had at least two episodes of severe CDI resulting in hospitalization. Potential subjects are to be fully informed as to this study's purpose, requirements, anticipated risks, etc., and are to be given the chance to review the informed consent form and receive satisfactory answers to all questions. Subjects must then sign the study-specific, IRB/REB-approved Informed Consent Form and HIPAA Form (if applicable), which is the point of study enrollment.

Subjects are eligible to receive study treatment upon confirmation that they have met all inclusion/exclusion criteria listed in Sections 4.1 and 4.2. Subjects must discontinue their antibiotics for 24-48 hours prior to receiving the first study enema.

4.4 Subject Withdrawal or Termination

A subject's study participation is considered complete after the final telephone call is conducted 24 months after completion of study treatment with RBX2660.

A subject may withdraw or be withdrawn from the study prematurely for the following reasons:

- Withdrawal of consent by subject
- Lost to follow-up
- Failure to comply with study requirements
- Termination of study by the sponsor
- Death
- Other (to be specified).

The reason for termination is recorded on the Study Exit eCRF. In the event that a subject withdraws from the study, every attempt should be made to have the subject return for an early withdrawal visit and to complete the 3-, 6-, 12-, and 24-month telephone calls. [redacted]

5.0 RBX2660

5.1 RBX2660 Description

RBX2660 microbiota suspension 50 g/150 mL in an enema bag. Each bag of RBX2660 provides one enema (one dose). [redacted] One study treatment consists of two doses of RBX2660 administered 7 ± 2 days apart.

5.3 Rebiotix Kit Description

[redacted]

5.4 Treatment Regimen

See Section 6.0 for the Schedule of Study Procedures and a full description of them.

5.5 Subject Compliance Monitoring

Subjects are assessed at an in-office visit at 8 weeks and via telephone calls from the study coordinator at 1, 2, 3, and 4 weeks and 3, 6, 12, and 24 months after completion of study treatment with RBX2660. See Section 6.0 for follow-up assessments and phone calls, and Section 4.4 for subject withdrawal/termination.

[redacted]

5.7 Preparation of RBX2660

Instructions for Use with detailed directions for RBX2660 preparation and administration are in each Rebiotix Kit.

5.8 Packaging

[redacted]

[redacted]

5.9.3 Dispensing and Administering RBX2660

Dispensing procedures vary from site to site, but each site's study investigator is responsible for delegating the administration of RBX2660 to a qualified and trained health care professional; [redacted]

[redacted]

[redacted]

6.0 Study Procedures

6.1 General Information

Study information is collected on study-specific electronic case report forms (eCRFs) by the site. Study monitoring occurs at regular intervals to ensure the protection of subject rights and safety, data integrity and accuracy, and proper study conduct in compliance with the protocol and applicable regulations including 21 CFR 50 and 312 and ICH E6 GCPs. The study-required procedures for treatment subjects are to be conducted as shown in Table 6-1 and illustrated in Figures 2 and 3.

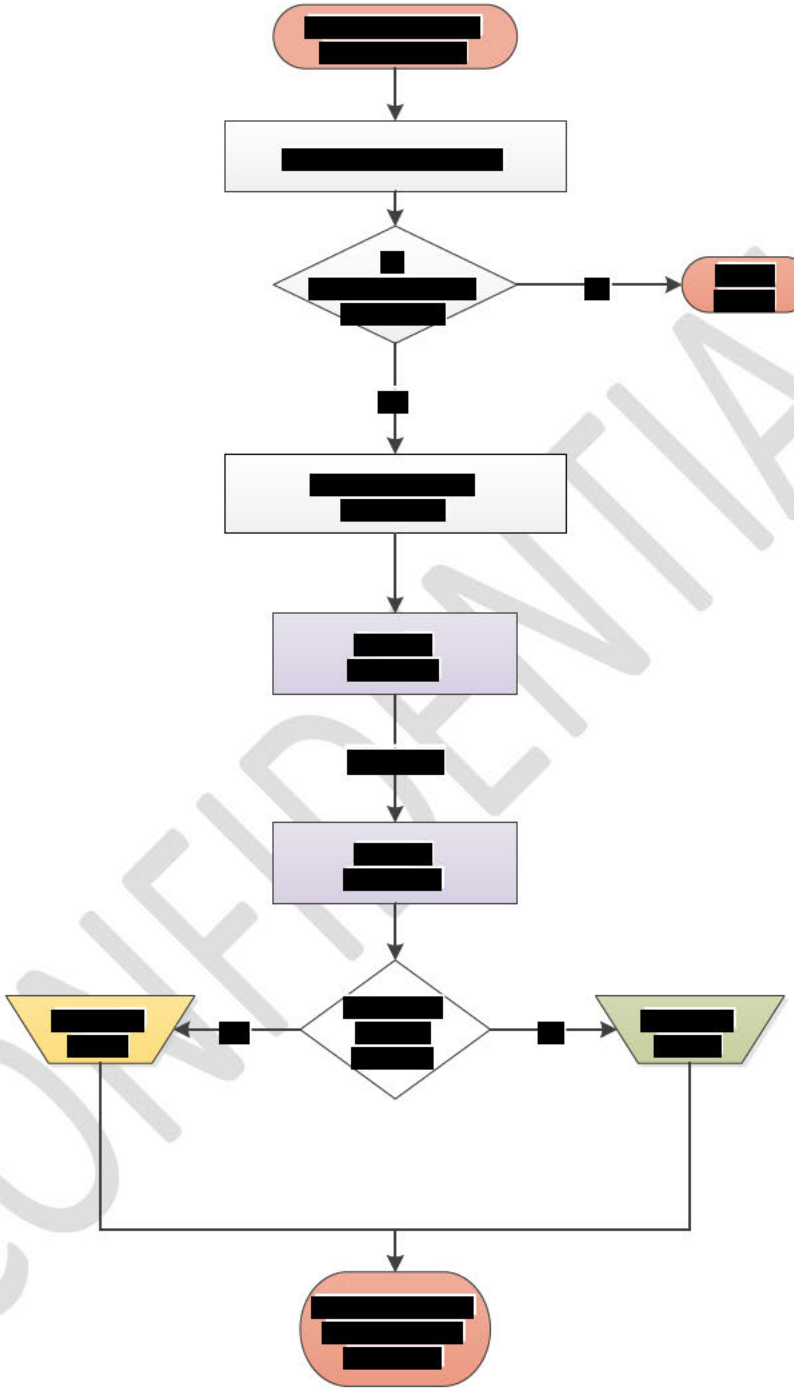

Figure 2: Study Design

Table 6-1: Schedule of Study Procedures

Activity	Visit 1 Screening	Visit 2 Baseline/ First Enema	Visit 3 Second Enema 7 ± 2 days after Visit 2	Visit 4 8-Week (± 7 days)	Phone Assessment (Weeks 1, 2, 3, and 4 (± 3 days))[2]	Phone Assessment at 3, 6, 12, and 24 months (± 7 days) after Visit 3[3]
Informed consent obtained	X					
Demographics, medical history	X					
Prescribe/continue antibiotics	X					
[redacted]		[redacted]				
[redacted]	[redacted]		[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]		[redacted]		
[redacted]		[redacted]				
Pregnancy testing performed	X	X	X			
Form SF-36 administered	X			X[6]		
Inclusion/exclusion criteria confirmed	X	X				
[redacted]		[redacted]				
RBX2660 administered		X	X			
Recurrence of CDI symptoms assessed		X	X	X	X	X
Vital signs assessed	X	X	X	X		
Subject Diary reviewed and collected[1]	X[1]	X[1]	X[1]	X[1, 5]		
Concomitant medications	X	X	X	X	X	X
AEs/SAEs assessed	X	X	X	X	X	X
Review of Pre-existing Conditions				X		X

[1]Solicited AEs are collected through 7 days after last treatment with RBX2660. Review the Diary at each visit and collect completed pages at Visits 2, 3, and 4.

[redacted]

Figure 3: Study Procedures and Timeline

6.2 Informed Consent

All subjects must sign the study-specific IRB/REB-approved Informed Consent Form and HIPAA Form (if applicable); see Section 4.3. The original is to be stored in the subject's medical record unless otherwise indicated by site procedures and a copy of the signed Form is to be placed in the subject's study file. A copy of the Form is to be given to the subject for his/her records.

6.3 Subject Diary

Subjects are to complete the Subject Diary from the time of enrollment until seven days after the last study treatment enema (second dose). The Diary serves as a tool for the subject to record pre- and post-treatment health and information that may indicate the reoccurrence of CDI and adverse events. The pre-treatment Diary information is recorded by the subject from the day of enrollment to the day before the first study treatment and is reviewed and collected at the baseline visit. The post-treatment Diary pages are given to the subject on the day of the first study treatment and are to be collected at the second enema visit (Visit 3) and at the 8-week office visit (Visit 4). The study coordinator reviews the contents of the Diary and records the relevant information in the study database. All pages of the Diary are required to be collected, reviewed and entered into the study database.

6.4 Visit 1: Screening Visit

At the screening visit, subjects are told about the study and informed consent (and HIPAA Form, if applicable) is (are) obtained. Standard demographics (gender, ethnicity, date of birth, etc.) and a detailed medical history are collected. Blood for the absolute neutrophil count is obtained. If applicable, a urine-dipstick pregnancy test is taken. Vital signs are assessed, concomitant medications are recorded, and AEs/SAEs are assessed. Completion of the Subject Diary is explained and how to complete it is demonstrated; see Section 6.3. Remind subjects to bring completed Diary pages with them at Visits 2, 3, and 4. Inclusion/exclusion criteria are assessed. A baseline Form SF-36 is administered. [redacted] Antibiotics for the control of CDI symptoms are prescribed or continued [redacted]

6.4.1 Stool Samples [redacted]

Subjects are requested to provide stool samples to Rebiotix at screening; in between Visits 2 and 3; at 1, 4, and 8 weeks after Visit 3; around the time of the 6-, 12-, and 24-month phone calls; if CDI reoccurs prior to 56 days after completing study treatment and if CDI occurs any time during the study participation. [redacted]

6.5 Visit 2: Baseline Visit and First Enema

The baseline visit is performed on the day of the first enema administration. A modified physical exam (no genitourinary exam unless medically indicated) is performed to establish baseline health status, vital signs, and document pre-existing medical conditions. Blood and stool samples are collected per Table 6-2 and are tested at the site's CLIA-approved lab to establish baseline health should an adverse event occur in the future. [redacted]

Table 6-2: [redacted]

[redacted]

The pre-treatment Subject Diary is reviewed and collected and a post-treatment Subject Diary is given to the subject; completion instructions are to be reviewed with the subject. Inclusion/exclusion criteria and completion of the washout period are confirmed. Recurrence of CDI symptoms is assessed, concomitant medications are recorded and AEs/SAEs are assessed. Prior to administration of each dose of RBX2660, a female subject of childbearing potential is required to undergo a urine-dipstick pregnancy test to confirm that she is not pregnant. RBX2660 is administered via enema per the Instructions for Use and standard site procedure by the authorized RBX2660 Administrator; see section 5.9.3. [redacted]

[redacted] The subject is sent home after a review of the Subject Diary instructions, instructions to call the study coordinator if CDI symptoms reoccur, and an appointment for the next enema of RBX2660.

6.6 Visit 3: Second Enema

The subject returns to the site for the second enema of RBX2660 7 ± 2 days after the first enema [redacted] Recurrence of CDI symptoms is assessed, concomitant medications are recorded and AEs/SAEs are assessed. Prior to administration of RBX2660, a female subject of childbearing potential is required to undergo another urine-dipstick pregnancy test to confirm that she is not pregnant. The Subject Diary is reviewed to ensure it is being completed properly and the first page of the post-treatment Diary should be collected if available. RBX2660 is administered via enema per the Instructions for Use and standard site procedure by the authorized RBX2660 Administrator. [redacted] The subject is sent home after a review of the Subject Diary instructions, instructions to call the study coordinator if CDI symptoms reoccur, and an appointment for the 8-week follow-up visit.

6.7 Weekly Telephone Assessments

Study coordinators will make weekly phone calls during weeks 1-4 after completing study treatment with RBX2660. The purpose of the calls is to assess for AEs including SAEs and the onset of new chronic diseases, recurrence of CDI and an update of concomitant medications. [redacted]

6.8 8-Week Office Visit (Visit 4)

Subjects return to the study site for the 8-week follow-up visit after completing study treatment with RBX2660. [redacted] At this visit, reoccurrence of CDI and adverse event information is assessed, a review of medication changes and pre-existing conditions is conducted, and the SF-36 Form is administered. The assessment of pre-existing conditions includes discussion with the subject to determine if the condition is improved, unchanged, worsened or resolved since completion of study treatment. The Subject Diary is collected and reviewed with the subject. [redacted] If CDI reoccurrence is suspected < 56 days after completing study treatment, the investigator assesses for recurrence using the four criteria in Section 3.6. The need for retreatment for *C. difficile* infection in the absence of a positive stool test for *C. difficile* will be made by the study investigator so that the appropriate treatment of the subject can be quickly administered in the case of failure to avoid serious adverse events, including hospitalization or death. If the subject does not have reoccurrence of CDI before 56 days after completing study treatment, s/he is considered a treatment success (see Section 3.5). [redacted]

6.9 3-, 6-, 12-, and 24-Month Telephone Assessments

Subjects receive a telephone assessment for adverse events, including serious adverse events and the new onset of chronic diseases at 3, 6, and 12 months; and an assessment of pre-existing conditions, serious adverse events and the new onset of chronic diseases at 24 months after completion of study treatment with RBX2660. These phone calls must occur within the window specified in Table 6-1; calls [redacted]
The assessment of pre-existing conditions includes discussion with the subject to determine if the condition improved, unchanged, worsened or resolved since completion of study treatment. Information on concomitant medications is updated as needed. [redacted] Adverse events and the new onset of chronic diseases are to be recorded and reported as required in Section 7.0 [redacted]

[redacted] The last phone call occurs at 24 months after completion of study treatment with RBX2660. [redacted]

6.10 Antibiotic-treated Historical Control

The efficacy of RBX2660 will be evaluated by comparing the recurrence-free rate observed in the study population to the recurrence-free rate identified from antibiotic-treated historical controls. A chart review will be conducted by Rebiotix or its designees to gather the historical control data to be used in the comparison. The historical control protocol can be found in Appendix 1 to this study protocol.

7.0 Assessment of Safety and Efficacy

7.1 Assessment of Safety

7.1.1 Definitions

- Adverse event: an adverse event (AE) is any untoward medical occurrence associated with the use of RBX2660, whether or not the event is considered product-related.
- Adverse reaction: Any adverse event caused by RBX2660.
- Suspected adverse reaction (SAR): any adverse event for which there is a reasonable possibility that RBX2660 caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the RBX2660 and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than "adverse reaction," which means any adverse event caused by RBX2660.
- Serious adverse event (SAE) or serious suspected adverse reaction: An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator, Medical Monitor or sponsor, it results in any of the following outcomes:
  - Death
  - Life-threatening adverse event
  - Inpatient hospitalization ≥ 24 hours or prolongation of an existing hospitalization

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect.

Important medical events that do not result in death, are not life-threatening, or do not require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

- Unexpected adverse event or unexpected suspected adverse reaction: an adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed.
- Life-threatening: An adverse event or suspected adverse reaction that places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.

7.1.2 Anticipated Adverse Events

The following is list of anticipated adverse events that may or may not be causally related to RBX2660, the enema procedure, or CDI:

- gas (flatulence)
- belching
- abdominal distension or bloating
- increased diarrhea
- abdominal cramping or pain
- constipation
- colitis
- fever ≥ 38.0° C (100.4°F)
- fatigue
- chills
- transmission of disease from the donor to recipient
- rectal irritation or pain
- rectal bleeding
- nausea
- vomiting
- hypotension
- puncture of the intestine

7.1.3 Solicited Adverse Events

The following list of anticipated adverse events are solicited from subjects via the Subject Diary from the date of enrollment through the 7th day after completing study treatment:

- gas (flatulence)
- abdominal distension or bloating
- rectal irritation or pain
- chills/severe shivering
- abdominal pain or cramping
- increased diarrhea
- constipation
- rectal bleeding
- nausea
- vomiting
- fever ≥ 38.0° C (100.4°F)

Through the completion of the Subject Diary, subjects are asked specific questions regarding frequency and severity of the solicited AEs.

7.1.4 Preexisting Condition

A preexisting condition, including abnormal physical exam findings, is one that is present at the start of the study and is to be recorded at the time of the screening or baseline visits. A preexisting condition is to be recorded as an adverse event if the frequency, intensity or the character of the condition worsens during study participation. They are also to be assessed at the 3-, 6-, 12-, and 24-month telephone assessments; See Section 6.9.

7.1.5 Adverse Event Reporting to the Sponsor

Sites are required to record and report all adverse events to Rebiotix via entry on the Adverse Event eCRF in the study database within **ten (10)** working days of discovery unless it is an SAE or serious suspected adverse reaction. Serious adverse events (SAEs) and serious suspected adverse reaction events must be reported within **three (3)** working days of discovery by recording them in the study database. Adverse event information is collected from the time the subject signs the Informed Consent Form and HIPAA Form (if applicable) through study termination. All adverse events, including serious adverse events and the new onset of chronic diseases, will be collected through 12 months; serious adverse

events and the new onset of chronic diseases will be collected through 24 months after completion of study treatment. [redacted] Neither a condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event if the hospitalization or prolonged hospitalization is for diagnostic or elective surgical procedures for a preexisting condition. Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful. Reoccurrence of CDI is *not* to be reported as an AE or SAE unless hospitalization ≥ 24 hours is required to treat it. If hospitalization is not required, it is to be recorded on the Follow-up eCRF and CDI History and On-study Episodes eCRF and reviewed as a component of efficacy assessment. If hospitalization for recurrent CDI is required, it is to be reported as an SAE within 3 working days of discovery in the database and on the CDI History and On-study Episodes eCRF. [redacted]

7.1.6 Grading Adverse Event Severity

Adverse Events are to be graded by severity by the site investigator; see Section 7.1.8. For the classification of adverse events, severity is not the same as serious, which is defined in section 7.1.1. Severity is an indication of the *intensity* or a specific event (e.g., mild, moderate or severe). Classification of an event as serious relates to an event's outcome or intervention criteria and is usually associated with events that pose a threat to a subject's life or functioning. An event can be severe but not serious, such as a migraine. [redacted] the site investigator will categorize the severity of an adverse event on the AE eCRF.

Table 7-1: Severity Grading Table*

Parameter	Grade 1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially Life-threatening
Flatulence	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	NA	NA
Belching (burping)	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	NA	NA
Abdominal distension or bloating	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	NA

Parameter	Grade 1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially Life-threatening
Increased diarrhea	Increase of ≤ 3 stools over baseline per 24-hour period	Increase of 4 – 6 stools over baseline per 24-hour period	Bloody diarrhea if not present at baseline OR increase of ≥ 7 stools over baseline per 24-hour period OR IV fluid replacement indicated if not indicated at baseline	Life-threatening consequences, e.g., hypotensive shock
Abdominal cramping/pain	Discomfort/pain causing no or minimal interference with usual social and functional activities	Discomfort/pain causing greater than minimal interference with usual social and functional activities	Discomfort/pain causing inability to perform usual social and functional activities	Disabling pain causing inability to perform basic self-care OR inpatient hospitalization ≥ 24 hours
Constipation	Occasional or intermittent symptoms, occasional use of stool softeners, laxatives, dietary modifications or enema	Persistent symptoms with regular use of laxatives or enemas indicated	Symptoms causing inability to perform usual social and/or functional activities	Life-threatening consequences, e.g., obstruction, toxic megacolon
Colitis	No symptoms, regardless of pathologic or radiographic evidence of inflammation	Abdominal pain, mucus or blood in the stool	Abdominal pain, fever, change in bowel habits with ileus; peritoneal signs	Life-threatening consequences, e.g., perforation, bleeding, ischemia, necrosis, toxic megacolon

Parameter	Grade 1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially Life-threatening
Fever	37.7 – 38.6°C (99.9 – 101.5° F)	38.7 – 39.3°C (101.6 – 102.8° F)	39.4 – 40.5°C (102.9 – 104.9° F)	> 40.5°C (104.9° F)
Fatigue/malaise	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	Incapacitating fatigue/malaise symptoms causing inability to perform basic self-care functions
Chills	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	NA
Rectal discomfort or irritation	No symptoms or symptoms not requiring medical intervention	Symptomatic with medical intervention (topical medications / treatments) indicated	Symptoms causing inability to perform usual social and functional activities or requiring medical intervention other than topical medications / treatments	NA
Rectal bleeding	Mild or intermittent without transfusion	Persistent without transfusion	Requires transfusion	Life-threatening consequences

Parameter	Grade 1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially Life-threatening
Nausea	Transient (≤ 24 hours) or intermittent nausea with nor or minimal interference with oral intake	Persistent nausea resulting in decreased intake for 24-48 hours	Persistent nausea resulting in decreased intake > 48 hours OR aggressive rehydration indicated, e.g., IV fluids	Life-threatening consequence, e.g., hypotensive shock
Vomiting	Transient (≤ 24 hours) or intermittent vomiting with nor or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR aggressive rehydration indicated, e.g., IV fluids	Life-threatening consequence, e.g., hypotensive shock
Hypotension	NA	Symptomatic, corrected with oral fluid replacement	Symptomatic, IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure

Parameter	Grade 1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially Life-threatening
Adverse event not identified elsewhere in this table	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death

*Adapted from *Division of AIDS Table for Grading of Severity of Adult and Pediatric Adverse Events* and *Addendum 3: Rectal Grading Table for Use in Microbicide Studies; May 2012.*

7.1.7 Causality

For all adverse events, the investigator must pursue and obtain adequate information to determine the outcome of the adverse event and to assess whether the AE meets the criteria for classification as an SAE, serious suspected adverse reaction, suspected adverse reaction, unexpected adverse event, or unexpected suspected adverse reaction. For all adverse events, sufficient information should be obtained by the investigator to determine the causality of the adverse event. For adverse events with a causal relationship to the RBX2660 or the enema procedure, follow-up by the investigator is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator.

The Investigator will make a causality assessment for all AEs and decide whether there is a reasonable possibility that the AE may have been caused by the study product or procedure, including an assessment of biologic plausibility, presence or absence of alternative causal explanations (such as continuation or exacerbation of the subject's

recurrent CDI symptoms), and temporal relationship to product administration and/or the procedure. Relatedness to RBX2660 or the enema procedure is defined as:

Unrelated: The event is due to an underlying or concurrent illness or effect of concomitant therapy and is not related to the study product or procedure (*e.g.*, has no temporal relationship to study product or procedure, or has a much more likely alternative etiology).

Possible: There is some temporal relationship between the event and the administration of the study product or procedure, and the event is less likely to be explained by the subject's medical condition or other therapies.

Probable: The temporal relationship between the event and administration of the study product or procedure is suggestive, and the event is unlikely to be explained by the subject's medical condition or other therapies.

Definite: The event follows a reasonable temporal sequence from administration of the study product or procedure, follows a known or suspected response pattern to the study product and/or procedure, improves upon stopping the study product, and reappears upon repeated exposure, if that occurs.

[redacted]

7.1.8 Medical Monitor

An independent Medical Monitor reviews SAEs and adverse events of interest to provide an objective, qualified judgment of the events. The Medical Monitor is a physician not participating as an investigator in this study. The Medical Monitor has the responsibility to review and evaluate the information relevant to product safety throughout the development and implementation of the protocol. The Medical Monitor performs the following functions:

- Reviews and adjudicates SAEs and AEs of interest;
- Reviews the study protocol and Investigator's Brochure for adequacy of safety oversight;

- Confers with site investigators and the Rebiotix Clinical Department regarding potential safety concerns as needed;
- Provides medical surveillance and provides appropriate recommendations for the conduct of the study as needed.

[redacted]

[redacted]

7.1.10 Sponsor Reporting of Adverse Events

The study sponsor is required to report certain study events in an expedited fashion to FDA and Health Canada. These written notifications of adverse events are referred to as expedited safety reports.

7.1.11.1 Temporal Reporting Requirements

The following describes the expedited safety reporting requirements by timeline for reporting and associated type of event:

- **Within 7 calendar days of event discovery by Rebiotix**
  Rebiotix will notify FDA and Health Canada of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than seven calendar days after the sponsor's initial receipt of the information.

- **Within 15 calendar days of event discovery by Rebiotix**
  Any study event that is:
  - suspected adverse reaction
  - serious
  - unexpected

  The AE must meet all three of these criteria for expedited reporting.

7.1.11.2 Additional Reporting Requirements

The sponsor will identify in IND safety reports all previous reports concerning similar adverse events and analyze the significance of the current event in light of the previous reports.

7.1.11.3 Reporting AEs to FDA and Health Canada

Rebiotix will report adverse events to FDA and Health Canada as required by applicable regulations. All adverse events will be reported in the annual and final clinical study reports.

7.2 Assessment of Efficacy

The primary efficacy parameter is treatment success, defined as the absence of CDI diarrhea without the need for retreatment with *C. difficile* anti-infective

therapy or FT through 56 days after the completion of study treatment (Visit 3). Some subjects may continue to have short bouts of diarrhea before the 56-day time point, but most if not all will be resolved within 30 days after receiving RBX2660. The efficacy assessment will occur at the 8-week office visit. [redacted] See Sections 6.10 and 8.0 of this protocol and the separate Statistical Analysis Plan for additional details on the efficacy assessment.

8.0 Statistical Considerations

8.1 Introduction

See the separate Statistical Analysis Plan for complete details of the statistical methodology and analyses that will be employed for this study. The primary analysis will be a comparison of the recurrence-free rate of CDI diarrhea in subjects treated with RBX2660 to the recurrence-free rate of CDI diarrhea in the antibiotic-treated historical control groups (see Appendix 1). [redacted] The study will enroll up to approximately 150 subjects to allow for loss-to-follow and additional safety numbers if needed. [redacted]

8.2 Endpoints

8.2.1 Primary Endpoint

Efficacy, measured by the recurrence-free rate of CDI diarrhea without the need for retreatment with *C. difficile* anti-infective therapy or fecal transplant through 56 days after completion of study treatment with RBX2660, will be evaluated by comparing the recurrence-free rate observed in the study population to the recurrence-free rate identified from antibiotic-treated historical controls.

8.2.2 Secondary Endpoints

1. The frequencies and severity grades of solicited AEs from the first day of assigned study treatment through seven days following the last enema of assigned study treatment will be collected.

2. Adverse events, including serious adverse events and the onset of new chronic diseases, will be collected at in-office visits at 1, 4 and 8 weeks and at 3-, 6-, and 12-month calls; they will be categorized by frequency, severity, seriousness, and causality.
3. Serious adverse events and new onset of chronic diseases will be collected at a telephone call at 24 months and will be categorized by frequency, severity, seriousness, and causality.
4. Frequencies of major complications of CDI including death, septic shock, toxic megacolon, colonic perforation, emergency colectomy, or ICU admission will be collected through the 24-month telephone assessment.
5. The effect of RBX2660 on pre-existing conditions other than recurrent CDI will be assessed by comparing the change in the condition throughout the course of the study.
6. SF-36 scores recorded at the Week 8 visit will be compared to scores recorded at the screening visit.
7. Efficacy of *C. difficile* anti-infection therapies administered to confirmed treatment failures will be compared to each other for the rates of success at resolving CDI diarrhea for at least 56 days after completion of the anti-infection therapy.

8.3 Analysis of Efficacy

The primary endpoint is: Efficacy, measured by the recurrence-free rate of CDI diarrhea without the need for retreatment with *C. difficile* anti-infective therapy or fecal transplant through 56 days after completion of study treatment with RBX2660. The primary analysis will compare the proportion of subjects in the treatment arm who are recurrence-free to the proportion of historical controls who are recurrence-free. [redacted] An efficacy and safety data-received-to-date analysis will be conducted on the treatment group subjects when approximately 100 of them

complete their 8-week assessment. [redacted]

8.4 Analysis of Safety

Summary statistics will be provided for the Safety Population (treated subjects) Adverse events that occur during the study. Analyses will include a listing of AEs by type and will include the seriousness, severity, and relatedness of the event. Adverse events will be summarized using counts of the number of events and frequencies (counts and percentages) of the number of subjects in the Safety Population. The frequency of each preferred term (PT) within the primary system organ class (SOC) as well as overall primary SOC frequency will be presented.

Adverse events will be recorded for each subject from the day of enrollment through the 12-month phone call. Serious adverse events (SAEs) and the new onset of chronic disease will be recorded for each subject from the day of enrollment through the duration of the study.

Solicited adverse events will be recorded in the Subject Diary from enrollment through the 7th day after the last study treatment and will be summarized utilizing frequencies, proportions and 95% CIs for categorical variables and using descriptive statistics.

The solicited adverse events that will be collected and summarized are:

- gas (flatulence)
- belching
- abdominal distension or bloating
- increased diarrhea
- abdominal cramping or pain
- constipation
- colitis
- fever
- fatigue
- chills
- transmission of disease from the donor to recipient
- rectal discomfort or irritation
- rectal bleeding
- nausea
- vomiting
- hypotension
- irritation or puncture of the intestine

Major complications of recurrent CDI include death, septic shock, toxic megacolon, colonic perforation, emergency colectomy, or ICU admission collected through the 24-month telephone assessment. Incidence of major complications of recurrent CDI will be summarized in tabular format.

8.5 Management of Missing Data

The safety assessment will be analyzed using all available data only. No imputation of missing data is planned for the safety assessment. Efficacy assessments will also be analyzed using all available data. [redacted]

[redacted]

9.0 Study Administration

9.1 Institutional Review Board / Research Ethics Board Approval

The protocol, Informed Consent Form and HIPAA Form (if applicable) must be reviewed and approved by the respective IRB/REB and Rebiotix before subject recruitment and enrollment begins.

Prior to subject enrollment, a signed copy of the IRB/REB approval letter addressed to the investigator and a full copy of the IRB/REB-approved Informed Consent Form and HIPAA Form (if applicable) must be submitted to Rebiotix, certifying trial approval.

Investigators are responsible for submitting and obtaining initial approval and continuing approval from the IRB/REB and forwarding copies of the approval letters to Rebiotix. The original letters are to be kept in the electronic Trial Master File (eTMF) designated for this study.

US Sites

Investigators are responsible for submitting and obtaining initial approval and continuing approval from the IRB/REB and forwarding copies of the approval letters to Rebiotix. The original letters are to be kept in the electronic Trial Master File (eTMF) designated for this study.

The investigator will notify the Rebiotix study manager within five (5) working days of withdrawal of IRB/REB approval.

Institutional Review Boards will operate in accordance to 21 CFR 56 and their own standard operating procedures.

Canadian Sites

Prior to subject enrollment, a signed copy of the REB approval letter addressed to the investigator, a full copy of the REB-approved Informed Consent Form, and completed Research Ethics Board Attestation must be submitted to [redacted], the Agent for Rebiotix in Canada, and to Rebiotix. Investigators are responsible for submitting and obtaining initial approval and continuing approval from the REC and forwarding copies of the approval letters to [redacted] Rebiotix. The original letters are to be kept in the electronic Trial Master File (eTMF) designated for this study.

The investigator will notify [redacted] Rebiotix within five (5) working days of withdrawal of REB approval.

Research Ethics Boards will operate in compliance with Health Canada regulations and their own standard operating procedures.

9.2 Form 1572 and Financial Disclosure

The principal investigator at each site (US and Canada) will complete and return a study-specific Form 1572 to the sponsor before beginning the study, as required by federal regulations. In the event of a change in study personnel, the site will complete and submit a new Form 1572 to Rebiotix within 60 days of the change. The investigator agrees to be responsible for conducting the investigational study in accordance with the protocol, applicable FDA regulations including reporting and record-keeping requirements, GCPs, local IRB/REB requirements, and controlling dispensation and administration of RBX2660. In addition, the investigator is responsible for ensuring that informed consent is obtained from

each subject prior to participating in the study, as well as protecting the rights, safety and welfare of participating subjects.

All investigators will be required to sign a Financial Disclosure form, which certifies the investigator's and his/her immediate family's financial interest in Rebiotix and study outcomes. Investigators must inform the Rebiotix of any changes to the information documented on the Financial Disclosure form throughout the course of the study and for a period of one year following completion of the study.

9.3 Canadian Sites: Qualified Investigator Undertaking Form

The principal investigator(s) at each Canadian site will complete and return the Qualified Investigator Undertaking Form to [redacted] before beginning the study, as required Health Canada. The investigator agrees to be responsible for conducting the investigational study in accordance with the protocol, applicable Health Canada regulations including reporting and record-keeping requirements, GCPs, local REB requirements, and controlling dispensation and administration of RBX2660. In addition, the investigator is responsible for ensuring that informed consent is obtained from each subject prior to participating in the study, as well as protecting the rights, safety and welfare of participating subjects.

9.4 Subject Confidentiality

All information and data sent to Rebiotix, and/or its designees concerning subjects and their participation in this study are considered confidential by Rebiotix and its designees (subcontractors or contract research organization). Only authorized Rebiotix personnel or approved contracted agents of Rebiotix have access to some portions of these confidential files and will act in accordance with applicable regulations. The IRBs, REBs, FDA and Health Canada also have the right to inspect and copy all records pertinent to this study. All data used in the reporting of the study will eliminate identifiable references to the subjects as much as possible.

9.5 Study Managers

The study managers have knowledge of Good Clinical Practices, pertinent laws and regulations, and documented training in standard operating procedures pertaining to study management and monitoring. The study managers are:

[redacted]
Rebiotix Inc
2660 Patton Road
Roseville Minn. 55113
[redacted]

[redacted]
Rebiotix Inc
2660 Patton Road
Roseville Minn. 55113
[redacted]

[redacted]
Rebiotix Inc
2660 Patton Road
Roseville Minn. 55113
[redacted]

9.6 Study Site Qualification

Investigational center qualification visits or phone calls will be conducted by Rebiotix prior to acceptance of a site into this study. The site qualification visit or phone call will be scheduled to include time with the study investigator, co-investigators, study coordinator and other study personnel. Areas of discussion include a review of personnel training, investigator qualifications, RBX2660 Administrator qualifications, adequacy of potential subject pool, FDA- or Health Canada-regulated study experience, this study's specific requirements for procedures and equipment, and a review of staffing availability and appropriateness. A written report of the qualification call/visit will be generated by the sponsor representative who conducts the call/visit. Resolution of any concerns and/or completion of any appropriate study activities identified during the qualification process will be documented and submitted to the study investigator.

9.7 Investigator / Site Training

The sponsor will provide appropriate training to each investigator, RBX2660 Administrator, study coordinator(s), and other site personnel who will be involved in the study prior to study initiation. Training will address topics including ordering, secure storage and administration procedures, Subject Diary instructions, follow-up visit and phone call requirements, adverse event reporting, and accurate data collection. Training will include a detailed review of the protocol, eCRF completion, study-specific procedures, monitoring logistics, and regulatory requirements.

9.8 Data Management

Electronic case report forms specifically created for this study will be used to collect study data. The study investigator or his/her designee at each site is responsible for recording all data onto the study eCRFs as noted on the Delegation of Authority Log. The investigator must review and electronically sign all eCRFs as instructed; these responsibilities cannot be delegated to another person. Ongoing data review will be performed according to the study-specific Data Management Plan.

9.9 Monitoring

This study is monitored according to the study-specific Clinical Monitoring Plan. The investigator must allocate adequate time for such monitoring activities. The investigator must also ensure that the monitor or other compliance or quality assurance reviewers are given access to all study-related documents and study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and have adequate space to conduct the monitoring visit. Study monitors and their activities are managed by the study manager or qualified designee; see Section 9.4. Study monitors may be from either Rebiotix or [redacted] Monitors follow Rebiotix' monitoring SOPs and the study-specific Monitoring Plan.

9.10 Direct Access to Source Data/Documents

The investigator is expected to facilitate study-related monitoring, audits, and inspections by the IRB/REB, sponsor and sponsor representatives, FDA, and Health Canada of all study-related documents including direct access to original source documents such as medical records and lab results, regulatory documents, study data, etc. The investigator will ensure access for the inspection of applicable study-related facilities, e.g., pharmacy, laboratory, exam rooms, etc.

Participation as a study investigator and study site in this study implies acceptance and support of inspections by FDA, Health Canada and/or Rebiotix or its designee(s).

9.11 Investigator Responsibilities

The investigator is responsible for ensuring that the study is conducted according to the protocol, applicable FDA or Health Canada regulations for investigational new drugs, HIPAA (US only), GCPs and local IRB/REC requirements. Specific responsibilities are listed in this protocol.

Study records and reports are kept in the electronic Trial Master File set up by Rebiotix. Paper files may be kept by the site but the official study file is the Rebiotix electronic Trial Master File; this is the file that will be reviewed during monitoring, close-out, and audit visits. Records and reports will remain on file for a minimum of two years (25 years for Canadian sites) after either the completion/termination of this study or the date RBX2660 receives market approval for the indication being studied, whichever is later. The study investigator must contact Rebiotix before destroying any records and reports pertaining to the study to ensure that they no longer need to be retained. Rebiotix must be contacted if the study investigator plans to leave the site to ensure that arrangements for a new investigator or records transfer are made prior to investigator departure.

The investigator will promptly report to the IRB/REB all changes in the research activity and all unanticipated problems involving risk to subjects or others, and that he/she will not make any changes in the research without prior written approval from the sponsor and IRB/REB. The investigator will adhere to all IRB/REB requirements imposed on this study.

9.12 Investigator Records

Records to be maintained by the investigator in the Rebiotix Trial Master File include:

- Protocol and all amendments
- Signed Form 1572
- Signed Financial Disclosure Form(s)
- IRB approval letter including consent and HIPAA (US sites)
- IRB Membership list or Letter of Assurance (US sites)
- Signed Qualified Investigator Undertaking Form (Canadian sites)

- REB approval letter including the Informed Consent Form (Canadian sites)
- REB Attestation (Canadian sites)
- REB membership list or Letter of Assurance (Canadian sites)
- All correspondence relating to the study between the site/investigator/coordinator and the IRB/REB, sponsor, [redacted]
- CVs and professional licenses for all investigators and key study personnel
- Delegation of Authority/Site Signature Log
- Product Accountability Log (or electronic pharmacy equivalent)
- Study Visitor Sign-in Log
- Blank set of each version of eCRFs
- Subject Screening/Enrollment log
- Reports submitted to Rebiotix and/or the IRB/REC

The following records must be maintained for each subject enrolled in the study:

- Signed Informed Consent Form and HIPAA Form (if applicable)
- Compete, accurate and current eCRFs and DCFs
- Adverse event reports and any supporting documentation
- Protocol deviations
- Complete medical records, including procedure reports, lab reports, professional notes, etc.
- Records pertaining to subject death during the investigation (including death records, death certificate, and autopsy report if performed).

Rebiotix reserves the right to secure data clarification and additional medical documentation on subjects enrolled in this study at any time.

9.13 Investigator Reports

Study investigators are required to submit the following reports at the times specified below:

- Adverse Events: report all AEs to the sponsor via eCRF within **ten (10) working days** of discovery.
- Serious Adverse Events and suspected adverse reactions: report to the sponsor via eCRF within **three (3) working days** of discovery.
- Progress and Final reports: the investigator will submit annual study progress reports and a final study report when the study is terminated at the site to Rebiotix and the IRB/REB.

9.14 Study Site Termination

Rebiotix reserves the right to terminate a study site for any of the following reasons:

- Failure to properly secure subject informed consent or HIPAA Authorization (if applicable) prior to study enrollment or the conduct of any study-required procedures/assessments.
- Failure to report adverse events as required in Section 7.0.
- Protocol deviations.
- Repeated failure to appropriately and accurately complete eCRFs.
- Failure to enroll an adequate number of subjects.
- Loss of or unaccounted product inventory.
- Administrative decision by the company.

9.15 Protocol Amendments

Neither Rebiotix, its designees (subcontractors or contract research organization) nor the study investigators may modify this protocol without obtaining written approval of the FDA, Health Canada, and/or IRB/REBs as required. No modifications may be made without prior written approval of Rebiotix.

9.16 Protocol Deviations

Any deviations from this protocol undertaken to protect the life or physical well-being of a subject in an emergency situation must be reported to Rebiotix within 48 hours of occurrence and to the respective IRB/REB as soon as possible, but in no event no later than five calendar days after the emergency occurs. Protocol deviations of any kind must be avoided as much as possible and those that do occur will be tracked in the clinical study database.

9.17 Auditing

The investigator will permit study-related auditing by the IRB/REB, sponsor, FDA and/or Health Canada to ensure compliance to applicable regulations, GCPs, and the study protocol.

10.0 Publication Plan

Rebiotix has unrestricted publication rights of the study data. The study site and the investigator are not entitled to publish or release any information pertaining to this study or its results without the prior written consent of Rebiotix. The decision as to whether to provide such consent shall be made once Rebiotix has had an opportunity to review the contents of any proposed publication or release regarding the investigation and, if

necessary, to delay any publication or release in order to protect the confidential or proprietary nature of any information contained therein. Merely the fact that the proposed publication or release contains statements unfavorable to Rebiotix shall not constitute grounds for prohibiting publication; however, all unfavorable statements must be based on adequate scientific evidence. Rebiotix reserves the right to give the data to third parties for publication or release and to name co-authors. Rebiotix retains the right to review and edit all proposed manuscripts, abstracts, publications, and presentations based upon this study or its results prior to submission to any organization, business, agency, person, publisher, society, or other entity.

11.0 References

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Appendix 1: Antibiotic-treated Historical Control Data Collection Protocol

A1. Description of the Historical Control Study

"An Open-label Efficacy and Safety Assessment of Rebiotix RBX2660 (microbiota suspension) for the Treatment of Recurrent *Clostridium difficile* Infection Protocol #2015-01" is a prospective, multicenter, open-label study assessing RBX2660 as an adjunct to antibiotics for the treatment of recurrent CDI. Efficacy of RBX2660, measured by the recurrence-free rate of CDI diarrhea without the need for retreatment with *C. difficile* anti-infective therapy or fecal transplant through 56 days after completion of study treatment with RBX2660, will be evaluated by comparing the recurrence-free rate observed in the study population to the recurrence-free rate identified from antibiotic-treated historical controls. A chart review will be conducted by qualified and trained site personnel to gather the historical control data to be used for the comparison. Safety data on the antibiotic-treated historical controls will also be gathered, but as it is expected that such data may be scant, they will not be used for comparison purposes but will be reported for informational purposes.

A2. Objective

The objective of the antibiotic-treated historical control is to obtain a closely matched data set of the standard of care antibiotic treatment for recurrent CDI comparison to provide an adequate and well-matched set of data for the efficacy analysis of RBX2660.

A3. Background and Rationale

Clostridium difficile infection (CDI) commonly occurs after use of broad-spectrum antibiotics for a variety of conditions. It is widely accepted that CDI occurs when the normal microbiota of the intestine, which hosts anywhere from 300-500 species of microorganisms, is perturbed by the use of antibiotics (Gough et al., 2011). Historically, CDI responded well to metronidazole or oral vancomycin therapy, but in the last 10-15 years, the incidence and severity of CDI have dramatically increased and responsiveness to these agents has decreased (Bakken et al, 2011; Drekonja et al., 2011; Gerding and Lessa, 2015; Venugopal and Johnson, 2012).

As the CDI epidemic continues to grow, the numbers of failed treatments and patients who experience relapses and/or recurrences are also increasing, complicated by the emergence of newer, more virulent, and more antibiotic-resistant strains; this includes BI/NAP1/027 (ribotype 027), among others

(Gerding and Lessa, 2015; Guo et al., 2012). Compounding the problem of increased CDIs is the increased incidence of recurrent CDI, which is an episode of CDI that occurs within eight weeks of a previous episode. It is estimated that the CDI recurrence rate after an initial response to antibiotic therapy ranges from 25-35% (Cammarota et al., 2014; Marsh et al., 2012), and that patients with one recurrence have a 40% chance of another relapse. The rate of recurrence after two relapses rises to 65% (Bakken, 2009; Brandt et al., 2012; Kelly et al., 2012; Surawicz et al., 2013).

There are no available therapies, i.e., approved or licensed in the United States, for the treatment of recurrent CDI, although various antibiotic regimens have been used with minimal success. RBX2660 has been studied in two human clinical trials, including the ongoing Phase 2B prospective, randomized, placebo-controlled, double-blinded study. Given the widespread use of unapproved fecal microbiota transplant drugs from unregulated stool banks, it has become exceedingly difficult to recruit suitable patients for a placebo-controlled study. Furthermore, there are ethical concerns regarding the use of placebo given the consistent rates of FT success for recurrent CDI reported in the literature (Bakken, 2015; Cammarota et al., 2014; Gough et al., 2011). Therefore, obtaining a carefully matched historical control data set to provide an efficacy comparator to RBX2660 results obtained from this clinical study is an ethical and feasible approach to obtain confirmatory evidence of the product's effectiveness as a treatment for recurrent CDI.

A4. Study Design and Methods

This is a retrospective chart review of patients treated with antibiotics for recurrent CDI who match key inclusion/exclusion criteria from the clinical study (see below). Patients will not be contacted. A waiver of Informed Consent/HIPAA authorization for the collection of chart data will be obtained from each site's IRB prior to beginning the chart review process. Site personnel will review their patient databases and identify patients with a diagnosis of multi-recurrent CDI, which is defined as a documented diagnosis of the return of CDI diarrhea ($\geq$ 3 watery stools in $\leq$ 24 hours for at least two consecutive days) that begins less than 8 weeks after completion of a CDI treatment regimen that resulted in the cessation of CDI symptoms. The diagnosis must include at least one positive *C. difficile* test on record. The identified charts will be accessed by the designated qualified and trained chart reviewer at the site. All patient records

on the list will be reviewed and a log will be kept documenting the review and why a record was excluded from the final historical control data set.

Data collection includes:

- demographics (age at the time of the start of data collection, gender, race, ethnicity);
- pre-existing conditions;
- concomitant medications;
- CDI history including each episode's start/stop dates, signs/symptoms, treatment(s) received, outcomes, and CDI-related hospitalizations;
- Blood and stool test results;
- Vital signs;
- Adverse events as recorded in the chart. (see Section 7.0 of the study protocol for all definitions). Adverse events will be adjudicated for seriousness, severity, and causality by the site investigator at the time of data collection as best as possible given the description of the event in the medical record.

[redacted]

All data and records will be kept in strict confidentiality as required by law and as stated in Section 9.4 of the study protocol.

A5. Sites

Approximately 2-5 US sites will be utilized for this historical control data collection.

A6. Inclusion / Exclusion Criteria for Historical Control Group

A6.1 Inclusion Criteria for Selection of Historical Control Group

All responses must be "yes" to include a record in the study:

1. ≥ 18 years old.
2. Medical record documentation of recurrent CDI including at least one positive *C. difficile* test at the time of original diagnosis and medical record documentation of: a) at least two recurrences after a primary episode and has completed at least two rounds of standard-of-care oral antibiotic therapy or b) has had at least two episodes of severe CDI resulting in hospitalization.

A6.2 Exclusion Criteria for Selection of Historical Control Group

All responses must be "no" to include a record in the study:

1. A known history of continued CDI diarrhea despite being on a course of antibiotics prescribed for CDI treatment.
2. Required continuous antibiotic therapy for a condition other than CDI.
3. Previous successful (resolution of CDI diarrhea) fecal transplant for recurrent CDI < 6 months prior to the date the most recent sequence of multi-recurrent CDI began.
4. Previous unsuccessful (recurrent CDI diarrhea was unresolved) fecal transplant.
5. Previous treatment with RBX2660.
6. Diagnosis of inflammatory bowel disease (IBD), e.g., ulcerative colitis, Crohn's disease, or microscopic colitis.
7. Diagnosis of irritable bowel syndrome (IBS) as determined by Rome III criteria.
8. History of chronic diarrhea due to causes other than *C. difficile*.
9. History of celiac disease.
10. Disease symptoms caused by a confirmed intestinal pathogen other than *C. difficile*.
11. Colostomy.
12. Intraabdominal surgery within 60 days prior to the date the most recent sequence of multi-recurrent CDI began.

[redacted]

18. Life expectancy of < 12 months at initiation of treatment for CDI.
19. Compromised immune system [redacted]

A7. Statistical Considerations

Approximately up to 200 subjects (minimum of 100) will be included in the historical control group to appropriately power the primary efficacy study endpoint (see Section 8.0 Statistical Considerations in the study protocol and the Statistical Analysis Plan).

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