Protocol No.:

GenePOC CDiff_clinical-01

Prospective Multi-Center Study for Clinical Validation of the Molecular-Based GenePOC CDiff system for the Detection of Toxin B gene from Toxigenic *Clostridium difficile (C. difficile)* Strains in unformed (soft or liquid) Human Stool Specimens.

Protocol version	Version date
V1	2016-Mar-01
V2	2016-May-16
V3	2016-Nov-18
V4	2017-May-12

FOR INVESTIGATIONAL USE ONLY

The performance characteristics of the GenePOC™ CDiff test have not been established.

To be used by qualified investigators only.

The GenePOC CDiff test must not be used as a diagnostic procedure without confirmation of the diagnosis by another medically established diagnostic product or procedure.

CONFIDENTIAL AND PROPRIETARY

REVISION SUMMARY

This revision summary is intended as an aid in understanding the changes being implemented. Review of the summary alone does not ensure complete understanding. The actual text must be reviewed to ensure full understanding of the change(s).

Version	Date	Section	Change(s) description	Reason for change (s)
V1	2016-Mar-01	NA	Original	NA
V2	2016-May-16	Whole document	Official Nomenclature of Device updated	To ensure uniformity in all GenePOC documents
		Changes made in Section 1, 4.1, 4.4, 4.5, 5, 6.1, 6.6.1, 6.6.2, 7.3, 7.5.1.1, 7.5.1.3, 7.5.2 and 13.2 *Complete list of changes is available upon request.*		Enhancement of clarity
		1	Changed in planned study period: study start date June to July	New information
		3.1 and 7.4	Within CCFA-HB: Cycloserine Cefoxitin and Fructose Agar plate with Horse Blood, HB and Horse-Blood removed	New information
			Added: BAP, GLC, MSI, QC and SOP acronyms	
			Changed: CMG: Cooked Meat Glucose Broth changed for Chopped Meat carbohydrate broth	
		6.2.2 and 6.2.4	Changed: No negative sample will be used for proficiency training. Instead 150 µL of SBT will be used as Negative Control.	New information
		6.2.2, 6.2.4, 6.6.2 and 7.5.1.1	Removed: (e.g. American Type Culture Collection, ATCC 43255)	Positive external control strain not yet determined, will be added to Study Site Manual
		6.3	6.3.1, added: “Unformed” stool 6.3.1, Deleted: Not currently under treatment for CDI; 6.3.2, Deleted: Patient under antibiotics treatment for CDI;	Information not available on leftover specimens
			6.3.1, Added: “At least 1.25ml of” unformed stool specimen (defined as specimen assuming the shape of its container);	To enhance clarity
		7.3.2 and 7.5.2	Completely changed	To match Selected Reference Method site
		7.5.1.3 and 10.2	Added: generic clinical trial email address	New information

V3	**2016-Nov-18**	**Whole document**	Cdiff **changed** for CDiff **Changed**: Stability of SBT and clinical specimens changed from 4 days to 7 days if kept at 2-8°C. **Changed**: freezing temperature from -70°C to -25°C or colder	**To match with official name of the Assay and PI**
			Changed in Clinical Study Manager: Catherine Lippe is replacing Rachelle Nadeau as Study Manager	**New information**
			Changed: Sample for specimen when applicable as described in section 3.2	**To match with official nomenclature**
			Changed: Sample Transfer Device (STD) for Disposable transfer tool (DTT)	**To match with official nomenclature**
		Minor changes made in other section for enhancement of clarity ***Complete list of changes is available upon request.***		
		1.0	**Changed in Clinical Trial Main Eligibility Criteria:** fresh specimens must be tested […] within **168** hours **(7 days)** […].	**New information**
		3.1	**Added:** NEC (Negative External Control) and PEC (positive external Control) **Added:** PrC, (new abbreviation for Process Control)	**To enhance clarity**
		3.2	**Changed:** Definition of sample changed and definition of specimen added.	**To match with official nomenclature**
		Protocol synopsis and 4.2	**Changed:** “test” for “assay” and “preparation” for “processing”	**To match with Package insert version 4**
		4.3	**Changed:** image of inoculating loop (2)	**To match with official name of the Assay and PI**
		4.4	**Added:**[…] dipped into the **homogenized** stool specimen […].	**To enhance clarity**
		Protocol synopsis and 6.1	**Changed:** up to seven (7) changed for up to (8)	**New information**
		6.2.1 and 6.2.2	**Changed**: Negative control will consist of 150 µL of SBT changed for BRU broth;	**New information**
		6.2.2	**Added**: data will be gathered (if available)	**To enhance clarity**
		6.2.4	**Changed**: SBT changed for NEC	**New information**
		6.5.1	**Changed:** Specimen unique number changed from CXnnnnA to CXnnnA	**Error in previous version**
		6.5.2	**Changed**: The operator will save the instrument data on a properly labeled USB keys on each testing day. Data saved on the USB key will be uploaded to the eClinical database. The USB key must be maintained as a backup at the Clinical Center.	**Paragraph re-written due to new information**

		6.6.1	**Deleted**: "and shipping to GenePOC once or twice per week";	**To match with new instructions in section 6.5.2**
			Deleted: "Plastic bags to hold/store and ship PIE"	**New information, PIE will not be kept**
		7.3	**Changed**: 1ml of stool specimen changed for 500 µL of stool specimen **Paragraphs rearranged to reflect the recommended workflow**	**New information**
		7.3.1.1.1	**Added** :A picture should be taken of the PIE and sent to the Study Manager (please refer to Study Site Manual section 9 for instructions). .	**New information**
		7.3.2.1	**Deleted**: "to 54" in: "The plate will be incubated at 35-37°C in anaerobic conditions for 48 ~~to 54~~ hours"	**New information**
		7.3.2.2	**Deleted**: .36 to. in: The tube will be incubated at 35-37°C in anaerobic conditions for ~~36 to~~ 48h." **Deleted**: to 54 in: "The CCFA plate will be incubated anaerobically at 35-37°C for 48 ~~to~~ ~~54~~h."	**New information**
		7.3.2.4	**Changed**: (incubated 48 -72h […]) changed for (incubated up to 48 h […]) **Added**: An isolate cultured on BAP will be preserved in broth with 10-20% glycerol. Preserved isolates will be stored at-70°C or colder for possible future use. **Deleted**: "An aliquot of the CMC broth will be frozen with 10-20 glycerol at".	**New information**
		7.4	**Changed 2nd bullet**: All Frozen stool specimen and CCMB-TAL Broth must be stored at -25°C or colder, and culture isolates at -70°C or colder and only specimens requested by the Study Manager will be shipped on dry ice to GenePOC; **Deleted**: stored between 2°C to 8°C in a plastic bag and shipped on icepacks to GenePOC as directed by Study Manager. **Added**: discarded in appropriate biological waste receptacle in accordance with country, federal, provincial, state and local regulations.	**New information**
		7.5.1.1	**Changed**: SBT for BRU Broth	**New information**
		9.1.1	**Deleted**: sent to GenePOC at least once per week. The Clinical Center must keep one of the instrument back-up media **Added**: kept on site. **Added**: Data obtained from GenePOC instrument will be forwarded to GenePOC through the EDC system.	**New information**
		10.1	**Deleted**: (UNR or other)	**Error in previous version**

		13.1	**Paragraph re-written:** Make1 copy daily of runs from the GenePOC instrument. Keep the copy at site for all Instrument backups. Transfer instrument data to sponsor through EDC database	**New information**
V4	**11-May-2017**	**Whole Document**	**Minor reformatting of the text to increase readability**	
		Protocol Acceptability	**Removed:** Rachelle Nadeau Dany Leblanc **Added:** Catherine Lippe Keith Chiasson	**New information**
		Sponsor Contacts	**Catherine Lippé's cell phone number was modified to +1 418 925-8597**	**New information**
		6.2	**Original Text:** consist of ~~*four (4)*~~ distinct segments: Proficiency testing, Clinical Accuracy testing, Discrepant testing (when required), and Reproducibility **Replaced by:** consist of five (5) distinct segments: Proficiency testing, Clinical Accuracy testing, Discrepant testing (when required), Frozen Specimen Testing and Reproducibility	**New information**
		6.2.5	**Added:** 6.2.5 Frozen Specimen testing In the event that additional results are needed, the frozen aliquots will be retested with the GenePOC CDiff assay on selected sites. The sites will be provided with a list of randomly selected specimens to thaw, and test again following the procedure in Section 7.3.1. The frozen aliquots will not be tested by the Reference Method.	**New Section**
		10.1	**Catherine Lippé's cell phone number was modified to +1 418 925-8597**	**New information**

PROTOCOL ACCEPTABILITY

Protocol Proposed by:

Catherine Lippe, MSc
Clinical Study Manager
GenePOC Inc.

Date (yyyy-mmm-dd)

Sponsor

Keith Chiasson, PhD
Director, Clinical Operations
GenePOC Inc

Date (yyyy-mmm-dd)

Acceptability

I have reviewed and understood this protocol and agree to its provisions. Any modifications must be made by written mutual agreement.

Clinical Center:

Address:

Principal Investigator Name

Principal Investigator Signature

Date (yyyy-mmm-dd)

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1 PROTOCOL SYNOPSIS

Clinical Trial Protocol Number	**GenePOC-CDiff_clinical-01**
Protocol Title	Prospective Multi-Center Study for Clinical Validation of the Molecular-Based GenePOC CDiff system for the Detection of Toxin B gene from Toxigenic Clostridium difficile (*C. difficile*) Strains in unformed (soft or liquid) Human Stool Specimens.
Sponsor	GenePOC Inc.
System Summary	The primary purpose of this clinical investigation is to verify the performance of the GenePOC CDiff test on the GenePOCTM instrument. This will be achieved by comparing the GenePOC CDiff test to the Toxigenic Culture (TC) and cell cytotoxicity neutralisation assay (CCNA), a conventional method considered as gold standard for detection of toxigenic *Clostridium difficile* in stool samples.
Study Locations	Up to eight (8) geographically distributed Canadian and United States Clinical Centers are targeted, with each site testing high volumes of specimens for toxigenic *C. difficile* combined with high prevalence rates. GenePOC CDiff test will be performed at each of the selected clinical centers. The reference method, the Toxigenic Culture (TC), is plan to be performed at a single reference laboratory.
Intended Use	The GenePOC CDiff assay performed on the GenePOC instrument is a qualitative in vitro diagnostic test that utilizes automated sample processing and real-time polymerase chain reaction (rtPCR) to detect the toxin B (tcdB) gene of toxigenic Clostridium difficile (*C. difficile*) in unformed (liquid or soft) stool specimens obtained from patients suspected of having *C. difficile* infection (CDI). The GenePOC CDiff assay is intended for use as an aid in the diagnosis of CDI in humans in conjunction with clinical and epidemiological risk factors.
Investigational Product (IUO)	The GenePOC CDiff test will be performed according to the Study Protocol using the GenePOC instrument. The GenePOC system, composed of GenePOC CDiff test and GenePOC instrument, used in conjunction with appropriate reagents, is capable of automated cell lysis, dilution of nucleic acids from multiple sample types as well as automated amplification and detection of target nucleic acid sequences.

Reference Method	Toxigenic Culture (TC) is defined as anaerobic culture to isolate a *C. difficile* strain, then, if present, followed by confirmation of toxigenicity of the isolate by a cell cytotoxicity neutralisation assay (CCNA) More specifically, the *C. difficile* bacteria, when present, will be isolated from unformed (soft or liquid) stool specimens using a direct and an enriched culture method. Toxigenicity of the isolated *C. difficile* will be determined using a tissue culture cytotoxicity assay. For this clinical trial, the Reference Method will be performed, by one selected site only, on stool specimens coming from all Clinical Center.
Phase	Development Phase - Qualification of investigational device (IUO).
Planned Study Period	Clinical investigation to begin in July 2016 at the earliest and to be completed within approximately three (3) months.
Planned Specimen Size	The purpose of the clinical investigation is to enroll sufficient specimens to obtain a total of 150 positive specimens for *C. difficile* based on the Reference Method final result. With an estimated prevalence of approximately 10-20%, up to 1500 specimens will be tested across all Clinical Centers. The performance obtained and the confidence interval will be monitored during the study in order to stop the enrollment when appropriate. A minimum of 20 positive results per site is expected but sites with a higher prevalence of *C. difficile* could contribute with more positive results to reach the required number.
Objectives	The primary objectives of this multi-site prospective investigational trial are: • To establish the performance characteristics of the GenePOC CDiff test for its use to detect Toxin B gene from toxigenic Clostridium difficile strains. Sensitivity and specificity will be established in comparison to the Reference Method. • To estimate the Positive and Negative Predictive Values (PPV and NPV) of the GenePOC CDiff test. • To estimate the rate of unresolved results for the GenePOC CDiff test due to Sample Processing Control failure (Unresolved sample results). • To determine the reproducibility of the GenePOC CDiff test between sites.

Study Design	Clinical Centers will be selected based on a number of criteria, such as investigator and site personnel availability, number of specimens of interest tested for Toxin B gene from Toxigenic Clostridium difficile isolates and CDI prevalence. The Site that will be selected to perform the Reference Method will have laboratory facilities where Toxigenic *C. difficile* isolates characterization methods are performed for routine, investigation or research purposes. This clinical investigation will be composed of the following segments: Proficiency testing, Clinical Accuracy, Discrepant testing (when required) Frozen Specimen Testing (at selected sites) and Reproducibility (at selected sites). There are no risks to the subject inherent to the execution of this investigation since it will: 1. Be performed on excess de-identified specimens only; and 2. Include parallel clinical routine testing by an approved and established method for reporting subject results. Study will be initiated from subjects suspected of having *C. difficile* infection (CDI) for whom diagnostic procedures are indicated and ordered. Unformed stool specimen will be tested as per clinical center standard test. Left over of this sampling will be collected by research personnel for the purpose of this clinical trial and tested on GenePOC CDiff system and sent to the selected Clinical Center for Reference method testing. Results obtained from testing using the GenePOC CDiff test will be compared to those obtained by the Reference Method. Investigators will ensure that, for a given specimen, the individuals performing the GenePOC CDiff test are masked from the result of the Reference Method and vice-versa. Electronic and/or paper Case Report Form(s) will be used to record pertinent specimen and laboratory information. No personal subject information will be collected by GenePOC. In cases of discrepant results between the Reference Method and the GenePOC CDiff test, GenePOC personnel will perform further characterization testing to elucidate the discrepancies.
Clinical Trial Main Eligibility Criteria	• Specimens from subjects suspected of having CDI for whom diagnostic procedures are indicated and ordered. • Only one (1) unformed stool specimen per subject will be included in the study. • Fresh specimens must be tested with the GenePOC CDiff test within 48 hours of collection if kept at 2-25oC or within 168 hours (7 days) of collection if kept at 2-8oC. Specimen kept in Anaerobe Transport Medium must be tested within 4 days of collection for Reference Method testing.

Clinical Study Statistical Methods	Results obtained with the GenePOC CDiff test will be compared to those obtained with the Reference Method. The statistical analyses will include all compliant specimens and will determine at least the following: • The sensitivity and specificity, PPV and NPV as well as exact 95% confidence interval. • The unresolved and indeterminate rates along with 95% exact confidence interval. • Poolability of the data. • Reproducibility percent agreements for qualitative and quantitative data.

2 SPONSOR CONTACTS

2.1 Sponsor

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2.2 Clinical Study Manager

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3 TERMINOLOGY

ADE	Adverse Device Effect
AE	Adverse Event
ATCC	American Type Culture Collection
BAP	Blood Agar Plate
bp	Base pairs
CCFA	Cycloserine Cefoxitin and Fructose Agar plate
CCMB-TAL	Cycloserine Cefoxitin Mannitol Broth with Taurocholate and Lysozyme
CCNA	Cell Cytotoxicity Neutralisation Assay
CDA	Confidentiality Disclosure Agreement
CDC	Centers for Disease Control and Prevention
CDI	*Clostridium difficile* Infection
C. difficile	*Clostridium difficile*
CLIA	Clinical Laboratory Improvement Amendments
CMC	Chopped Meat carbohydrate broth
CRA	Clinical Research Associate
CTA	Clinical Trial Agreement
CTI	Clinical Trial Incident
DNA	Deoxyribonucleic Acid
EC	External control
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
GLC	Gas Liquid Chromatography
IEC	Independent Ethics Committee
INC	Inconclusive
IND	Indeterminate
IRB	Investigational Review Board
IUO	Investigational Use Only
LOD	Limit of Detection
MDR	Medical Device Reporting
MSI	Microbiology Specialist Inc.
NEC	Negative External Control
NPV	Negative Predicative Value
PC or PrC	Process Control
PCR	Polymerase Chain Reaction
PI	Principal investigator
PEC	Positive External Control
POC	Point of Care
PPV	Positive Predictive Value
QC	Quality Control
REB	Research Ethic Board
rtPCR	Real time Polymerase Chain Reaction
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SBT	Sample Buffer Tube
SOP	Standard Operating Procedures
STD	Sample Transfer Device *(also called Disposable transfer tool (DTT))*
TAT	Turn-Around Time
TC	Toxigenic Culture
tcdB	Toxin B gene
UNR	Unresolved
US	United States of America

3.1 Definitions

Clinical Center(s)	Site(s) that will perform the GenePOC CDiff test and/or the Reference Method for the clinical study.
GenePOC CDiff system	The system is composed of the GenePOC CDiff test which is used in conjunction with the GenePOC instrument.
PCR Operator	Participating laboratory technician who has successfully completed the proficiency testing
(CDiff) PIE	*C. difficile* disposable microfluidic cartridges (describe in this document as PIE because of the shape of the cartridge).
Principal Investigator (PI)	A person who actually conducts the clinical investigation, under whose immediate direction the test article (device) is used involving a sample derived from a human subject; or, in the event of an investigation conducted by a team of individuals, is the responsible leader of that team.
Reference Method	The Reference Method for this study will be Toxigenic Culture then, if *C. difficile* strain is present, it will be followed by confirmation of toxigenicity of the isolate by a cell cytotoxicity neutralisation assay (CCNA)
Sample	SBT inoculated with left over of unformed stool (soft or liquid) specimen
Specimen	Left over of unformed stool (soft or liquid) specimen intended for regular *C. difficile* diagnostic testing.

4 INTRODUCTION

4.1 Protocol Scope

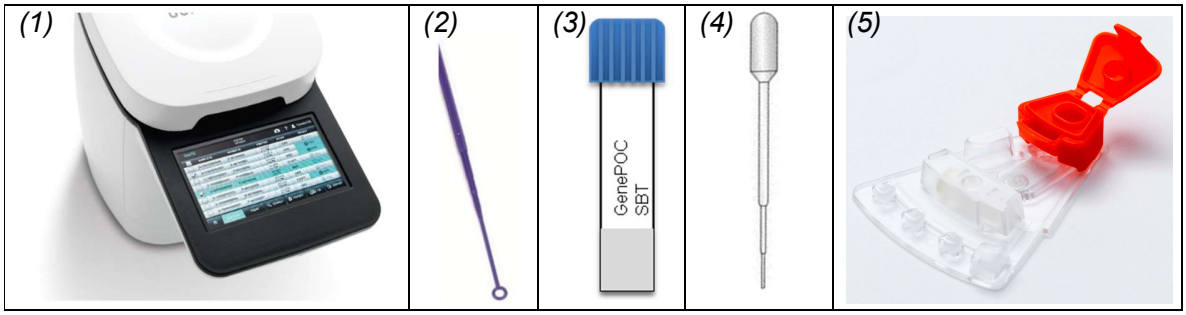
The purpose of this clinical investigation is to establish the performance characteristics of the GenePOC CDiff test for its use in determining the presence of toxigenic *C. difficile* in human unformed stool specimens. Sensitivity and Specificity will be established in comparison to the Reference Method. For this trial the Reference Method will be Toxigenic Culture (TC) then, if *C. difficile* is present, it will be followed by confirmation of toxigenicity of the isolate by a Cell Cytotoxicity Neutralisation Assay (CCNA), gold standard method for CDI diagnosis.

4.2 Intended Use

The GenePOC CDiff assay performed on the GenePOC™ instrument is a qualitative *in vitro* diagnostic test that utilizes automated sample processing and real-time polymerase chain reaction (rtPCR) to detect the toxin B (*tcdB*) gene of toxigenic *Clostridium difficile* (*C. difficile*) in unformed (liquid or soft) stool specimens obtained from patients suspected of having *C. difficile* infection (CDI). The GenePOC CDiff assay is intended for use as an aid in the diagnosis of CDI in humans in conjunction with clinical and epidemiological risk factors.

4.3 Device Description

The GenePOC CDiff test will be performed using the GenePOC instrument (**(1)** below). The GenePOC instrument, used in conjunction with appropriate reagents, is capable of automated lysis cells, dilution of nucleic acids from multiple sample types as well as automated amplification and detection of target nucleic acid sequences.



The GenePOC CDiff test kits consist of:

Components		Packaging
(2)	Transfer Loop	Disposable 5µL inoculating loop to dip into the unformed stool specimen and transfer into the SBT
(3)	Sample Buffer Tube (SBT)	A 4.5mL tube containing 1mL of sample buffer
(4)	Disposable transfer tool (DTT)	Plastic disposable transfer pipette for transferring inoculated sample buffer into the PIE.
(5)	CDiff disposable microfluidic cartridges (PIE)	Fully integrated disposable microfluidic cartridge for detection of *C. difficile*.

4.4 Test Principle

An unformed (soft or liquid) stool specimen is collected using standard stool collection device. Using a disposable 5µL inoculating loop (transfer loop) dipped into the homogenized stool specimen, stool material is transferred into SBT and vortexed.

The sample processing (addition to buffer, mixing) is simple, but not automated. The GenePOC instrument automates and integrates nucleic acid purification and amplification, and detection of the target sequence in complex samples using real-time Polymerase Chain Reaction (rtPCR). The system consists of an instrument with embedded software for running tests and displaying the results. The system requires the use of single-use disposable cartridges (PIE) that contain the Polymerase Chain Reaction (PCR) reagents and host the amplification and detection process. Because the cartridges are self-contained, cross-contamination concerns are minimized.

The GenePOC CDiff test includes reagents for the simultaneous detection of the target *C. difficile* DNA and a process control (PC) to monitor processing, amplification, and the absence of reaction inhibitors. The *C. difficile* primers and probe detect a target region of 263 base pairs (bp) of the toxin B gene (*tcdB*) of *Clostridium difficile*. The results are interpreted by the system from measured fluorescent signals and embedded calculation algorithms. Results may be viewed, be printed transferred and/or store by the user.

4.5 Clinical Benefits and Justification for the Use of a New Technology

C. difficile is a naturally occurring bacteria in the gut of a small percentage of the population, prevalence of approximately 1-3% in healthy adults (Goudarzi, et al., 2014). In most cases, it does not cause any health problem because it lives in balance with the microorganisms which normally colonize the intestine of human beings.

C. difficile is the leading cause of infectious diarrhea in hospitalized patients (Ghantoji, et al., 2010) (Barbut, et al., 2014). According to the Center for Disease Control and Prevention (CDC), the annual incidence of *C. difficile* Infection (CDI) in the United States (US) is approaching 500,000 cases with a mortality rate of between 1 and 2,5%, which would have resulted in around 29,000 deaths in 2011 (Lessa, et al., 2015). 15-25% of nosocomial CDI would be consecutive to an antibiotic treatment (Shroeder, et al., 2014).

In addition to the associated morbidity and mortality, CDI significantly increase health care costs due to the increased length of stay and readmissions frequency. In the US, the economic burden of CDI is estimated between 750 million and 3.2 billion (Ghantoji, et al., 2010). In Europe, the economic burden is estimated at 3 billion Euros (€) (Bouza, 2012). In fact, a meta-analysis of costs associated with CDI revealed that the care and treatment of a primary case of CDI costs is 2,871 to 4,846 US$2008 while for a recurring infection, costs increase at 13,655 to 18,067 US$2008 per case (Ghantoji, et al., 2010).

A recent econometric study, (Shroeder, et al., 2014) demonstrated that rapid diagnosis of CDI is more effective and less costly than traditional methods recommended by guidelines. In most environments, molecular diagnosis on request would be favorable economically in view of limiting false negatives diagnosis.

Moreover, a recent feasibility and acceptability study of rapid *C. difficile* diagnostic at Point of Care (POC) revealed that in addition to allowing the decrease of the median waiting time results from 18 to just under 2 hours, the GeneXpert of Cepheid technology was well accepted by nurses and technical staff of two London hospitals (Goldenberg, et al., 2014).

Early detection of CDI would directly and indirectly decrease the morbidity and mortality rate of this infection, directly by more effective management of patients and indirectly by a reduction in

nosocomial transmission attributable to the presence of an infected patient (not diagnosed) on the wards (Surawicz, et al., 2013).

However, it is imperative that screening is performed only in infection symptomatic patients having produced ≥ 3 unformed (liquid or soft) stools in 24 hours (Bagdasarian, et al., 2015). The definitive diagnosis of CDI must be accompanied by a positive result for toxigenic *C. difficile* or one of its toxins or pseudomembranous colitis evidences.

In an impact assessment carried out in the UK, study comparing the test Xpert *C. difficile* cytotoxin in the neutralization assay of *C. difficile*., it has been shown that the fastest PCR diagnosis (average TAT 1.5 hours vs. 22.5 to 46.5 hours for the positive/negative) reduces the length of stay for patients and generate savings of around £ 2,300 (~ 4500 CAD) per patient suspected to have a CDI (Sewell, et al., 2014). Rapid diagnosis would also allow better control of the empirical antibiotic therapy (Barbut, et al., 2014).

Several molecular diagnostic tests are commercially available for the early detection of *C. difficile* (Le Guern, et autres, 2013). However, the GenePOC CDiff system is developed to enable the decentralized diagnosis of CDI, ideally at point of care (POC).

5 TRIAL OBJECTIVES

The objectives of this multi-center prospective clinical study are:

- To establish the performance characteristics of the GenePOC CDiff test for its use to detect Toxin B gene from toxigenic *C. difficile* in unformed (liquid or soft) stool specimens obtained from patients suspected of having *C. difficile* infection. Sensitivity and specificity will be established in comparison to the Reference Method.
- To estimate the Positive and Negative Predictive Values (PPV and NPV) of the GenePOC CDiff test.
- To estimate the rate of unresolved results for the GenePOC CDiff test due to Sample Processing Control failure (Unresolved sample results).
- To determine the reproducibility of the GenePOC CDiff test between sites.

6 STUDY DESIGN

6.1 Clinical Centers Requirements

Up to eight (8) Clinical Centers (with a minimum of 3 sites (1 Canada and 2 US) will participate in this clinical study. Clinical centers will be selected for this study based on a number of criteria, such as investigator and site personnel availability, number of specimens of interest tested for *C. difficile,* CDI prevalence, familiarity with the study Reference Method (for the selected site only), and experience with clinical investigations.

For this clinical trial, the Reference Method will be performed by one selected site only, on unformed (liquid or soft) stool specimens coming from all Clinical Centers.

Site(s) will have a laboratory where *C. difficile* characterization methods are performed for routine and/or research purposes. Results from clinical centers routine method will be collected.

The Clinical Centers regulatory documents requirements are detailed in Appendix A.

6.2 Overview of the Evaluation

The Clinical investigation will consist of five (5) distinct segments: Proficiency testing, Clinical Accuracy testing, Discrepant testing (when required), Frozen Specimen Testing (on selected sites) and Reproducibility (selected sites).

6.2.1 Proficiency Testing

Each PCR operator who will participate in the GenePOC CDiff clinical trial will be trained by a GenePOC Study Manager (or CRA) on handling and performing the GenePOC CDiff test according to the Investigational Use Only (IUO) Package insert, the Study Site Manual and the GenePOC system User's Manual.

A test panel containing negative samples and *C. difficile* positive samples will be used to assess each new participating technician proficiency with the protocol test methods. This panel will be provided in a sufficient number. Negative control will consist of 150 µL of BRU broth. A participating technician failing to achieve the qualification criteria on initial testing of the GenePOC CDiff test must repeat the training panel, and will be qualified if he/she meets the acceptance criteria for the repeated run. If the participating technician fails to meet the retest criteria, he/she cannot be qualified for participation in the GenePOC CDiff clinical study as a PCR operator for the GenePOC CDiff system unless re-training occurs and the participating technician is able to pass the proficiency test.

To be qualified for participation in the GenePOC CDiff clinical trial as a PCR operator, each participating technician must achieve a correct answer for all positive samples and true negative (no target) samples in the molecular method using the provided proficiency panel.

If a new participating technician is included in the study after the initial training and proficiency testing occurs, the training of this person can be performed by a GenePOC representative or by an authorized proficient PCR operator on-site. The new participating technician must also pass the proficiency test to participate in the study.

6.2.2 Clinical Accuracy Testing

This clinical trial is designed in such a way that only one site may perform the Reference Method, which requires that the selected Clinical Center (for reference method) has laboratory facilities

where *C. difficile* characterization methods are performed for routine, investigation or research purposes.

Specimens will be a leftover of unformed stool. Specimen testing will be performed according to Section 7 of this Protocol. Results obtained from testing of specimens using the GenePOC CDiff test will be compared to those obtained by the Reference Method. These results will serve to verify the GenePOC CDiff test performance.

Investigators will ensure that, for a given specimen, the individuals performing the GenePOC CDiff test are masked from the results of the Reference Method and vice-versa.

The purpose of the clinical investigation is to enroll sufficient specimens to obtain approximately 150 positive specimens for *C. difficile* based on the Reference Method final result. With an estimated prevalence of approximately 10-20%, it is projected that up to 1500 specimens will be needed across all participating Clinical Centers.

The Study Manager and/or CRA will monitor specimen enrollment and prevalence of *C. difficile* across all participating Clinical Centers. It may be necessary to increase the enrollment rate or to prematurely stop enrollment at one or more participating centers, depending upon the total number of specimens and the positive specimens obtained across all Clinical Centers.

At least three (3) lots of the GenePOC CDiff test kits will be used for this Clinical trial. Each Clinical Center with test a minimum of two (2) lots.

Electronic Case Report Form (eCRF) will be used to record all pertinent information. No personal subject information will be collected but the following general demographic data will be gathered (if available):

- Age category and
- Specimen origin (i.e. In-patient for intensive and non-intensive care units, as well as long term care facility, out-patient, emergency).

Once per day, a reference toxigenic *C. difficile* strain bearing the *tcdB* gene will be used as a positive external control (PEC) while 150µL of BRU broth will be used as a negative external control (NEC). If either or both of the ECs fail to give the expected result, all samples tested in that run will be repeated from the remaining SBT stored at 2-8°C containing the specimen along with new NEC and PEC.

6.2.3 Discrepant Testing (when required)

In cases of discrepant results between the Reference Method and the GenePOC CDiff test, further characterization testing may be requested by GenePOC to be performed by the Clinical Center and possibly at GenePOC to attempt to resolve the discrepancy. See Appendix C for description of the minimal discrepant testing. An electronic or paper Discrepant Results Form will be used for recording discrepant testing information.

6.2.4 Reproducibility Testing (for selected sites)

Reproducibility study will be performed with a panel of samples spiked at 3 different concentrations of *C. difficile*: Negative, Low positive (1-2x LOD) and Moderate positive (2-3x LOD). Each panel will consist of 3 samples with 3 replicates of each concentration.

The site-to-site reproducibility will test the same reproducibility panel at three (3) designated sites (2 external sites and GenePOC laboratories) for the within-run (3 replicates), between-run (2 runs per day from two different operators) and between-day (5 days, consecutive or not) variance with multiple operators and instruments. A single lot of the GenePOC CDiff test will be

tested by all sites according to the training manual instructions and the IUO CDiff test Package Insert.

The reproducibility panel will contain randomized moderate positive, low positive and true negative.

Once per day, a reference toxigenic *C. difficile* strain bearing the *tcdB* gene will be used as a positive external control (EC) while 150µL of NEC will be used as a negative EC. If either or both of the ECs fail to give the expected result, all samples tested in that run will be repeated from the remaining SBT containing the sample stored at 2-8°C along with new positive and negative ECs.

6.2.5 Frozen Specimen testing

In the event that additional results are needed, the frozen aliquots will be retested with the GenePOC CDiff assay on selected sites. The sites will be provided with a list of randomly selected specimens to thaw, and test again following the procedure in Section 7.3.1. The frozen aliquots will not be retested with the Reference Method.

6.3 Study Criteria

Note: For this trial, specimens will not be collected for the express purposes of this evaluation. Excess de-identified specimens will be used.

6.3.1 Inclusion criteria

- Unformed Stool specimens from patients suspected of having CDI for whom diagnostic tests are indicated and ordered;
- At least 1.25mL of unformed stool specimen (defined as specimen assuming the shape of its container);
- Only one (1) specimen per patient will be included in the study;
- Materials use within their expiration date;
- Transport, storage times, and conditions (e.g. room temperature and/or refrigerated) within requested indications.

6.3.2 Exclusion Criteria (left over)

- Specimens from patients for whom CDI diagnostic tests have not been ordered;
- Transport and storage times and conditions that exceed these Study Protocol requirements;
- Formed or hard stool specimens or rectal swabs.

6.4 Specimen Size Justification

The purpose of the clinical investigation is to enroll sufficient specimens to obtain a total of 150 positive specimens for *C. difficile* based on the Reference Method final result. With an estimated prevalence of approximately 10-20%, up to 1500 specimens will be tested across all Clinical Centers. The performance obtained and the confidence interval will be monitored during the study in order to stop the enrollment when appropriate.

A minimum of 20 positive results per site is expected but sites with a higher prevalence of *C. difficile* could contribute with more positive results to reach the required number. A potential

minimum of 150 specimens and a potential maximum of 300 total specimens will be obtained at each clinical center.

The performance obtained and the statistical confidence intervals will be monitored during the study in order to stop the enrollment when deemed appropriate.

6.5 Data Collection

The principal investigator (PI) will ensure that each designee and the scope of the designee's delegated authority are recorded on the Site Signature and Delegation of Authority Log. The designee's signature, initials, start and end dates and list of study related tasks, must be recorded on the form.

6.5.1 Case Report Forms

All Clinical Centers will use electronic data capture (EDC) through an electronic Case Report Forms (eCRF). In case the eCRF would not be available for any given reason, a paper case report form will be available and sent electronically to Study Manager, eCRF will have to be completed once available again.

An eCRF will be completed for each specimen enrolled in the study in order to record relevant demographic, clinical and laboratory information. These forms must be completed as described in the Study Site Manual.

Subjects whose specimen will be included in the study will not be identified by name or by hospital medical record number on the eCRF. A field is available for a study-specific subject identifier code which will uniquely identify each subject. This code will be assigned by a designated individual at the Clinical Center, who is not directly involved in any other aspects of the study. This code will be a series of:

- 5 digit XX-YYY (X being the site number and Y the specimens number attributed sequentially
- And three letters FML: (“F” being the first letter of the First Name; “M” being the first letter of the Middle Name or a dash (-) if the person does not have a middle name; “L” being the first letter of Last name). **As per local regulations, initials can also be a series of three letters determined by clinical center.**

This code will have no relation with protected health information, and will be used only to track subject enrollment. A Study Subject Log associating each subject with its subject identifier code will be maintained in a secure on-site location by the designated individual assigning the codes to the subjects.

Each enrolled specimen will be assigned a unique Study Number, which will be identified by bar code labels to be provided by GenePOC. Study Numbers will conform to seven (7) characters in a "CXnnnA" format: where "C" is a letter identifying the test (CDiff), "X" is a number identifying the site, "nnn" is an incrementing number identifying each specimen within a study segment and "A" is a letter denoting the study segment, ("A" for Accuracy or "D" for Discrepant testing). For panel members provided by GenePOC for testing, the same study number pattern will be used and the study segment will be identified as follows: "P" for Proficiency, “R” for Reproducibility and "W" for Workflow Practice.

6.5.2 GenePOC Data

The operator will save the instrument data on a properly labeled USB keys on each testing day. Data saved on the USB key will be uploaded to the eClinical database. The USB key must be maintained as a backup at the Clinical Center.

6.6 Study Materials

6.6.1 Provided by GenePOC

- GenePOC instrument
- GenePOC sample Racks;
- GenePOC system User's Manual;
- Tube Vortexer;
- Instructions and Training Materials for the Study (*Study Site Manual*);
- GenePOC CDiff test IUO kits (including PIE, SBT, transfer loop and disposable transfer tool);
- CRFs and other required electronic and printed forms;
- Barcode labels with study numbers for all specimens;
- Masked and randomized proficiency and reproducibility (if applicable) panel of characterized samples;
- Anaerobic Transport Medium (Anaerobe Systems);
- Shipping materials: shipping boxes, dangerous good shipping labels, GenePOC pre-addressed FedEx waybills, shipping forms and other material and/or equipment needed for shipping;
- Freezer boxes, plastic microtubes and screw-caps;
- USB keys for instrument data daily backup;
- Swabs for environmental testing (as described in section 7.5.1.3);
- Listing for Study binder(s) indicating essential information for archiving purposes at the closing of the study;
- Technical and engineering support as needed;
- Miscellaneous supplies as agreed upon.

6.6.2 Provided by Investigator

- Specimens meeting the eligibility criteria outlined in Section 6.3 above;
- Copies of the laboratory's procedures for the *C. difficile* collection, routine diagnostic method and quality control;
- Material for routine testing of *C. difficile*:
- Facilities, time and personnel necessary to perform the evaluation according to this Study Protocol, and manage the study records according to the Study Site Manual, the GenePOC instructions and Regulatory Requirements;
- Import permit provided by the CDC (into US only) for pathogens including at least *Clostridium species*.
- Trained personnel in the preparation and shipment of biological substances per CDC (USA sites only), Transportation of Dangerous Goods Regulations 14 (TDGR) (Canadian sites only), and IATA guidelines (all sites);
- Anaerobic workstation at 35-37°C (For Reference Method site only);
- Secure room temperature storage space for the GenePOC CDiff test kits (as required by package inserts and Study Site Manual);

- Adequate refrigerator (between 2°C and 8°C) for the storage and fresh specimens;
- Adequate storage space in a -25oC freezer or colder to hold frozen stool specimen, SBT, broths and panels;
- Control organisms for Reference Method,
- Dry ice to send frozen stool specimen and/or SBT;
- Site policies or procedures to prevent the release of personal information about subjects to the investigator;
- Miscellaneous supplies as agreed upon.

7 LABORATORY TESTING PROCEDURES

7.1 Specimen Collection

- Collect unformed stool specimen in a clean, dry container or bedpan not contaminated with urine, residual soap, toilet paper, water, or disinfectants.
- Transfer appropriate volume of deidentified stool specimen to:
  - An aliquot for specimen freezing for further testing (if needed);
  - An Anaerobe Systems anaerobic transporter;
  - To sample buffer tube (SBT)

7.2 Specimen Storage and Handling

Specimens should be transported to the laboratory according to the hospital's policies.

- Specimens that meet the study criteria should be labelled with a barcode study number provided by GenePOC;
- All relevant information regarding the specimen will be recorded on the appropriate Specimen Handling worksheet and data thereafter transferred to eCRF;
- Fresh specimens stored at 2-25°C can be tested with the GenePOC CDiff system within 48 hours or within 7 days of collection if kept at 2-8°C. Specimen kept at room temperature in Anaerobe Transport Medium must be tested within 4 days of collection for Reference Method testing.
- Frozen specimens will be provided by GenePOC, and the detailed testing procedure will be provided as an addendum to the Study Site Manual.

7.3 Testing Algorithm

Results obtained with the GenePOC CDiff test will be compared to those obtained with the Reference Method. All Clinical Centers will be shipping the stool specimen for Reference Method testing. The Reference Method is described in detail in Section 7.3.2 of this protocol.

The unformed stool specimen will follow the normal hospital path up to the Hospital routine test, of which deidentified specimen left over will be divided in three parts. At least 1,25 mL will be needed for protocol requested analysis.

First, approximatively 500 µL of stool specimen will be transferred into an Anaerobe Transport Medium kept at room temperature for shipment to the Selected site for Reference Method testing. The Reference method is toxigenic culture (TC) that is defined as anaerobic culture to isolate *C. difficile*, followed by evaluation of toxigenicity of the isolates through Cell Cytotoxicity Neutralisation Assay (CCNA).

Second, a minimum of 250-500 µL of the homogenized stool specimen will be transferred in a collection tube to be frozen at -25°C (or colder) for further testing (if needed).

Third, using a transfer loop, 5 µL of the homogenized stool specimen will be transferred to an appropriately labelled Sample Buffer Tube (SBT). The SBT will be closed and vortexed as described in the GenePOC CDiff test Package insert and/or Study Site Manual. The SBTs will be placed at 2-8°C in the event repeat testing is required. If the sample does not require repeat testing, the SBT will be frozen at -25°C or colder.

7.3.1 GenePOC CDiff test

The GenePOC CDiff test will be performed according to the test Package insert and Study Site Manual.

7.3.1.1 *Non-Reportable Results*

Samples that initially produce a non-reportable result (Unresolved, Indeterminate or External Control failure) will be repeated as described below. Samples that have a reportable (Positive, Negative) result upon repeat testing will be included in the data analyses. Repeated samples that do not give a reportable result will not be included in data analyses. They will be reported separately as non-reportable samples.

7.3.1.1.1 Unresolved Samples (UNR)

An Unresolved Sample is when the process control fails (no detection) and the target reaction (*tcdB*) also shows no detection. A picture should be taken of the PIE and sent to the Study Manager (please refer to Study Site Manual section 9 for instructions).

An Unresolved sample will be repeated from the refrigerated SBT. These samples may be repeated alone, with other samples to be repeated, or included in a new run with fresh specimens. In cases where the sample is Unresolved again, no additional repeat testing will be performed. Please refer to section 7.3.3 for action to be taken with remaining SBT.

7.3.1.1.2 Indeterminate Result (IND)

For an Indeterminate (IND) result due to an instrument failure, testing of the sample(s) will be repeated from the refrigerated SBT and may be tested with other samples that require repeat testing, or included in a new run with fresh samples. For any IND result, the Study Manager should be contacted and the incident must be documented on the eCRF. In cases where the sample is Indeterminate again, no additional repeat testing will be performed. Please refer to section 7.3.3 for action to be taken with remaining SBT.

7.3.1.1.3 External control failure

In case of a failure of either or both external control, the testing of all clinical samples and any repeated samples included in the run will be repeated from the refrigerated SBTs along with new external control (see Section 7.5 for details). In cases where external control fails again, the Study Manager should be contacted.

7.3.2 Reference Method

The *C. difficile* bacteria, when present, will be isolated from soft or liquid stool specimens using direct and enriched culture method.

Microbiology Specialists Incorporated (MSI) is the selected center for Reference Method. Their Standard Operating Procedures (SOP) will be utilized as summarized below.

The specimens will be inoculated onto a standard cycloserine cefoxitin and fructose agar plate (CCFA) (direct culture, Section 7.3.2.1) and cycloserine cefoxitin mannitol broth with taurocholate and lysozyme (CCMB-TAL) (enriched culture, Section 7.3.2.2). After incubation, the broth will be sub-cultured to another CCFA plate. Colonies morphologically resembling *C. difficile* will be identified as described in Section 7.3.2.3. The isolate will be growing in chopped meat carbohydrate broth (CMC) for identification confirmation by gas liquid chromatography (GLC). If the isolated strain is identified as *C. difficile*, the same CMC will be used for toxigenicity assessment. The toxigenicity of the isolates using a tissue culture cytotoxicity assay will be determined as described in Section 7.3.2.4. The detailed methods are described in the Reference Method Laboratory's SOP.

7.3.2.1 *Direct Culture (As per MSI SOP No. ST041 dated 2006/Jul/17)*

- A commercially available standard CCFA plate will be inoculated using a swab of the stool specimen from the Anaerobic Transport Medium and streaked for isolation.
- The plate will be incubated at 35-37°C in anaerobic conditions for 48 hours.
- The plate will be examined for colonies characteristic of *C. difficile.*

Note: Due to the very high sensitivity of *C. difficile* to oxygen, the plate should be put in aerobic conditions only when necessary and for a short period of time.

7.3.2.2 *Enriched Culture (As per MSI SOP No. ST041 dated 2006/Jul/17)*

- The same swab that is utilized to inoculate the CCFA plate will be used to inoculate a CCMB-TAL broth tube.
- The tube will be incubated at 35-37°C in anaerobic conditions for 48h.
- The broth is then sub-cultured to another CCFA plate. The CCFA plate will be incubated anaerobically at 35-37°C for 48h.
- The plate will be examined for colonies characteristic of *C. difficile.*
- The enriched culture can be stopped as soon as a toxigenic *C. difficile* is confirmed in the direct culture for a given specimen

Note: Due to the very high sensitivity of *C. difficile* to oxygen, the plate and broth should be put in aerobic conditions only when necessary and for a short period of time.

7.3.2.3 *C. difficile Identification (As per MSI SOP No. ST041 dated 2006/Jul/17)*

Colonies morphologically resembling *C. difficile* from the Direct or Enriched plates will be confirmed as follows:

- Gram stain,
- Characteristic barnyard-like odor,
- Aero intolerance on chocolate agar plates, in CO2 and a blood agar plate (BAP) under anaerobic conditions. The BAP plate contains a vancomycin (5 mcg) disc.
- Gas liquid chromatography (GLC)

All tests above are required to confirm that the isolated strain is *C. difficile.*

7.3.2.4 *Toxigenicity Assessment (As per SOP No. ST040 dated 2012/Feb/24)*

Toxigenicity Assessment will be proceeding once *C. difficile* identification is confirmed by the presence of iso acids by GLC. The same CMC broth (incubated up to 48h at 35-37°C in anaerobic atmosphere) used for iso acids identification is used to perform the toxin assay.

The procedures will follow the SOP noted above.

An isolate cultured on BAP will be preserved in broth with 10-20% glycerol. Preserved isolates will be stored at-70°C or colder for possible future use. The remaining of the CMC broth will be kept at room temperature to inoculate a new CMC broth in case a repeat of the test is required.

7.3.2.5 *Inconclusive Result*

For an Inconclusive (INC) result in the Reference Method due to a non-interpretable cytotoxicity test result (partial rounding of the cells, stretching instead of rounding), specimen(s) will be retested from a newly inoculated and incubated CMC broth. The CMC broth kept at room temperature should be used to inoculate a new CMC broth (using a dilution factor of around 1/10) that should be processed as directed in Section 7.3.2.4, Toxigenicity Assessment.

Specimens that have a reportable (Positive, Negative) result after the initial or repeated testing will be included in the data analyses. Repeated specimens that do not give a reportable result will not be included in data analyses. They will be reported separately as non-reportable specimens.

7.3.3 Discrepant Testing

In cases of discrepant results between the Reference Method and the GenePOC CDiff test, further characterization testing may be requested by GenePOC to be performed by the Clinical Center.

If results from Reference Method and GenePOC CDiff test remains discrepant after retesting, frozen stool specimen, CCMB-TAL Broth and/or frozen SBT coming from discrepant result specimen may be sent to GenePOC for further testing.

During the course of the trial, the GenePOC Study Manager will inform the sites which specimens require to be shipped to GenePOC.

7.4 Storage & Shipping

- SBT samples must be kept at 2-8°C after testing for a maximum of seven (7) days. When it is confirmed that no repeat is necessary, they will be frozen at -25°C or colder for possible future discrepant testing;
- All Frozen stool specimen and CCMB-TAL Broth must be stored at -25°C or colder, and culture isolates at -70°C or colder and only specimens requested by the Study Manager will be shipped on dry ice to GenePOC;
- All PIEs (containing amplified product) must be discarded in appropriate biological waste receptacle in accordance with country, federal, provincial, state and local regulations.
- Detailed shipping instructions will be provided in the Study Site Manual.
- At the end of the trial, clinical center will be instructed by Study Manager to destroy, as per their institution policy, specimens that did not require shipment to GenePOC.

7.5 Quality Control (QC) Testing

In addition to the quality assurance and quality control programs of each laboratory under their respective certification (e.g. CLIA, ISO 15189), the minimum quality control described below must be performed during the course of this study.

7.5.1 GenePOC CDiff test

7.5.1.1 *External Controls*

For each day in which samples are processed, appropriate positive and negative external control (ECs) will be tested. These external controls results will need to be kept in the Site File and forwarded to GenePOC.

External control materials are provided by GenePOC. A reference toxigenic *C. difficile* strain bearing the *tcdB* gene will be used as a positive external control (EC) while 150 µL of BRU broth will be used as a negative EC. If either or both of the ECs fail to give the expected result, all samples tested in that run will be repeated from the remaining SBT containing the specimen stored at 2-8°C along with new positive and negative ECs.

Preparation of these controls will be detailed in the Package Insert and in the Study Site Manual.

7.5.1.2 *Sample Process Control*

The Process Control (PC) is incorporated into GenePOC CDiff PIE and is intended to monitor for the effectiveness of liquid displacement, sample treatment (Lysis) and heating during the sample processing steps. The PC also monitors the integrity of the PCR reagents, the thermal cycling, and for the presence of inhibitory substances during the amplification and detection steps. A failed PC renders the sample Unresolved in absence of *tcdB* DNA target.

7.5.1.3 *Monitoring for the Presence of DNA Contamination*

At the request of the GenePOC Study Manager, the work area and equipment will be monitored for the presence of toxigenic *C. difficile* bearing the *tcdB* gene contamination using the GenePOC CDiff test. Should contamination arise, the Study Manager will instruct the site on the measures to be taken prior to testing additional specimens.

7.5.2 Reference Method

7.5.2.1 Media QC

All media verified for physical appearance and sterility for each lot shipment and/or at the time of use. In addition, culture media and vancomycin disk performance as assessed at a minimum for each lot shipment and periodically as described in the package inserts and clinical laboratory standard using the recommended and/or required strains.

Media Performance Quality Control details

Media or Reagent	Strains Tested for Performance	QC frequency
CCMB-TAL	*C. difficile*	Each lot or shipment
CCFA	*C. difficile* *C. perfringens*	Each lot
Vancomycin disk (5mcg)	*C. perfringens* *B. fragilis*	Each lot and weekly
CMC	*P. anaerobius*	Each lot and weekly
Chocolate Agar	*H. influenzae* *N. aonorrhoeae*	Each lot or shipment
Brucella Agar	*C. difficile* *B. fragilis*	Each lot or shipment
Blood Agar plate	*S. pyogenes* *S. pneumoniae* *E. faecalis*	Each lot or shipment

7.5.2.2 GLC QC

For GLC testing, quality control is performed each day of testing. A commercially available volatile acid standard mix is injected into the column. All volatile acids must be present in order to pass QC.

7.5.2.3 Tissue Culture Cytotoxicity Assay QC

Tissue Culture Cytotoxicity Assay QC will be performed as described in MSI SOP ST041 each testing day.

8 Clinical Trial Quality Assurance

The following measures will be taken to assure the quality of the trial conduct and data.

8.1 Accountability of Investigational Materials

The Clinical Center will account for all GenePOC CDiff system investigational materials received from GenePOC. GenePOC clinical trial team will monitor site records for investigational materials accountability. All materials will be handled and disposed of according to Package Insert instructions, Study Site Manual or as directed by the GenePOC Study Manager. Upon completion of the study, all remaining supplies provided by GenePOC will be returned to GenePOC unless otherwise directed by the GenePOC Study Manager in writing.

8.2 Process for Protocol Changes and Deviations

8.2.1 Protocol Amendment

A Protocol Amendment is a change to, or clarification of, the Study Protocol which may impact the conduct and potential benefit of the study, or participant safety. For each protocol amendment, changes must be reviewed, approved and signed by the GenePOC Study Manager, a representative of GenePOC and the Principal Investigator. All changes to the Protocol must be submitted to the Clinical Center IEC for examination and approval (if required). The Principal Investigator will ensure that protocol changes are implemented after written approval by all parties (including the IEC if needed), that all personnel using the protocol at the Clinical Center receive training for the change, and that the new protocol is maintained in the study records at the Clinical Center. Examples of such changes include, but are not limited to: the study objectives, study design, specimen sizes, study procedures, or administrative aspects.

8.2.2 Protocol Deviations

Each protocol deviation reported to the GenePOC Study Manager by the Clinical Center, observed by the GenePOC Study Manager or CRA during monitoring contacts or visits, or observed through review of eCRFs will be documented by the GenePOC Study Manager, CRA or Clinical Center personnel on a Protocol Deviation Form. The Protocol Deviation Form will contain the description of the deviation, the impact on the trial or subject enrollment, the impact to the affected data and any corrective action taken to conform to the protocol. All Protocol Deviation Forms will be reviewed and signed by the GenePOC Study Manager or CRA and the Principal Investigator (if the Clinical Center was involved or concerned). A copy of each Protocol Deviation Form will be kept at the Clinical Center and the original at GenePOC.

Examples of such deviations include, but are not limited to:

- not completing the testing required by the protocol;
- enrolling inappropriate specimens;
- failure to follow Package Insert instructions for use and storage of the investigational device;
- failure to follow the Reference Method (e.g. use of expired materials);
- failure to perform EC as directed;
- not respecting the time to perform testing or retesting.

Protocol deviations might render a data not eligible to be included in the final data analysis.

8.3 Trial Consistency and Integrity: Monitoring and Auditing

Monitoring will be accomplished either by on-site visit or through remote monitoring as detailed in the Clinical Monitoring Plan and recorded on Monitoring Visit Report.

Periodic checks may be made with the Principal Investigator and/or his/her staff. It will include (but not limited to) availability of materials, subject and specimen enrollment, product accountability, and clarification of any questions. These checks may be accomplished via telephone conversations and/or written communication (emails or letters/memos) and documented on a Site Contact Report.

Telephone conversations will be documented by the GenePOC Study Manager and/or CRA (recorded on the Site Contact Report). Critical study communications imparted by telephone to Clinical Center personnel (including but not limited to new instructions or training, identification of protocol deviations, corrective actions, or incidents) will be followed up with a written communication to the Clinical Center. Copies of relevant written communication (including e-mails) will be kept by the GenePOC Study Manager and the Clinical Center.

Monitoring may include review of IEC review status, laboratory accreditation, subject enrollment status, regulatory requirements, study compliance, eCRF and source data, adverse event and incident reporting, and study materials storage and disposition. Protocol Deviation documentation should be reviewed as well as eCRFs and applicable source data. Follow-up actions will be documented in writing to the Clinical Center.

The GenePOC CRA or Study Manager will arrange for correction of discrepancies, missing data, or omissions on eCRFs by the Principal investigator or designee as appropriate.

The GenePOC Study Manager will maintain close liaison to clarify any problems that may arise, and to ensure that the study is being carried out according to this protocol and the applicable regulatory requirements. The Clinical Center shall permit the GenePOC CRA, and other GenePOC personnel as indicated, to visit the Clinical Center and audit or inspect the study records and materials, to determine Principal Investigator’s compliance with the protocol, relevant guidelines and regulations for clinical trials.

9 DATA COLLECTION, MANAGEMENT AND REPORTING

9.1 Clinical Center Responsibility

9.1.1 General Instructions on Recording and Sending Data to GenePOC

An Electronic Data Capture (EDC) system will be used by all Clinical Centers.

Individual instructions for completing paper source documents, eCRFs and other forms will be provided by GenePOC in the Study Site Manual.

Electronic media (USB key) containing instrument data should kept on site. Data obtained from GenePOC instrument will be forwarded to GenePOC through the EDC system.

Test results should be entered in the eCRF promptly i.e. within 2 business days of the test results are confirmed.

The following paper forms, but not limited to (refer to Appendix A), will be completed as needed and kept on site during the study with a copy at GenePOC. The original will be kept at GenePOC at the end of the study with copies kept at the Clinical Center:

- Site Signature and Delegation of Authority Log (All persons authorized to complete, review and/or sign study documentation, including CRFs, will sign this Log.
- Study Visit Log;
- Reference method external control forms for culture media will be completed and copies will be sent to GenePOC with originals kept at the Clinical Center at the end of the study.
- Site Contact Log forms will be completed and maintained at the Clinical Center.
- Paper source documents will be completed first then the data will be transferred to the eCRF.

9.1.1.1 *Electronic Data Capture (EDC)*

Electronic CRFs (eCRF) will have to be completed immediately (usually within 2 business days of specimen enrollment) following completion of the testing procedure.

The Principal Investigator must designate, in writing, any individuals authorized to complete and review the eCRFs. Only the Principal Investigator will have the right to sign the eCRFs.

9.1.2 Final Report

The Principal Investigator, with input from the laboratory personnel, will be asked to provide GenePOC with a final report of special study issues, findings, assessment of ease-of-use and workflow, and any problems or recommendations, within 30 days of completion or termination of the study. This final report may include the convenience (ergonomics, performance, utility, rapidity, etc.) of the GenePOC CDiff system for integration into routine laboratory use and potential use near the subject.

9.2 GenePOC Responsibility

A termination letter or notice will be sent by GenePOC to the Principal Investigator notifying the Clinical Center of the successful completion of the study terms by the Clinical Center. Terms and notifications for other causes of study termination will be addressed in the Clinical Trial Agreement.

9.3 Data Confidentiality

GenePOC will maintain the security and confidentiality of all trial data sent to GenePOC. GenePOC and the Clinical Center may be required to provide regulatory agencies access to trial data and records. GenePOC trial databases will not be shared with any third party. GenePOC provides an assurance that the de-identified data obtained in this study will be safeguarded and not used for unauthorized purposes. All other agreements by GenePOC, the Principal Investigator, and the Clinical Center in regards to confidentiality may be found in the Confidential Disclosure Agreement and the Clinical Trial Agreement.

9.4 Data Verification and Validation Rules

Data captured will be entered into the database through the EDC system. Data related to eCRF and accountability logs will be transferred from the source documents to the eCRFs. Data related to the Reference Method, the discrepancy testing and EC will be transferred directly from the laboratory worksheets (considered as source documents) to the eCRFs.

Data will be managed and controlled following GenePOC Quality Procedures and stored in a secure database with limited access and with electronic audit trails.

Data validation (confirming the correctness, completeness, and compliance of the data) will be performed both during and after data entry. Validation during data entry will be performed using both visual inspection and programming incorporated into the forms.

10 CLINICAL TRIAL INCIDENTS, RISK MANAGEMENT AND ADVERSE EVENTS

10.1 Clinical Trial Incidents

A Clinical Trial Incident (CTI) is defined as any problem involving the investigational device, Reference Method, procedures, human subjects, or operators as a result of execution of this Study Protocol. CTIs may adversely (or potentially adversely) affect human safety, the integrity of the evaluation data, or the operation of devices.

All serious occurrences which affect the health or safety of human subjects or operators involved in this evaluation are considered a Clinical Trial Incident, and may also be determined to be an Adverse Event (AE). AEs are addressed specifically in Section 10.4 below. Since subjects are not directly affected by the diagnostic device under evaluation and results are not used for clinical decisions, virtually all CTIs are not considered AEs.

The Clinical Trials Incident Report Form is used to capture problems or observations arising during the use of the investigational product or in the execution of this Study Protocol and should be reported to the GenePOC Study Manager. The form should be filled out in the eCRF with a full description of the incident, and notification of the incident form may be sent by email to the GenePOC Study Manager. Incidents might include but are not limited to such occurrences as: instrument failure (IND or other), run incomplete, damage or deterioration of the GenePOC CDiff system, damage to devices or packaging caused by shipping or handling, or other incidents deemed to be failures or problems with the product or the execution of this Study Protocol.

10.2 Reporting of Incidents

Any incidents which occur during the use of the GenePOC CDiff system or execution of this Study Protocol must be reported immediately to GenePOC.

The primary contact will be the Study Manager.

Catherine Lippe Study Manager	Phone Cell Phone e-mail	(418) 650-3535 #269 (418) 925-8597 catherine.lippe@genepoc.ca clinical_trial@genepoc.ca

In case of a problem with the GenePOC™ instrument, the second line of support will be:

Sébastien Chapdelaine Vice President Research & Development	Phone Cell Phone e-mail	(418) 650-3535 #221 (418) 803-0650 sebastien.chapdelaine@genepoc.ca

The Clinical Center must identify itself as a GenePOC CDiff clinical trial site and by providing it site number

In case of a problem with the EDC System, contact EDC System support line (phone # 888.500.4247). If this support line does not provide a satisfactory response, contact the first line of support listed above who will either address the problem or dispatch the problem, as needed.

All incidents must be documented on a Clinical Trial Incident report form.

10.3 Risk Management

10.3.1 Potential Risks to the Subject

There are no risks to the subject inherent to the execution of this evaluation; the investigational testing will:

a) Be performed on excess de-identified specimens only; and,
b) Include parallel clinical routine testing by an approved and established method for reporting subject results.

10.3.2 Potential Risks to the Personnel operating the Product

To reduce the risk of exposed personnel, all processing, testing, and culturing of potentially infectious specimens must always be performed according to Standard Precautions, CDC Guidelines, Standard Guidelines, and the Clinical Center's own Laboratory Safety procedures and policies.

10.4 Adverse Events

- An **Adverse Event** (AE) is defined as any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory finding) in subjects, users or other persons (investigational devices only) which is associated with the use of an investigational product or participation in an investigation, whether or not related to the investigational medical device. This includes event not seen at baseline and event that if present at baseline have worsened in intensity.
- **Anticipated Adverse Events** are defined as adverse events that are already known to occur from past experience. However, as cited in Section 10.1 above, there are no anticipated adverse events associated with this Study Protocol.
- An **Adverse Device Effect** (ADE) is defined as an adverse event related to the use of an investigational medical device.
- A **Serious Adverse Device Effect** (SADE) is defined as any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device.
- An **Unanticipated Serious Adverse Device Effect** is a Serious Adverse Device Effect for which the effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects. If an Unanticipated Serious Adverse Device Effect occurs, it must be reported immediately by the Principal investigator to GenePOC and to the IEC (when required). GenePOC may additionally be required to report the occurrence to regulatory authorities.
- **Medical Device Reporting** (MDR) - Any commercially marketed device used in the trial which causes or contributes to a death or serious injury must be additionally reported to the commercial manufacturer as an MDR reportable event, under US federal regulations and Health Canada. An occurrence of serious injury during the trial that resulted from the use of a marketed device may require reporting as both an Adverse Event (to the IEC and Sponsor of the trial) and as an MDR (to the commercial manufacturer of the device).

11 REGULATORY AND ADMINISTRATIVE INFORMATION

11.1 Institutional Requirements

The Study Protocol must be submitted for review to the Clinical Center's IEC prior to the start of the study. In addition, this committee will inform the Principal investigator and the sponsor as to whether a waiver of informed consent has been granted.

This must be done in accordance with Part 56 (Institutional Review Boards) of the Code of Federal Regulations Title 21 (USA) and Section 81(k) of the Canadian Medical Device Regulations.

A copy of the IEC acknowledgment of review or approval from each institution must be forwarded to the study sponsor, GenePOC.

11.2 Ethical Conduct and Good Clinical Practice

The procedures set forth in this Study Protocol are designed to ensure that GenePOC and the clinical investigators abide by the principles of Parts 50 and 56 of the Code of Federal Regulations Title 21 (USA), the Tri-Council's Code of Ethical Conduct for Research involving Humans (1998) and Part 3 of the Medical Devices Regulations (Canada), the Declaration of Helsinki (USA and Canada), and the Good Clinical Practices (ICH: USA, Canada and Europe), in the conduct, evaluation and documentation of this study.

11.3 Investigator Responsibilities

The investigator responsibilities are defined in the present Study Protocol. The Financial Disclosure certification must be filled out in accordance with Part 54 of the Code of Federal Regulations, Title 21.

11.4 Subject Information Confidentiality

All information will be treated with the utmost confidentiality by the hospital and the study sponsor, GenePOC. Only de-identified data will be obtained. Subject names or medical record numbers will not be transferred to the study sponsor.

The specimens will be used strictly for isolation of *C. difficile* strain bearing the *tcdB* gene, and for no other purposes. Results from this study may be used by GenePOC to fulfill regulatory requirements of Health Canada, the US Food and Drug Administration (FDA), the European Parliament (providing the CE marking) and rest of the world.

11.5 Data Maintenance and Disclosure

Study binder(s) will be provided to each Clinical Center to organize the required study documentation. Each Clinical Center is directly responsible for the maintenance and organization of the study documentation.

Any corrections and/or changes made to entries on paper forms or logs, by the Principal Investigator or designees, must be crossed out with a single line leaving the initial entry legible. The correction must be dated and initialed. Incorrect entries must not be covered with correction fluid, obliterated, or made illegible in any way. If the reason for the change is not obvious, an explanation for the change must be written next to the modification.

Any change performed on an electronic form will be audited by the system and, when required, justified before electronic signature.

The investigator is obligated to provide the study sponsor with complete test results and all data derived from the study. Any information that is unclear will be brought to the attention of the Principal Investigator and/or laboratory contact for prompt resolution.

11.6 Clinical Center Compliance

11.6.1 Investigator Responsibility

Data generated from this evaluation will be used to support regulatory submissions. The Principal Investigator is expected to ensure that the Clinical Center and its personnel comply with Good Clinical Practices and pertinent regulations governing clinical research.

11.6.2 Accountability of Materials

All materials will be handled and disposed of according to manufacturer's instructions. All investigational devices and their use and disposal will be accounted for in writing. Upon completion of the study, all remaining devices provided by GenePOC will be returned to GenePOC unless otherwise directed by the GenePOC Study Manager in writing.

11.6.3 Retention of Records

The Principal Investigator will retain trial related documents as required by the applicable regulatory requirements or by an agreement with GenePOC. The Principal Investigator should take measures to prevent accidental or premature destruction of these documents. Essential documents should be retained for:

- A period of five years after the last approval or a marketing application to regulatory agencies.
- A period of five years has elapsed since the formal discontinuation of development of the investigational product.

11.7 Trial management

11.7.1 Study Initiation

The Clinical study is anticipated to start in June 2016 at the earliest. Specimen enrollment will begin upon signed approval of all contract trial agreements, receipt of written IEC acknowledgment of review of this Study Protocol and demonstration of proficiency. The study is expected to be conducted over a period of three months depending upon the enrollment rate of diagnostic specimens and the prevalence of *C. difficile* strain bearing the *tcdB* gene.

11.7.2 Study Extension

The evaluation may be extended beyond the estimated duration under terms agreed upon by the Clinical Center and GenePOC if more data are required for product development, or if the study is redirected or re-initiated. Any redirection of the study or re-initiation after a period of suspension would be subject to the re-negotiation of terms between GenePOC and the Clinical Center.

11.7.3 Rules for Discontinuation

GenePOC retains the right to terminate or curtail the study if this study protocol is not followed, or if the device under evaluation requires further development to meet the intended clinical objectives.

The Clinical Center shall terminate the study upon any of the following conditions:

- Completion of the agreed maximum number of positive results or the agreed maximum number of total specimens;
- Reaching the date of maximum study duration;
- Withdrawal of approval from the Clinical Center Administration or IEC.
- Other reasons and conditions for study termination could be addressed in the Clinical Trial Agreement.

12 REFERENCES

Bagdasarian N, Rao K and Malani PN Diagnosis and treatment of Clostridium difficile in adults: a systematic review [Article] // JAMA 33. - 2015. - pp. 398-408.

Barbut F [et al.] Does a rapid diagnosis of Clostridium difficile infestion impact on quality of patient management? [Article] // Clin Microbiol Infect 20. - 2014. - pp. 136-144.

Bouza E Consequences of clostridium difficile infection: understanding the healtcare burden [Article] // Clin Microbiol Infect 18 suppl.6. - 2012. - pp. 5-12.

Ghantoji SS [et al.] Economic healthcare costs of Clostridium difficile infection: a systematic review [Article] // J Hosp Infec 74. - 2010. - pp. 309-318.

Goldenberg SD [et al.] Point-of-care testing for clostridium difficile infection: a real-world feasibility study of a rapid molecular test in two hospital settings. [Article] // Infect Dis Ther DOI 10.1007/s40121-014-0038-6. - 2014.

Goudarzi Mehdi [et al.] Clostridium difficile Infection: Epidemiology, Pathogenesis, Risk Factors, and Therapeutic Options [Article] // Hindawi Publishing Corporation. - 2014. - Vol. 214.

Le Guern R et Wallet F Diagnostic de l'infection à Clostridium difficile au laboratoire [Article] // Ann Biol CLin. - 2013. - 71. - pp. 395-400.

Lessa FC [et al.] Burden of Clostridium difficile infection in the United States [Article] // New England Journal of Medicine 372. - 2015. - pp. 825-834.

Sewell B [et al.] Cost and impact on patient length of stay of rapid molecular resting for Clostridium difficile [Article] // Infect Dis Ther. - 2014. - 3. - pp. 281-293.

Shroeder LF [et al.] Economic evaluation of laboratory testing strategies for hospital-associated Clostridium difficile infection [Article] // J Clin Microbiol 52. - 2014. - pp. 489-496.

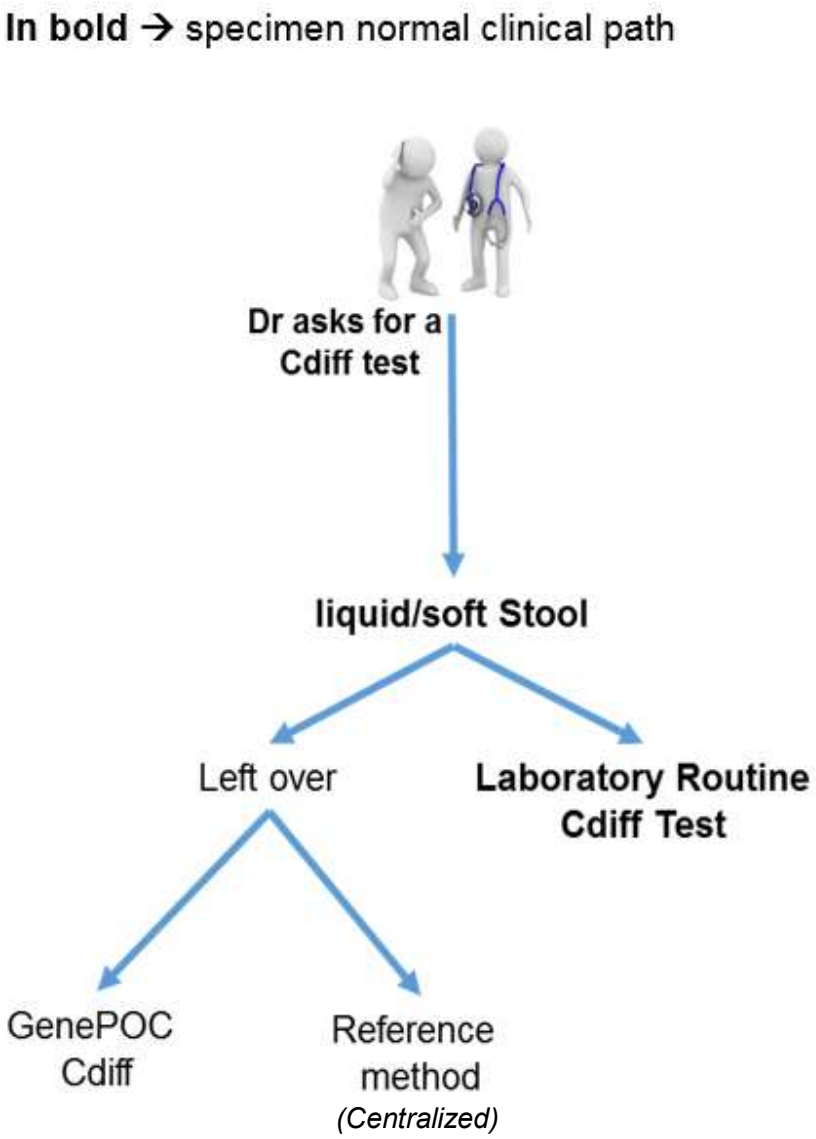
Surawicz CM [et al.] Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections [Article] // Am J Gastroenterol 108. - 2013. - pp. 478-498.

13 APPENDIX

13.1 APPENDIX A: Regulatory Documents Requirements

Trial Requirements	Investigator	Sponsor
Confidential Disclosure Agreement: (CDA)	Original (signed and dated)	Original (signed and dated)
Curriculum vitae of principal investigator, co-investigators and other key site personnel	Copy	Copy
Study training records	Copy	Original
Laboratory Certification	Copy	Copy
Protocol, including any revisions and changes	Original (signed and dated)	Original (signed and dated)
CTA and Financial Agreement	Original (signed and dated)	Original (signed and dated)
IEC composition, correspondence Or IEC acknowledgment of review	Original	Copy
Financial Disclosure Agreement and Updates	Original (signed and dated)	Original (signed and dated)
Site Contact Log	No Copy	Original
Site Signature and Delegation of Authority Log and Study Visit Log	Original (during the study) Copy (at the end of the study)	Copy (during the study) Original (at the end of the study)
Clinical Trial Incident Report form	Original (signed and dated)	Copy
Laboratory Worksheets	Copy	Original
Reference method external control forms	Original	Copy
Investigational Material Accountability Log	Copy	Original
Relevant Communications	Copy (printed or electronic)	Copy (printed or electronic)
USB Keys for runs	Make1 copy daily of runs from the GenePOC instrument. Keep the copy at site for all Instrument backups. Transfer instrument data to sponsor through EDC database	Transfer electronically onto server and keep on a CD after.

13.2 APPENDIX B: Testing Algorithm



13.3 APPENDIX C: Discrepant Testing Algorithm