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Pivotal Study of a Single-Use, Point-of-Care Molecular Diagnostic Device for the Detection of *Neisseria gonorrhoeae* (NG), *Trichomonas vaginalis* (TV), and *Chlamydia trachomatis* (CT) in Women

DMID Protocol Number: 18-0024

Sponsored by:
Division of Microbiology and Infectious Diseases
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Statement of Compliance

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

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11
- International Conference on Harmonisation: Good Clinical Practice (ICH E6); 62 Federal Register 25691 (1997); and future revisions
- Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- National Institutes of Health (NIH) Office of Extramural Research, Research Involving Human Subjects, as applicable
- National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance

The study is a nonsignificant risk device study and must comply with the Exempted Investigational Device Exemption (IDE) requirements under 21 CFR §812.2 (c).
All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protections Training.

Signature Page

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

Site Investigator:_____

Signed: _____ Date: _____
Name/Title

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List of Abbreviations

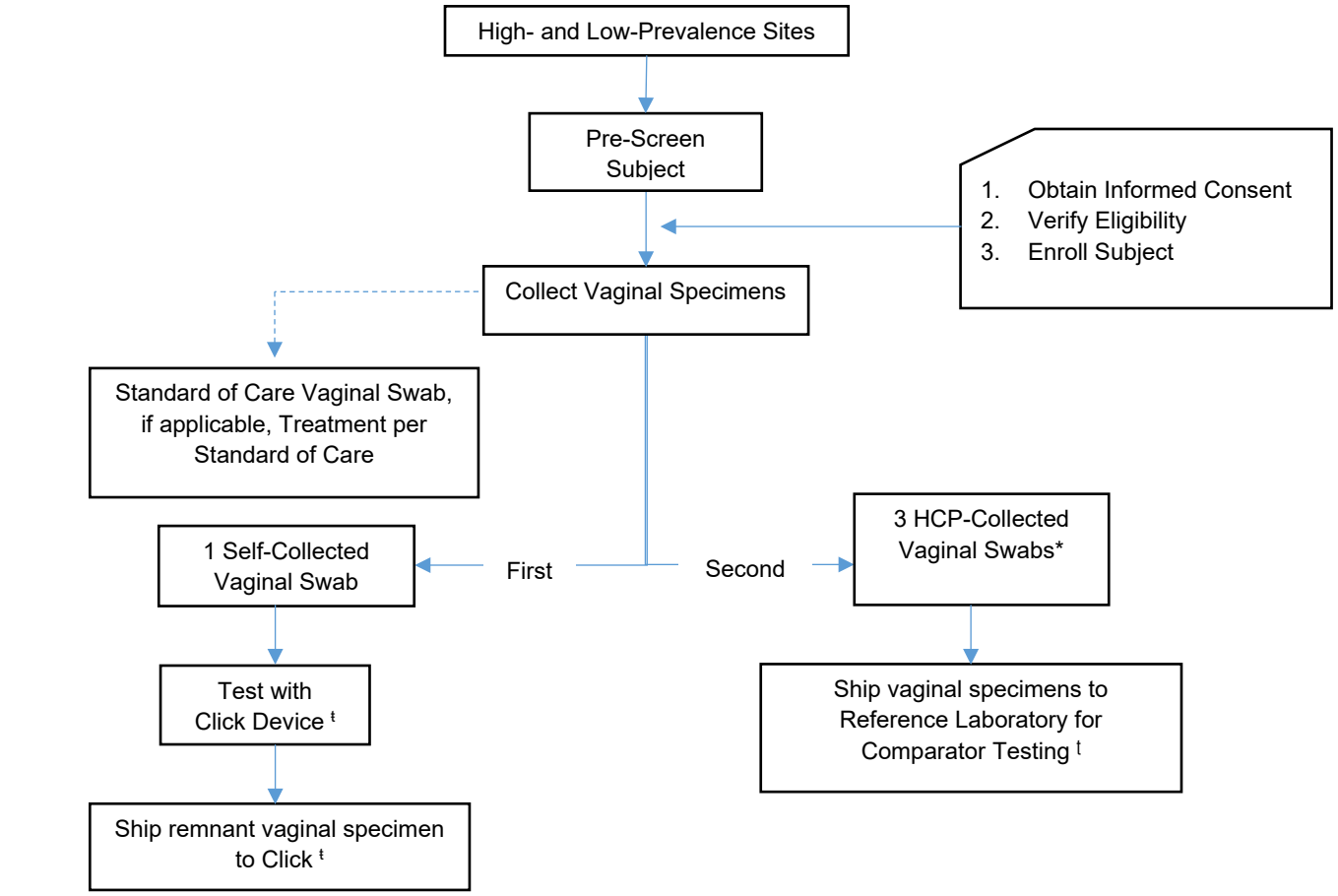
AE	Adverse Event
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CLIA	Clinical Laboratory Improvement Amendments
CRF	Case Report Form
CT	*Chlamydia trachomatis*
DHHS	U.S. Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
FDA	U.S. Food and Drug Administration
FWA	Federalwide Assurance
GCP	Good Clinical Practice
HCP	Health Care Provider
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IRB	Institutional Review Board
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NAAT	Nucleic Acid Amplification Test
NG	*Neisseria gonorrhoeae*
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PCR	Polymerase Chain Reaction
PHI	Personally Identifiable Information
PI	Principal Investigator
PIS	Patient Infected Status
POC	Point-of-care
PRN	“As Needed” (Latin: *pro re nata)*
SAE	Serious Adverse Event
SDCC	Statistical and Data Coordinating Center
SOC	Standard of Care
SOP	Standard Operating Procedure
STI	Sexually Transmitted Infection
TV	*Trichomonas vaginalis*
UADE	Unanticipated Adverse Device Effect

Protocol Summary

Title:	Pivotal Study of a Single-Use, Point-of-Care Molecular Diagnostic Device for the Detection of *Neisseria gonorrhoeae* (NG), *Trichomonas vaginalis* (TV), and *Chlamydia trachomatis* (CT) in Women
Population:	Approximately 1750 female subjects ≥14 years of age in the United States.
Number of Sites:	At least three clinical sites across diverse geographic areas in the United States; approximately one-third of sites will have a patient population with low prevalence.
Study Duration:	Approximately 9 months after enrollment of the first subject.
Subject Duration:	One visit, approximately 60 minutes.
Primary Objective:	To assess the performance of the Click Diagnostics point-of-care, single-use device for the detection of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), and *Trichomonas vaginalis* (TV) in self-collected vaginal specimens as compared to Patient Infected Status (PIS) determined by three approved comparator assays using vaginal specimens collected by a qualified health care provider (HCP) as defined by state/local regulatory authorities, in support of obtaining FDA clearance and a Clinical Laboratory Improvement Amendments (CLIA) waiver.
Estimated Time to Complete Enrollment:	Approximately 9 months or until evaluable sample size targets are met.

Schematic of Study Design for Symptomatic and Asymptomatic Subjects

Study Visit 1



* Order of HCP-collected vaginal swabs will be randomized according to a provided randomization list.
t All study leftover comparator vaginal liquid media will be kept frozen at the Reference Laboratory and may be shipped per DMID instructions.
t Used Click devices and leftover Click specimens will be returned to Click Diagnostics at intervals defined in the MOP.

1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Sexually transmitted infections (STIs) are persistent and expanding problems. The US Centers for Disease Control and Prevention (CDC) estimates that nearly 20 million new STIs occur every year and account for almost $16 billion in health care costs. STIs affect men and women alike, and are especially common among young people ages 15-24 years [1].

Chlamydia (*Chlamydia trachomatis*, [CT]) and gonorrhea (*Neisseria gonorrhoeae*, [NG]) are the most frequently reported bacterial STIs in the United States. These infections are called “silent infections,” as most infected people are asymptomatic and lack abnormal physical examination findings. The CDC estimates 2.86 million infections of chlamydia and 800,000 gonorrheal infections occur annually in the United States but not more than half are reported because most people are asymptomatic and do not seek testing [2,3]. Figures from 2015 support this assumption with only 1.5 million cases of chlamydia and 400,000 cases of gonorrhea reported to the CDC. Almost two-thirds of new chlamydia infections occur among people aged 15-24 years, and about 1 in 20 sexually active women aged 14-24 years has chlamydia [3]. More than half of new gonorrhea infections per year are estimated to occur among people 15-24 years of age. Many cases of chlamydia and gonorrhea often co-exist, and continue to go undiagnosed and unreported [1,4]. CT can cause urethritis, epididymitis, proctitis, cervicitis, acute salpingitis, and pelvic inflammatory disease. CT infections are often asymptomatic in both males and females. Children born to CT-infected mothers are at higher risk for conjunctivitis and chlamydial pneumonia [5]. Gonococcal infections are more likely to be symptomatic and may cause lower and upper genital tract infections, and occasionally disseminated infections with gonococcemia with a characteristic rash, tenosynovitis, and arthritis [6]. The immunologic response triggered by lower genital tract infections with CT and NG leads to significant inflammation of the cervico-endometrial tissue [3]. Due to infection and the associated chronic inflammatory response, several important sequelae may result from these infections, including pelvic inflammatory disease, ectopic pregnancy, and infertility [7-10].

A third STI, trichomoniasis (*Trichomonas vaginalis* [TV]), is considered the most common curable STI but is not a notifiable disease. In the United States, an estimated 3.7 million people have the infection, but only about 30% develop symptoms of trichomoniasis [11]. Infection is more common in women than in men, and older women are more likely than younger women to be infected. Further, trichomoniasis can increase the risk of contracting or spreading other STIs [5]. Infection of TV is often asymptomatic [12-14]. However, even asymptomatic infections are of public health concern. TV has been associated with a more than a 2.7-fold increase in the risk of HIV acquisition [15-17], a 1.3-fold increase in preterm labor, and a 4.7-fold increase in pelvic inflammatory disease [18-19]. The U.S. CDC Sexually Transmitted Disease treatment guidelines recommend TV screening for all HIV-infected women when care is initiated, then at least annually, as well as diagnostic testing in symptomatic women presenting with vaginal discharge [10].

If left untreated and undiagnosed, CT, NG, and TV can result in serious, permanent damage to the reproductive system, pelvic inflammatory disease, ectopic pregnancy, and infertility [20].

Diagnostic testing for pathogens of CT, NG, and TV is currently performed at a variety of sites: public health, private, and hospital laboratories, physician's offices/physician office laboratories, and some pharmacies. Sample collection is performed at the physician's office. The sample is then transported and processed in a qualified laboratory. After testing, the results are transferred by a laboratory information system, or manually entered into patient records. Competition in these sectors ranges from very high quality testing and large test menus in the laboratories to a very limited menu of low quality rapid tests at the physician's office. While laboratory-based tests for chlamydia and gonorrhea have high accuracy, they are far removed from patients and performed on an average sample-to-result-to-patient timeline of 3 to 5 days. Trichomoniasis testing is conducted using wet mount microscopy in the physician's office, but has a poor sensitivity of 50-70% [21]. Nucleic acid amplification tests (NAATs) for TV have provided highly sensitive and specific diagnoses, but require laboratory infrastructure [22-25].

Agencies such as the CDC understand that NAATs are the most sensitive tests and can be performed on easily obtainable specimens such as vaginal swabs (either health care provider [HCP]- or patient-collected) or urine [3]. However, at this time, no simple, rapid NAAT test exists for physicians to test male and female samples for these three pathogens at the same time at the physician's office.

The amount of time until diagnosis, the lack of symptoms in most people, and the subsequent lack of treatment result in preventable disease spread. Thus, there is an urgent need for point-of-care STI diagnostic devices that provide rapid and highly accurate results. The performance of such diagnostics should be similar to the gold standard of NAATs [26,27].

The hypothesis of this clinical study is that the Click Diagnostics Sexual Health Test performs substantially equivalent to the NAAT predicate system.

2.1.1 Description of the Click Diagnostics Sexual Health Test System

The Click device is a single-use, disposable, fully integrated, rapid, compact device containing a polymerase chain reaction (PCR)-based NAAT for accurate, qualitative detection and differentiation of deoxyribonucleic acid (DNA) from CT, NG, and TV organisms. The device is intended to provide a highly accurate, simultaneous diagnosis of chlamydia, gonococcal, and trichomonas infections that can be used in a variety of environments without the need of a complex instrument. The Click device is intended for use with specimens from females 14 years of age and older.

The Click device integrates sample preparation, PCR amplification, DNA detection, and visible result indication into a single disposable test unit. The device uses 650 µL of sample, which is introduced into the device using a transfer pastette. The Sample Preparation module heat inactivates the sample.

PCR amplification is achieved by combining the inactivated sample with lyophilized PCR reagents, and then thermocycling. PCR primers are designed to target CT, NG, and TV. The output from PCR flows to the detection module where enzymatic processes provide a visually apparent colorimetric signal for multiplexed detection of the target pathogens. Amplified pathogenic target (if present) is hybridized to specific locations along a flow channel. This flow channel is configured to facilitate an enzymatic reaction that utilizes horseradish peroxidase (HRP) and a color-producing substrate. This will result in an observable color change for a positive reaction. The basic controls strategy relies on a positive control spot on the flow cell. If all elements in the Click device are functioning properly, the positive control spot will produce color. The positive control monitors effective sample prep, PCR amplification, and detection.

The presence of any targeted pathogen in the sample leads to a purple color change in the corresponding viewing window of the device. This allows for easy visual discrimination of positive and negative test results as well as verification of a successful positive control, as directed by the Quick Start Instructions.

2.1.2 Reference Method

The Patient Infected Status (PIS) provides the reference for this study. This algorithm incorporates results from three recognized FDA-cleared methods for the CT and NG pathogens and from three recognized FDA-cleared methods for TV (Table 1). NAAT 1, NAAT 2, and NAAT 3 refer to BD Probetec, Hologic Aptima, and BD MAX, respectively.

BD ProbeTec™ CT/GC Qx Amplified DNA Assays – BD Molecular Diagnostics
BD ProbeTec™ Trichomonas vaginalis Qx Assay – BD Molecular Diagnostics
APTIMA COMBO 2 Assay – Hologic, Inc.
APTIMA Trichomonas Vaginalis Assay – Hologic, Inc.
BD MAX™ CT/GC/TV – BD Molecular Diagnostics

Table 1: PIS Algorithm

	NAAT1	Positive				Negative				Equivocal (EQ)*			
	NAAT2	**POS**	**EQ**	**NEG**	**INVALID**	**POS**	**EQ**	**NEG**	**INVALID**	**POS**	**EQ**	**NEG**	**INVALID**
NAAT3													
POS		**NA[a]**	**POS**	**POS**	**POS**	**POS**	**IND**	**NA**	**IND**	**POS**	**IND**	**IND**	**NA**
NEG		**NA**	**IND[b]**	**NEG**	**IND**	**NEG**	**NEG**	**NA**	**NEG**	**IND**	**IND**	**NEG**	**NA**
UNR[c]		**NA**	**IND**	**IND**	**IND**	**IND**	**IND**	**NA**	**IND**	**IND**	**IND**	**IND**	**NA**
INVALID[d]		**NA**	**IND**	**IND**	**IND**	**IND**	**IND**	**NA**	**IND**	**IND**	**IND**	**IND**	**NA**

[a]NA = not applicable and indicates that no tie-breaker test is required because the first two reference tests were in agreement**.**
[b]IND = indeterminate and indicates that the true patient status cannot be determined based on the results obtained from the three reference tests.
[c]UNR = unresolved.
[d]For the BD MAX CT/GC/TV assay (NAAT 3), indeterminate and incomplete test results as reported by the instrument should be interpreted as INVALID using this composite comparator algorithm.

The diagnostic variable of interest for this study is the binary infected/not infected findings for CT, NG, and TV on the Click Diagnostics Sexual Health Test as compared to PIS determined by existing comparator tests. The designation of a subject as being positive, negative**,** or indeterminate will be based on the combined results of the reference assay tests. A subject will be considered “Positive” where two reference assays are positive. A subject will be considered “Negative” where two reference assays are negative. If the results of the first two assays are discordant, the PIS will be determined by the third assay (tie-breaker). Any other potential combination of results will be considered an “Indeterminate” result. The testing methods utilized in the study are summarized in Table 2.

Table 2: Organism Type Testing Methods

Organism	Self-Collected/ Click Device	HCP-Collected/Comparator Methods		
	Vaginal Swab	Vaginal Swab/ NAAT 1	Vaginal Swab/ NAAT 2	Vaginal Swab/ NAAT 3 (Tie-breaker)
CT	X	X	X	X
NG	X	X	X	X
TV	X	X	X	X

2.2 Scientific Rationale

Click Diagnostics has developed a small, single-use, fully disposable point-of-care (POC) diagnostic device that is capable of being used to rapidly and accurately detect any form of infectious disease. Through dramatic miniaturization, optimization, and cost reductions, Click has reinvented PCR technology. Commercialization of this technology would dramatically expand the reach of molecular diagnostic testing, moving it from centralized test laboratories and large healthcare facilities to small clinics and outreach settings. The device in this clinical study focuses on detecting three of the most common sexually transmitted infections in women that cause significant morbidity.

Current laboratory-based STI tests have an average sample-to-result-to-patient timeline of 3 to 5 days. This is a significant problem as many patients are lost to follow up before the test result is available, and without treatment, patients may continue to spread the infections and risk complications such as pelvic inflammatory disease, infertility, and perinatal complications. Women could benefit from the Click device because they suffer the most serious consequences of STIs, which could be curtailed with single visit POC diagnosis and treatment.

The intended use of the Click device is for the qualitative detection and differentiation of DNA from CT, NG, and TV to aid in the diagnosis of chlamydial, gonorrheal, and trichomonas infections, respectively. The assay is to be used with vaginal swabs obtained by self-collection from asymptomatic and symptomatic females age 14 or older.

Note that females younger than 14 years will not be included in the study, as they may not be able to follow directions to provide a self-collected vaginal specimen.

3 HYPOTHESIS, OBJECTIVES, AND OUTCOME MEASURES

3.1 Hypothesis

The identification of each organism (*Chlamydia trachomatis* [CT], *Neisseria gonorrhoeae* [NG], and *Trichomonas vaginalis* [TV]) in self-collected vaginal swabs by women using the Click device will agree with the PIS with a high sensitivity and specificity.

3.2 Study Objectives

3.2.1 Primary Objective

To assess the performance of the Click device for detection of CT, NG, and TV in self-collected vaginal specimens as compared to PIS determined by three approved comparator assays using vaginal specimens collected by a qualified HCP in support of obtaining FDA clearance and a Clinical Laboratory Improvement Amendments (CLIA) Waiver.

3.2.2 Secondary Objectives

3.2.2.1 To assess the performance of the Click device for detection of CT, NG, and TV in self-collected vaginal specimens among symptomatic subjects as compared to PIS determined by three approved comparator assays using vaginal specimens collected by a qualified HCP.

3.2.2.2 To assess the performance of the Click device for detection of CT, NG, and TV in self-collected vaginal specimens among asymptomatic subjects as compared to PIS determined by three approved comparator assays using vaginal specimens collected by a qualified HCP.

3.2.3 Exploratory Objective

To assess the usability by non-laboratorian operators of the Click device for detection of CT, NG, and TV with subject self-collected vaginal specimens.

3.3 Study Endpoints and Outcome Measures

3.3.1 Primary Endpoint

The primary endpoint is the diagnostic accuracy reported as calculated point estimates for percent sensitivity and percent specificity along with their associated two-sided Wilson Score 95% Confidence Intervals (CIs) of the Click device as compared to PIS.

3.3.2 Secondary Endpoints

Secondary endpoints include calculated point estimates for percent sensitivity and percent specificity along with their associated two-sided Wilson Score 95% CIs of the Click device as compared to PIS reported separately for the symptomatic and asymptomatic sub-populations within the study cohort.

3.3.3 Exploratory Endpoints

3.3.3.1 Ease-of-Use questionnaire results from study operators at CLIA-waived sites will be summarized to assess the usability (ease of understanding of instructions, operations, and visual interpretation) of the Click device. In this guidance, intended study operator (user) refers to a test operator with limited or no training or hands-on experience in conducting laboratory testing (e.g., medical assistant, nurse, doctor, or an individual with no medical training).

3.3.3.2 Prevalence (reported as a percentage) of each infection will be reported across the entire study cohort and individually within the symptomatic and asymptomatic sub-populations. Sensitivity, specificity, and prevalence will be reported for each individual testing site.

3.3.4 Primary Outcome Measures

3.3.4.1 The i) percent sensitivity and ii) percent specificity of Click device for detection of *Chlamydia trachomatis* in self-collected vaginal specimens as compared to PIS using vaginal specimens collected by a qualified HCP.

3.3.4.2 The i) percent sensitivity and ii) percent specificity of Click device for detection of *Neisseria gonorrhoeae* in self-collected vaginal specimens as compared to PIS using vaginal specimens collected by a qualified HCP.

3.3.4.3 The i) percent sensitivity and ii) percent specificity of Click device for detection of *Trichomonas vaginalis* in self-collected vaginal specimens as compared to PIS using vaginal specimens collected by a qualified HCP.

3.3.5 Secondary Outcome Measures

3.3.5.1 The i) percent sensitivity and ii) percent specificity of Click device using self-collected vaginal swabs for detection of *Chlamydia trachomatis* from self-collected vaginal specimens as compared to PIS

using vaginal specimens collected by a qualified HCP among symptomatic subjects.

3.3.5.2 The i) percent sensitivity and ii) percent specificity of Click device for detection of *Neisseria gonorrhoeae* in self-collected vaginal specimens as compared to PIS using vaginal specimens collected by a qualified HCP among symptomatic subjects.

3.3.5.3 The i) percent sensitivity and ii) percent specificity of Click device for detection of *Trichomonas vaginalis* in self-collected vaginal specimens as compared to PIS using vaginal specimens collected by a qualified HCP among symptomatic subjects.

3.3.5.4 The i) percent sensitivity and ii) percent specificity of Click device for detection of *Chlamydia trachomatis* in self-collected vaginal specimens as compared to PIS using vaginal specimens collected by a qualified HCP among asymptomatic subjects.

3.3.5.5 The i) percent sensitivity and ii) percent specificity of Click device for detection of *Neisseria gonorrhoeae* in self-collected vaginal specimens as compared to PIS using vaginal specimens collected by a qualified HCP among asymptomatic subjects.

3.3.5.6 The i) percent sensitivity and ii) percent specificity of Click device for detection of *Trichomonas vaginalis* in self-collected vaginal specimens as compared to PIS using vaginal specimens collected by a qualified HCP among asymptomatic subjects.

3.3.6 Exploratory Outcome Measures

3.3.6.1 Usability will be measured by 5-point Likert scale responses, yes/no questions, and open-text questions from study operators on questions around three domains of interest: i) instructions, ii) operations, and iii) visual interpretation for the Click device for detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* with patient self-collected vaginal specimens.

3.3.6.2 The percentage of subjects with *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* overall and within the symptomatic and asymptomatic sub-populations.

4 STUDY DESIGN

This study is a multi-center study with a minimum of three CLIA-waived intended operator sites in the United States; approximately one-third of the total number of sites will have a patient population of low NG, CT, or TV prevalence. Low-prevalence sites are defined as sites with a prevalence ≤2% for any of the three targets. Sites with a prevalence higher than 2% for all of the three targets will be defined as high prevalence sites. The study will enroll approximately 1750 female subjects. The study will continue enrolling subjects until the targets for positive specimens are met (as outlined below). Approximately one-third of the total subjects shall be recruited from low-prevalence sites for the infections of interest.

Female subjects seen at the participating sites for any reason will be evaluated for enrollment in this study. All subjects will be managed per standard of care as applicable. Subjects who meet the inclusion/exclusion criteria will be offered enrollment into the study. Subjects who are enrolled in the study will provide clinical and demographic information, perform self-collection with a vaginal swab for the Click device, and allow the HCP to collect three additional vaginal swabs for the comparator methods. Subjects will complete the study in a single visit. The order of the swabs for comparator methods will be randomized per subject to account for the possibility that the yield of the swab is affected by previous swabs at the same anatomic site. The study specimens shall be collected according to institutional procedures as well as the manufacturer's instructions. The self-collected specimen shall be collected according to the Click self-collection instructions.

The study will include typical CLIA-waived collection and testing sites. At least nine different study operators among the study sites will perform the Click device testing, with each study operator testing a minimum of five positive and five negative specimens to support CLIA-waived status for CLIA categorization. Selected study operators should have non-laboratory background education and must not have certification for moderate or high-complexity testing to ensure representation of intended study operators to evaluate the use of the Click device.

Recruitment goals for the 510(k) phase of the study are at least 100 specimens positive for CT, 100 specimens positive for TV, and 45 specimens positive for NG as determined by PIS. Recruitment goals for the CLIA waiver phase of the study are 120 positive specimens for CT and TV (prospective), and 45 positive specimens for NG. In order to achieve the required precision for sensitivity of NG detection, additional specimens may be required. Banked NG positive specimens may also be used and will be provided to the testing sites for Click device testing. Thus, the maximum target for the number of PIS-positive NG samples is 85. At specified enrollment targets, the sensitivity of the Click device with respect to NG will be estimated. If the NG estimate meets the requirements for the 510(k) and CLIA waiver phases at any of the interim points and the enrollment targets for CT and TV have been met, collection of samples will stop. Otherwise, collection of samples will continue. See section 7 for details on the interim reviews. At least 30% of total samples must be from sites considered low prevalence. All comparator tests will be performed by a reference laboratory.

5 STUDY POPULATION, ENROLLMENT, AND DISCONTINUATION

5.1 Selection of the Study Population

The study population comprises female subjects ≥14 years of age visiting any of the participating clinics for any reason, who may be symptomatic or asymptomatic for STIs at locations including but not limited to: OB/GYN and primary care offices, as well as sexually transmitted disease, teen, public health, and family planning clinics. Any subject who meets the inclusion/exclusion criteria is eligible to participate in this study. No exemptions are granted on Subject Inclusion and Exclusion Criteria in Division of Microbiology and Infectious Diseases (DMID)-sponsored studies. Subject Inclusion and Exclusion Criteria must be assessed by a study clinician licensed to make medical diagnoses and designated by the Investigator of Record.

5.1.1 Minimum information required for each subject

- Date of birth
- Sex at birth
- Sexual health and reproductive history within 7 days prior to enrollment
- Recent (within 7 days prior to enrollment) genital tract infections or vaginal discharge
- Pregnancy status (self-report or results), if available
- Use of concomitant medications or genital products within 7 days prior to enrollment

5.1.2 Symptomatic and asymptomatic subject status

- Subjects will be classified as symptomatic if they report any of the following symptoms within 7 days prior to enrollment: change in vaginal discharge, abnormal bleeding/spotting, lower abdominal pain/pelvic pain, painful urination, increased urinary frequency, vaginal irritation such as itching, burning and/or soreness, pain or bleeding with sex/intercourse.
- Subjects will be classified as asymptomatic if they do not report any of the above symptoms.

5.2 Inclusion Criteria

Subjects must meet all of the following inclusion criteria in order to be eligible to participate in this study:

1. Willing and able to give voluntary written informed consent (or the parent/legal guardian will provide parental permission) before any study-related procedure is performed.
2. Female at birth. (Pregnant and breastfeeding women are eligible.)
3. Age ≥14 years at the time of enrollment.
4. Able to read and understand the procedural information provided for the study.

5. Able and willing to follow all study procedures, including performing self-collection of one vaginal swab and permitting a licensed HCP to collect three additional vaginal swabs.

5.3 Exclusion Criteria

Subjects meeting any of the following criteria at enrollment will be excluded from this study:

1. Have a medical condition, serious intercurrent illness, or other circumstance that, in the Investigator's judgment, could jeopardize the subject's safety, or could interfere with study procedures.
2. Enrollment in this study previously.
3. Use of antiperspirants and deodorants or the following vaginal products: douches, washes, lubricants, vaginal wipes, vaginal moisturizers, or feminine hygiene spray in the genital area, within 48 hours prior to enrollment.

5.4 Eligibility Criteria for Banked Specimens Obtained from Known Positive NG Subjects

Frozen NG banked specimens may be used and will be provided to the testing sites for Click testing as per the criteria in section 4. Positive NG-known subjects providing banked vaginal specimens should meet the following inclusion and exclusion criteria:

5.4.1 Inclusion Criteria

1. Vaginal specimen is from a female age ≥14 years at the time of specimen collection.

2. A minimum of 2.0 mL of frozen sample taken from well-characterized specimens collected from known NG subjects not being treated since testing positive within the past 4 months.

3. NG comparator results available.

4. Vaginal specimen is obtained from a collection wherein the subject consented/assented to future unspecified research use of her specimen.

5.4.2 Exclusion Criteria

1. Previously enrolled in this study.

5.5 Subject Recruitment, Screening, and Enrollment

Subjects arriving at the study sites for any reason who may meet the study inclusion/exclusion criteria will be evaluated for enrollment in this study. It is each site's responsibility to enroll only subjects who satisfy the inclusion/exclusion criteria and to obtain parental/legal guardian permission when required. Sites must adhere to local

policies and regulations that govern enrollment of either minors or pregnant women.

Sites may use any system for recruitment and enrollment that meets the study objectives and is IRB-approved. Recruitment can be by word-of-mouth, flyers, advertisement online, or any other means, subject to approval by the associated IRB. Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents and recruitment materials by the IRB.

5.6 Randomization

Each subject will be randomized to a particular order of HCP swab collection, allocating subjects equally to each of the possible swab orders. The list of randomized assignments will be prepared by statisticians at the Statistical and Data Coordinating Center (SDCC). Each subject's randomization assignment for the order of swab collection will be documented. Details of the randomization process is further specified in the Manual of Procedures (MOP).

5.7 Subject Discontinuation

Subjects are considered enrolled in this study once they meet all inclusion/exclusion criteria and sign an informed consent and assent form, as applicable. It is recommended that every subject remain in the study until completion of all study procedures; however, a subject's participation in the study may be discontinued. Should this occur, the reason for discontinuation must be documented in the source documentation. Reasons for discontinuation may include, but are not limited to, the following:

- *Subject withdrawal*: Subject participation in this clinical study is voluntary and the subject may discontinue participation at any time without loss of benefit or penalty.
- Investigator termination: Investigator may terminate the subject's participation without the subject's consent if the Investigator believes it is medically in the subject's best interest.
- *Adverse events (AEs)*: Investigator may terminate the subject's participation for reasons of adverse events associated with study procedures.
- *Termination of study*: Although the study Sponsor has every intention of completing the study, the Sponsor reserves the right to terminate the study at any time for clinical or administrative reasons. Reasons for termination include, but are not limited to, study closure at the discretion of DMID.
- *Other*: Subject may be terminated without the subject's consent for various other reasons including, for example, Sponsor's decision.

6 STUDY PROCEDURES/EVALUATIONS

6.1 Site Selection and IRB Approval

At least three geographically diverse clinical sites will collect vaginal swab specimens for the study and perform testing using the Click device. At least one reference laboratory will test the specimens obtained by the collection sites with the comparator methods. Each clinical site shall be assessed by the Sponsor, or a representative designated by the Sponsor prior to the start of the study, to ensure that the proper procedures, equipment, and personnel are in place to conduct the clinical study in accordance with the approved protocol, and that the site abides by the principles of good clinical practice (GCP) and in compliance with the principles enunciated in the Belmont Report.

All study procedures must be performed at an investigational site, under the direction of a principal investigator (PI) or designee, and at which this protocol has been approved by an IRB or IEC. Any amendments to the protocol or consent materials must also be approved before they are placed into use. No subject's personally identifiable information (PII) will be provided to the Sponsor. Additional sites may be included to meet sample size and testing requirements.

6.2 Training

Site personnel will be trained prior to performing any study procedures. All investigators, co-investigators, and site personnel involved in the study will be required to attend a training session, which may be conducted at a site initiation visit (SIV) by Sponsor personnel or by an agent of the Sponsor.

Training includes the protocol, case report form (CRF) completion, study personnel responsibilities, specimen documentation, data recording, and reporting. All investigators or study personnel who are trained will sign a training log. No investigator or study personnel will perform any study-related procedures prior to completion and documentation of required training.

Study operators of typical CLIA-waived testing sites will receive no additional instructions (e.g., written or verbal training, coaching, or prompting) beyond providing them with the Click Quick Start Instructions. Operators will not know the SOC results of the subject's specimens. Operators will be instructed not to discuss the test with other operators or otherwise coach or observe each other. Study operators will also be instructed not to discuss the test with clinic staff.

6.3 Visit 1: Eligibility, Enrollment, and Vaginal Swab Collection and Testing

The investigator or designee will assess potential subjects and ascertain suitability to qualify for the study based on the inclusion and exclusion criteria. The potential subject will be fully informed of the study design and procedures. If the subject is interested in participating, written informed consent from the adult subject or from the parent/legal guardian as well as assent from a minor will be obtained as applicable. After the

inclusion and exclusion criteria are satisfied and informed consent (or assent) has been provided, subjects will be considered to be enrolled into the study. All subjects meeting the eligibility criteria shall be included, without further selection, until the required sample sizes are achieved. A subject can be enrolled only once in the study. Each clinical site is required to keep track of potential subject screening and enrollment using a Screening and Enrollment Log. All subjects who complete the informed consent process will be assigned a unique subject ID number from the Screening and Enrollment Log that will be used to identify the specimens during the study. The duration of the study for each eligible and enrolled subject will be one visit, which will last about 60 minutes. Study enrollment is expected to be completed in approximately 9 months or until evaluable sample size targets are met. The following will be performed for subjects who give informed consent:

6.3.1 Study Procedures for Symptomatic and Asymptomatic Subjects

1. Following informed consent, collect subject demographic and complete relevant sections of the CRF.
2. Determine eligibility and subject enrollment.
3. Collect sexual health and reproductive history.
4. Collect subject-reported symptoms that occurred within 7 days prior to enrollment.
5. Collect and review any medications or supplements taken within 7 days prior to enrollment.
6. Collect all genital products (including deodorant or antiperspirant) used within the last 7 days.
7. If the clinic has a rule regarding SOC specimen collection, then the site may abide by that rule.
8. Provide subject with the Click Self-Collection Instruction Sheet, self-collection materials, and answer any questions the subject may have.
9. Subject will perform one (1) self-collection of a single vaginal swab. The specimen will be tested on the Click device. The self-collection will be performed first in a private setting; the subject will collect the vaginal swabs according to the Click Self-Collection Instructions. The subject will place the self-collected vaginal swab in its own swab transport tube following collection.
    a. NOTE: The self-collected vaginal swab will be tested by study operators.
    b. NOTE: All used Click devices will be shipped to Click Diagnostics at intervals defined in the MOP.
10. HCP will perform three (3) vaginal swab collections for comparator devices according to the order generated from the randomization assignment.
    a. NOTE: Specimens should be taken without the use of a speculum. Using a speculum to collect specimens will not be considered a protocol deviation; however, it must be recorded on the CRF, along with the name of the lubricant, if applicable.
11. Record any adverse events on the CRF.

12. Study operators and HCPs will process collected swabs in the collection media as appropriate. Click specimen must be tested on the Click device within two (2) hours of collection. The three (3) vaginal specimens for comparator tests will be processed and prepared for shipment/transport to a reference laboratory.
13. Study operators will test the specimens according to the Quick Start Instructions. The study operator will take a photograph of the Click device upon completion of test (camera or equivalent) and record results on the appropriate CRF.
14. Click specimens shall be stored frozen at -20^{o}C or below after testing is complete**.** Leftover self-collected specimens will be shipped to Click Diagnostics at intervals defined in the MOP.
15. HCP-collected vaginal swabs will be collected, transported, and tested according to the manufacturer's instructions at a reference laboratory designated by the Sponsor. All vaginal swab specimens shall be stored according to the MOP.
16. The remaining HCP-collected vaginal liquid media will be stored according to the MOP at the reference laboratory once all testing is performed. The disposition of those remaining HCP-collected vaginal liquid media samples may be shipped per DMID instructions.

6.4 Controls

One external positive control (positive for all infections) and one negative control (negative for all infections) will be run at each investigational site each month of testing and with each new shipment or lot of the Click device to monitor the performance of the Click device, or to qualify a new study operator. The external controls for the comparator testing will be assayed in accordance with the laboratories' standard operating procedures.

The study operator should follow the Click Quick Start Instructions to prepare and test the controls, and ensure the controls give a valid result prior to testing subject specimens. If any of the controls gives an "ERROR"**,** "INVALID"**,** or "INCORRECT" (negative control gives a positive result or positive control gives a negative result), as defined in the Quick Start Instructions, the failed control shall be retested using a new device and a new external control vial. Retests will be labeled according to the MOP. All specimens and retest results will be provided to the Sponsor according to the MOP.

If both control results are valid, the study operator shall proceed with testing subject specimens. If the retest does not give a valid result, call the Click representative (see contact information in the MOP).

6.5 Selection of Study Operators at CLIA-Waived Sites

Prior to study start, the following information will be collected for each study operator participating in the study:

- Education level
- Certification status, if applicable

- Employment status at location (full-time, part-time, PRN, etc.)
- Title at location
- Years of employment at location at current title and previous titles, if applicable
- Summary of daily duties at location

All study operators (chosen and not chosen) identified by the PI will be documented on a List of Potential Study Operators form. A reason for the study operators not chosen shall be provided.

Any new chosen study operators will run a set of controls and document the results on a self-training record form. The study operators will receive no help other than using the Quick Start Instructions as part of the test system.

At the conclusion of the study, each study operator will complete the Click Ease-of-Use Questionnaire containing 5-point Likert Scale questions and yes/no questions designed to assess usability of the Click device.

6.6 Click Diagnostics Sexual Health Test

The Click devices utilized in this study represent R&D manufactured devices. The devices will carry the label “For Investigational Use Only. The performance characteristics of this product have not been established.”

6.7 Click Device Testing

The Click device testing will be performed by study operators according to the Quick Start Instructions. If the initial Click test result is “INVALID” or “ERROR”, a single retest will be performed according to the Quick Start Instructions, provided enough specimen is available for retesting. Retest will be labeled according to the MOP. All specimen test results and retest results will be provided to DMID according to the MOP.

A valid result (e.g., positive for chlamydia) obtained upon retesting will be compared to the PIS result and classified as a true or false result. Specimens that remain unresolved after one retest will be recorded as such.

The number and percentage of the Click device test that produce an “INVALID” or “ERROR” result will be tallied by the site per Click device overall and reported.

6.7.1 Device Accountability

Click Diagnostics will provide the Click devices.

Each clinical site will be responsible for documenting the receipt, storage, use, and disposition of Investigational Use Only devices throughout the study. Records must be available for inspection by the DMID monitoring contractors. Used devices will be returned to Click per the MOP. The Monitors will not physically check the used Click devices. They may be returned prior to

monitoring of the device accountability logs, but must be logged according to the MOP. At the discretion of Click, remaining unused study supplies provided by Click may be returned at the conclusion of the study.

6.8 Study Procedures for Click Device Testing of Banked Specimens Collected from Known NG-Positive Subjects

If additional NG+ samples are needed, Click will provide banked specimens to the clinical sites according to the MOP.

6.9 Duplicate Subject Enrollment

If a subject is accidentally enrolled twice, the first result will be used in the analysis. The result from the second enrollment will be excluded from analysis.

6.10 Laboratory Evaluations for Comparator Methods

6.10.1 Laboratory Evaluations/Assays

Specific instructions for specimen preparation, handling, and storage are described in the MOP.

6.10.2 Specimen Collection, Preparation, and Handling

Specimens will be collected and handled for comparator testing by HCPs according to manufacturer's instructions/package insert. Handling of all specimens will be described in the MOP.

6.10.3 Instructions for Specimen Storage and Shipping

Shipping of clinical vaginal swab specimens for comparator testing will be performed according to the manufacturer's instructions. Shipping and storage of all specimens will be described in the MOP.

6.10.4 Instructions for Comparator Testing

Comparator testing will be performed according to the manufacturer's instructions. If the initial comparator test result for any of the three target organisms is "INVALID", "ERROR", "EQUIVOCAL", "INDETERMINATE", or "UNRESOLVED", a single retest will be performed according to the manufacturer's instructions, provided enough specimen is available for retesting. Retest will be labeled according to the MOP. All comparator test results and retest results will be provided to DMID according to the MOP.

A valid result (e.g., positive for chlamydia) obtained upon retesting will be used to determine the PIS result and classified as a true or false result. Specimens that remain unresolved after one retest will be recorded as such.

7 STATISTICAL CONSIDERATIONS

7.1 Study Hypothesis

This study will assess the ability of the Click device to identify each of the CT, NG, and TV organisms. The device's ability to identify each organism will be measured by its sensitivity and specificity compared to the PIS. The use of sensitivity and specificity assumes that PIS is the "true" status of each subject and indicates whether each organism is truly present or absent. Thus, the primary measures of interest are the device's true positive (correctly identifying the presence of the organism) and true negative rates (correctly identifying the absence of the organism). Formal hypothesis testing will not be performed; instead, the study is designed to estimate the device's sensitivity and specificity with a certain amount of precision (see next section).

7.2 Sample Size Considerations

Recruitment goals for the 510(k) phase of the study are at least 100 specimens positive for each of CT and TV, and at least 45 specimens positive for NG, as determined by PIS. Recruitment goals for the CLIA waiver phase of the study are 120 positive specimens for each of CT, TV, and 45 NG. Approximately 585 subjects will be recruited from low-prevalence sites (one third of the 1,750) and approximately 1165 subjects will be recruited from high prevalence sites. The average expected proportion of samples positive via PIS for CT, TV, and NG among subjects enrolled at the high prevalence sites are, respectively, 12.4%, 19.5%, and 8.1%. The corresponding estimates for the low-prevalence sites are 1.7%, 1.0%, and 1.4%. Based on these prevalence estimates, it is expected that the enrollment targets will be met for the three organisms. Thus, the study as designed is expected to meet the 510(k) and CLIA waiver recruitment goals for all three organisms. If NG prevalence is lower than expected, banked NG+ samples may need to be leveraged to meet the recruitment goals for NG. As indeterminate PIS results or invalid or failed Click device results are expected for a small number of cases, enrollment may extend beyond 1750 to replace these results. The duration of enrollment may be extended if the actual prevalence of any of the organisms is lower than predicted.

For the estimation of the sensitivity and specificity of the Click device, we assume both the sensitivity and specificity is at least 95%. For 510(k) clearance, the study aims to demonstrate that sensitivity to each of CT and NG is at least 95%, with a lower bound of the two-sided 95% Wilson Score confidence intervals at least 90%, and to demonstrate that sensitivity to TV is at least 95%, with a lower bound of the two-sided 95% Wilson Score confidence intervals at least 85%. Regarding specificity, the study aims to demonstrate specificity to each of CT, TV, and NG is at least 95%, with a lower bound of the two-sided 95% Wilson Score confidence intervals at least 90%. For the CLIA waiver, the study aims to demonstrate that both the sensitivity and specificity to each of CT, TV, and NG is at least 95%, with a lower bound of the two-sided 95% Wilson Score confidence intervals at least 89%.

To demonstrate this for CT and TV, 95% Wilson Score confidence intervals were calculated for a range of sample sizes, number of PIS-positive samples, and sensitivity/specificity estimates. Given the uncertainty in the exact number of subjects enrolled or PIS-positive samples, the following parameter ranges were explored: total sample sizes of 1500 to 2000, 100 to 200 PIS-positive samples, and sensitivity/specificity estimates of 95% to 99%. Wilson Score confidence intervals were calculated for each combination of sample size, number of PIS-positive samples, and sensitivity/specificity. As an example, Table **3** below displays the lower confidence bound using a sample size of 1750 and a select subset of the number of PIS-positive samples.

Table **3**: Lower 95% Wilson Score Confidence Bounds (%) for Various Number of PIS-Positive Samples and Sensitivity and Specificity Values

Number of PIS-Positive Samples	**Sensitivity (%)**					**Specificity (%)**				
	95	**96**	**97**	**98**	**99**	**95**	**96**	**97**	**98**	**99**
100	88.8	90.2	91.5	93.0	94.6	93.9	94.9	96.1	97.2	98.4
101	88.9	90.3	91.6	93.1	94.6	93.9	95.0	96.1	97.3	98.4
102	89.0	90.3	91.7	93.1	94.7	93.9	95.0	96.1	97.3	98.4
103	89.1	90.4	91.8	93.2	94.7	93.9	95.0	96.1	97.3	98.4
104	89.2	90.5	91.9	93.3	94.8	93.9	95.0	96.1	97.3	98.4
105	89.3	90.6	91.9	93.3	94.8	93.9	95.0	96.1	97.3	98.4
106	89.4	90.7	92.0	93.4	94.8	93.9	95.0	96.1	97.3	98.4
120	89.5	91.7	92.9	94.1	95.4	93.9	94.9	96.1	97.2	98.4
121	89.6	91.8	93.0	94.2	95.5	93.9	94.9	96.1	97.2	98.4
122	89.7	91.9	93.0	94.2	95.5	93.9	94.9	96.1	97.2	98.4
123	89.8	91.9	93.1	94.3	95.5	93.9	94.9	96.1	97.2	98.4
124	89.8	92.0	93.1	94.3	95.6	93.9	94.9	96.1	97.2	98.4
125	89.9	91.0	93.2	94.4	95.6	93.8	94.9	96.1	97.2	98.4
126	90.0	91.0	93.2	94.4	95.6	93.8	95.0	96.1	97.2	98.4

Over the entire ranges of parameter values explored, the lower bound of the specificity confidence interval never fell below 93.6%. The lower bound of the sensitivity confidence intervals never fell below 88.8% and was greater than or equal to 89% for all combinations of parameter values except for the following scenario:

- Sample Size = Any, Number of PIS-positive samples = 100 or 101, Sensitivity = 95%

The lower bound of the sensitivity confidence intervals was greater than or equal to 90% for all combinations of parameter values except for the following scenarios:

- Sample Size = Any, Number of PIS-positive samples = 100 to 112, Sensitivity = 95%
- Sample Size = Any, Number of PIS-positive samples = 120 to 126, Sensitivity = 95%

Thus, apart from a few scenarios, assuming the sensitivity/specificity of the Click device is at least 95%, the study as planned should be able to meet the confidence interval estimate requirements for 510(k) clearance and CLIA waiver for CT and TV.

For NG, the minimum number of PIS-positive samples required for the 510(k) and CLIA Waiver reports is 45. The planned maximum sample size for NG-positive samples is 85. Due to the small 510(k) and CLIA Waiver enrollment targets for NG and the difficulty in recruiting NG-positive subjects, reviews of the sensitivity of the Click device with regard to NG detection will occur at specified enrollment targets to ensure adequate precision of the estimate is achieved while allowing for the collection of samples to stop early if further NG sample collection is not necessary and the enrollment targets for CT and TV have already been met.

The first enrollment target at which sensitivity will be estimated is 45 PIS-positive NG samples. If the 510(k) and CLIA Waiver estimate requirements are met and the enrollment targets for CT and TV have already been met, then collection of NG samples will stop. If the estimate requirements are not met, then collection of samples may proceed to the next enrollment target, as described in Table 4.

Note that, per Table 5 in section 7.3, an interim report will be provided to the FDA after the collection of 50 PIS-positive CT samples, 50 PIS-positive TV samples, and 20 PIS-positive prospectively collected NG samples. The results presented in this interim report will not impact the operations of the study.

Table 4: NG Sensitivity Estimates for Various Interim Sample Sizes

Enrollment Target (Number of PIS-positive NG samples)	Number of False Negatives	Sensitivity Estimate (Lower 95% confidence bound)
45	0	100% (92.1%)
	1	97.8% (88.4%)
	2	95.6% (85.2%)
	3	93.3% (82.1%)
	>3	≤91.1% (≤79.3%)
55	1	98.2% (90.4%)
	2	96.4% (87.7%)
	3	94.5% (85.2%)
	>3	≤92.7% (≤82.7%)
70	2	97.1% (90.2%)
	3	95.7% (88.1%)
	>3	≤94.3% (≤86.2%)
85	3	96.5% (90.1%)
	4	95.3% (88.5%)
	>4	≤94.1% (≤87.0%)

Assessment of the specificity requirement for NG follows similarly to our investigation of specificity for CT and TV above; the study as planned should be able to meet the confidence interval estimate requirements for 510(k) clearance and CLIA waiver for NG.

7.3 Planned Interim Reports

Interim reports may be generated during the course of the study. The reports will only be made available to the Sponsor team and Click Diagnostics for the purpose of submission to the FDA. The study operators and laboratory staff that run the assays will not have access to the reports. The study statistician will provide the reports, which will contain summaries from analyses outlined in section 7.4 and detailed in the separate Statistical Analysis Plan.

The reports will be generated after the data are entered in the database; the approximate milestones for the generation of each report are provided in Table 5 below:

Table 5. Types of Study Reports and Schedule

Report Type	Milestone
Interim Report[1]	After the collection of 50 PIS-positive CT samples, 50 PIS-positive TV samples, 20 PIS-positive prospectively collected NG samples
510(k) Report	After the collection of 100 PIS-positive CT samples, 100 PIS-positive TV samples, ≥45 PIS-positive prospectively collected NG samples
CLIA Waiver Report	After the collection of 120 PIS-positive CT samples, 120 PIS-positive TV samples, ≥45 PIS-positive prospectively collected NG samples

[1]The need for this interim report depends on the rate of enrollment of subjects with PIS-positive NG samples. The report will be generated if it is possible to do so without halting enrollment and/or losing momentum in accrual or if the rate of enrollment of subjects who are PIS-positive NG is such it is expected that multiple NG milestones will not be met in a short period of time.

It is not anticipated that the study or its operations will be impacted by the analyses performed for the interim report (e.g., early termination of the study), but instead the report fulfills a regulatory requirement and provides the FDA an update on the progress of the trial. As such, adjustments for the multiple interim calculations of interval estimates are not planned.

The 510(k) and CLIA Waiver reports will be generated once the NG enrollment has finished per the enrollment procedure outlined in section 7.2. Though it is possible that NG sensitivity will be estimated multiple times, no adjustments for the multiple calculations are planned.

7.3.1 Ongoing Investigative Product Performance Tracking

The primary analysis of agreement of Click Sexual Health Test results compared to PIS will be performed as described in section 7.4.3 and as shown in the example 2 x 2 contingency table depicted in Figure 6 of this protocol on an ongoing basis during the course of the study. These analyses will track performance of the investigative device and thereby serve to inform the Sponsor team and Click Diagnostics as to the likelihood of successful completion of the study with performance conforming to acceptance criteria, or conversely, to eventual futility of the study for failure to achieve acceptable performance. In no case shall the results of these ongoing analyses initiate an early termination of the study for

benefit, i.e. based upon early achievement of acceptable performance.

In accord with DMID Policy, analysis results shall be distributed only to the Sponsor team and to Click Diagnostics and the receiving personnel shall not further distribute the results. No results shall be supplied to study site personnel or to study monitors, and neither the raw data nor the analysis of these ongoing analyses shall be submitted to FDA. Study updates will only be provided to the FDA at timepoints listed in Table 5 above. The results of these ongoing analyses shall not translate into study design changes, protocol amendments, or investigative device design changes. As such, no adjustments for the multiple analysis will be made.

7.4 Analysis Plan

A separate statistical analysis plan document will be generated that will contain the details of all interim and final analyses. This section outlines the major components of all analyse*s*.

7.4.1 Analysis Populations

The following analysis populations will be defined separately for each report (Interim, 510(k), CLIA Waiver). The statistical analysis plan will include the further details on the definitions of the report-specific analysis populations.

Enrolled Population: This population includes all participants enrolled at the time of the data cut-off for the report.

Evaluable Population: This population includes all samples available at the time of the data cut-off for the report, which provide an evaluable Click Diagnostics Sexual Health Test result and an evaluable PIS result for each tested pathogen (CT, NG, TV). There will be a separate Evaluable population for each organism.

A sample provides an evaluable result if both the Click device and the PIS provide a valid positive or negative result.

The primary outcome measure of sensitivity and specificity of the Click Diagnostics Sexual Health Test will be evaluated in the evaluable population.

7.4.2 Baseline Characteristics and Subject Disposition

Baseline and demographic characteristics and subject disposition, including but not limited to the number of subjects who contributed vaginal swabs and reasons for withdrawn samples, will be summarized overall and by clinical site. For both continuous and categorical variables, appropriate summary statistics will be applied. For continuous variables, descriptive statistics will include the number of non-missing values, mean, standard deviation, median, minimum, and maximum. For categorical variables, descriptive statistics will include counts and percentages per category.

7.4.3 Primary and Secondary Analyses

Sample proportions of subjects who are PIS-positive for each of the three organisms among all enrolled subjects and by symptomatic status will be estimated overall and by clinical site. Point estimates and 95% Wilson Score confidence intervals will be generated.

Table 6 below presents a 2 x 2 contingency table displaying the potential performance outcomes for the Click device in comparison to PIS, Where TP = Number of True Positives, TN = Number of True Negatives, FP = Number of False Positives, and FN = Number of False Negatives.

Table 6: 2 x 2 Contingency Table for Click Diagnostics versus PIS

Click Diagnostics Sexual Health Test	PIS: Infected	PIS: Not Infected
Infected	TP	FP
Not Infected	FN	TN

Sensitivity, specificity, positive predicted value (PPV), and negative predicted value (NPV) will be estimated along with their 95% Wilson Score confidence interval. The following formulae will be used to estimate the performance characteristics:

- Sensitivity (%) = TP/(TP + FN) * 100
- Specificity (%) = TN/(TN + FP) * 100
- PPV (%) = TP/(TP + FP) * 100
- NPV (%) = TN/(TN + FN) * 100

The above estimates, along with the number of true/false positives/negatives, will be calculated overall, by clinical site, and by symptomatic status. Subject listings of test results will be generated.

7.4.4 Exploratory, Ancillary, and Other Analyses

Responses to individual items on the Ease-of-use questionnaire will be summarized. For each item on the questionnaire, the proportion of study operators who select each level of the response will be summarized. In addition, the distribution of each item's responses (e.g., mean, standard deviation, median, range) will be calculated.

Multiple ancillary analyses, which support the primary and secondary analyses, will be performed and included in the interim and final reports. The statistical analysis plan will include full details of the planned analyses; below are high-level summaries of the analyses.

The number of invalid or missing results from and failed tests of the Click device will be summarized overall and by clinical site. The different combinations of results among the PIS assays will be tabulated overall, by organism, and by symptomatic status.

Using the observed sensitivity and specificity of the Click device, hypothetical PPV and NPV values will be calculated across a range of hypothetical prevalence rates given the following formulae:

- PPV = sens * p / [Sens * p – (1 – spec)*(1 - p)] * 100
- NPV = spec * (1 – p) / [(1 – sens) * p – spec*(1 - p)] * 100

Where Sens = Sensitivity, Spec = Specificity, and p = prevalence.

Additional supplemental and exploratory analyses, including sensitivity analyses, which assess the impact of indeterminate/invalid/failed/missing results on the analyses, may be performed and will be specified in the statistical analysis plan.

8 ASSESSMENT OF SAFETY

8.1 Risks/Benefits and Adverse Events Reporting

No adverse reactions are anticipated in this study, and the study procedures present no anticipated risks beyond the low risks of vaginal swab collection, i.e., risk of pain, discomfort, and/or vaginal bleeding. It is anticipated that the participants may experience discomfort when vaginal swab specimens are being collected; however, the risks and discomfort in this study are not greater than those ordinarily encountered during the routine collection of the vaginal swabs at a clinician's office. There are no known risks to operators while handling the device. All adverse events (AEs) should be recorded by the investigator. An AE is any untoward medical occurrence in a patient or clinical investigation subject using an investigational device and that does not necessarily have a causal relationship with this device. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational device, whether or not related to the investigational device. The Sponsor will collect AE information via the CRF for the clinic visits. AEs will be collected from the time of swab collection to the time that the subject leaves the clinic. Such data collection will include the start and stop dates of the event, causality with the investigational device (not related, related), outcome (ongoing, recovered, recovered with sequelae, not recovered, fatal), code for seriousness (SAE, UADE, not serious), and intensity (mild, moderate, or severe). The definitions of mild, moderate, or severe are as follows:

1 = Mild (awareness of a symptom but the symptom is easily tolerated)
2 = Moderate (discomfort enough to cause interference with usual activity)
3 = Severe (incapacitating; unable to perform usual activities; requires absenteeism or bed rest)

Unanticipated adverse device effects (UADEs) means "any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects" (21 CFR § 812.3). Adverse events of sufficient intensity and causality to the investigational device to meet the definition of a UADE will be investigated by the Sponsor as outlined in 21 CFR § 812.46(b) and reported as outlined in 21 CFR § 812.150. The Sponsor shall report the results of the UADE investigation to FDA and to all reviewing IRBs and participating investigators within 10 working days after the Sponsor first receives notice of the effect. Thereafter, the Sponsor shall submit such additional reports concerning the effect as FDA requests.

A serious adverse event (SAE; experience) or reaction is any untoward medical occurrence that: results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect. SAEs must be recorded by the investigator.

This study is performed entirely in vitro, and the result of the Click device and the study reference tests will not be used for patient management and will not be provided to health care providers or subjects. Therefore, there are no direct benefits to subjects who participate in this study. There may be benefits to future patients in the application of the Click Diagnostics Sexual Health Test in clinical care.

8.2 Reporting for Studies

Any AEs will be collected from the first study enrollment through study completion. The site investigator is responsible for documenting and reporting all AEs that are reported during the study, regardless of their relationship to study procedures.

All SAEs will be followed to adequate resolution or stabilization. Resolution of an SAE is defined as the return to pre-enrollment status or stabilization of the condition with the expectation that it will remain chronic. Time of resolution/stabilization of the event will be collected for device procedure-related SAEs.

The Sponsor will notify all participating site PIs (i.e., all PIs to whom the Sponsor is providing study device) in a safety report of serious risks from clinical trials or any other source, as soon as possible, but in no case later than 10 working days after the Sponsor is notified of the UADE or SAE determines that the information suggests a causal relationship or association with the study device. Relevant follow-up information to a safety report will be provided as soon as the information is available.

The investigator must report SAEs to the Sponsor, the reviewing IRB, and FDA within 24 hours after the investigator first learns of the event.

9 CLINICAL MONITORING

9.1 Site Monitoring Plan

Site monitoring is conducted to ensure that the human subject protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet Sponsor requirements, ICH/GCP guidelines, and applicable regulations, and that the study is conducted in accordance with the protocol, protocol-specific MOP, and applicable Sponsor standard operating procedures. DMID, the sponsoring agency, or its designee, will conduct site-monitoring visits as detailed in the clinical monitoring plan.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, electronic case report forms (eCRFs), informed consent forms, source documents necessary to support the documentation entered into the CRFs, and protocol and GCP compliance. Site monitors will have access to the study site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with site PIs to discuss any problems and actions to be taken and will document visit findings and discussions.

10 DATA HANDLING AND RECORD KEEPING

The site PI is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. Data collection forms will be derived from the eCRFs and provided by the SDCC to the site to record and maintain data for each subject enrolled in the study as a source document. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Permanent ink is required to ensure clarity of reproduced copies. When making a change or correction, the original entry should be crossed out with a single line, and the change should be initialed and dated. Do not erase, overwrite, or use correction fluid or tape on the original.

The DMID and/or its designee will provide guidance to investigators on making corrections to the source documents and eCRF.

10.1 Data Management Responsibilities

It is the responsibility of the PI to ensure that all team members handle data and related documentation appropriately. All non-operator completed data collection forms must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. The Click study operator's interpretation of Click test results should not be discussed with the operators nor should the operator's interpretation of Click test results be changed after the assessment has been completed (see MOP). Data collection is the responsibility of the study staff at the site under the supervision of the site PI. During the study, the site PI must maintain complete and accurate documentation for the study.

The SDCC for this study will be responsible for data management, quality review, analysis, and reporting of the study data. The SDCC will implement quality control (QC) procedures beginning with the data entry system and generate data QC checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for prompt clarification and resolution.

Data Capture Methods

All clinical (including, but not limited to, medical and sexual history, symptom status) and endpoint data will be entered into a 21 CFR 11-compliant Internet data entry system provided by the SDCC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the data collection forms/source documents completed by the study personnel.

10.2 Types of Data

Data for this study will include clinical, laboratory, demographic, behavioral variables, and outcome measures, including the operator questionnaire.

10.3 Timing/Reports

A final report will be prepared following the availability of all the clinical and laboratory data. During the course of the study, interim statistical reports may be generated and made available to DMID and Click Diagnostics to submit to the FDA.

10.4 Study Records Retention

Study records and reports, including, but not limited to, CRFs, source documents, informed consent forms (except for future use informed consent forms), laboratory test results, and device inventory records, shall be retained for 2 years after the last marketing application is approved for the device and until there are no pending or contemplated marketing applications. If an application is not approved for the device, study documents should be retained at least 2 years after clinical development of the device for investigational use is discontinued and the FDA has been so notified. The site must contact DMID for authorization prior to the destruction of any study records. No records will be destroyed without the written consent of the Sponsor. It is the responsibility of the Sponsor to inform the investigator when these documents no longer need to be retained. Informed consent forms for future use will be maintained as long as the sample exists.

10.5 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or MOP requirements. The noncompliance may be on the part of the subject, the investigator, or the study personnel. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. These practices are consistent with ICH E6: 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3 as well as 5.1, Quality Assurance and Quality Control, section 5.1.1 and Noncompliance, sections 5.20.1, and 5.20.2. It is the responsibility of the site PI and other study personnel to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via The Emmes Corporation's Advantage eClinical[SM].

All deviations from the protocol must be addressed in study subject case report forms. A completed copy of the DMID Protocol Deviation (PD) Form must be maintained in the regulatory file, as well as in the subject's CRF. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site PI and other study personnel are responsible for knowing and adhering to their IRB/IEC requirements.

11 SUBJECT CONFIDENTIALITY

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the Sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating subjects, and all study subject information will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

Applicable regulatory authorities, such as the FDA, or other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the investigator. The clinical study site will permit access to such records.

Results from this study will be de-identified prior to transfer to the Sponsor in order to protect subject confidentiality. Subjects will have code numbers and will not be identified by name. No subject's PII will be provided to the Sponsor.

In the event that a subject revokes authorization to collect or use PII, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PII, attempts should be made to obtain permission to collect at least vital status (i.e., that the subject is alive) at the end of their scheduled study period.

Certificate of Confidentiality

To further protect the privacy of study subjects, we have a Certificate of Confidentiality from the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research subjects, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping to ensure confidentiality and privacy to subjects.

A Certificate of Confidentiality does not prevent the subject from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

The Certificate of Confidentiality does not prevent the researchers from reporting, without the subject's consent, information that would identify the subject as a participant in the research project regarding matters that must be legally reported including: child and elder abuse, sexual abuse, or wanting to harm themselves or others.

11.1 Future Use of Stored Specimens

Click Diagnostics may store excess vaginal liquid media collected from this study indefinitely for the purposes of future testing and medical research related to CT/NG, TV, or other diseases or conditions. Each sample will be encoded (labeled) only with a barcode and a unique tracking number to protect the subject's confidentiality. Reports about future research done with the subject's samples will NOT be kept in the subject's health records. Results will not be provided to subjects. At the time of informed consent, subjects may choose to withhold permission for storage of their specimens and may continue in the study if they decline permission. Specimens from subjects not consenting to storage and future research will be destroyed at the end of the study.

12 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, each participating site(s) and its subcontractors are responsible for conducting routine quality assurance and QC activities to internally monitor study progress and protocol compliance. The site PI will provide direct access to all study-related sites, source data/data collection forms, and reports for the purpose of monitoring and auditing by the Sponsor, and inspection by local and regulatory authorities. The site PI will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The SDCC will implement QC procedures beginning with the data entry system and generate data QC checks that will be run on the database. Any missing data or data anomalies will be communicated to the participating site(s) for clarification and resolution.

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 Ethical Standard

The site PI will ensure that this trial is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research (National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research [April 18, 1979]), and codified in 45 CFR 46, 21 CFR 50, and 21 CFR 56, as applicable. The PI will also ensure conformity with ICH E6 GCP, and applicable federal regulations, guidance, and guidelines for GCP and clinical trials with humans.

13.2 Institutional Review Board

Each site PI will obtain IRB approval for this protocol to be conducted at his/her research site(s), and send supporting documentation to the DMID before initiating recruitment of subjects. The investigator will submit applicable information to the IRB/IEC on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP, and, as applicable, 21 CFR 56 (Institutional Review Boards) and 21 CFR 50 (Protection of Human Subjects), other federal, state, and local regulations. The IRB/IEC must be registered with Office of Human Research Protection (OHRP) [*OHRP-only* or *OHRP/FDA*] as applicable to the research. DMID must receive the documentation that verifies IRB/IEC approval for this protocol, associated informed consent documents, and upon request any recruitment material and handouts or surveys intended for the subjects, prior to the recruitment and enrollment of subjects.

Any amendments to the protocol or consent materials will be approved by the IRB/IEC before they are implemented. IRB/IEC review and approval will occur at least annually throughout the enrollment and follow-up of subjects, and may cease if annual review is no longer required by applicable regulations. The investigator will notify the IRB/IEC of deviations from the protocol and reportable SAEs, as applicable to the IRB/IEC policy.

Each institution engaged in this research will hold a current Federalwide Assurance (FWA) issued by the OHRP for federally funded research.

The IRB/IEC will determine that adequate provisions are made for soliciting the permission of each child's parent(s) or legal guardian, including whether permission of one parent is sufficient for research or whether permission is to be obtained from both parents.

13.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual’s agreeing to participate in the study and continuing throughout the individual’s study participation. Extensive discussion of risks and possible benefits of participation in this study will be provided to the subjects and their families. Consent forms describing in detail the study procedures and risks are given to the subject, and written documentation of informed

consent is required prior to enrolling in the study. Subjects will be informed of the NIH Certificate of Confidentiality and the extent of confidentiality of subjects' records the certificate covers. For enrollment of non-English speakers, a translated consent document will be available, and an appropriate person will conduct the consent process. Consent forms will be IRB approved and the subject will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. The subjects will sign the informed consent document prior to being enrolled in the study. The subjects should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent orally or in writing at any time and for any reason throughout the course of the study. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. The consent process will be documented in the research record.

The written informed consent form and any other written information to be provided to subjects should be revised whenever important new information becomes available that may be relevant to the subject's consent. Any revised written informed consent form and written information should receive the IRB/IEC's approval/favorable opinion in advance of use. The subject should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information should be documented.

13.3.1 Informed Consent/Assent Process (in Case of a Minor or Others Unable to Consent for Themselves)

In the cases of minors or others unable to consent for themselves, subjects may only be enrolled in the study with the consent of the subject's parent(s) and/or legal guardian. The subject should be informed about the study to the extent compatible with the subject's understanding. If capable, the subject should assent by signing and personally dating the written consent form. A separate IRB-approved assent form, describing (in simplified terms) the details of the study, study procedures, and risks may be used. Assent forms do not substitute for the consent form signed by the subject's parent(s), legal guardian, and/or legally authorized representative. The IRB may have specific institutional policies regarding enrollment of subjects who are unable to provide informed consent for themselves.

Where parental permission is to be obtained, the IRB may find that the permission of one parent is sufficient for research to be conducted. The IRB may also determine that both parents must give their permission unless one parent is deceased, unknown, incompetent, or not reasonably available, or when only one parent has legal responsibility for the care and custody of the child.

If non-English speakers will be enrolled, a translated consent document will be available and an appropriate person will conduct the consent process. The study will include women, minorities, and adolescents 14-17 years old, per site

demographics. Females under 14 will not be enrolled, as is done similarly for pregnancy test studies.

13.4 Exclusion of Women, Minorities, and Children

The study will include women, minorities, and adolescents 14 years of age or older who meet the subject inclusion/exclusion criteria, regardless of religion, sex, or ethnic background. The demographic and ethnic distribution of the study population will be generally reflective of the distribution of subjects within the facilities in the US geographic locations of the study sites. The NIH has mandated that children (defined as anyone under the age of 21) be included in research trials when appropriate. This study will enroll children aged 14 to 20 who are able to give informed consent or parental/guardian permission granted as deemed by the IRB. Females under 14 will be excluded, as is done similarly for pregnancy test studies.

14 PUBLICATION POLICY

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH-funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.
Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH Office of Extramural Research (OER) Grants and Funding, http://grants.nih.gov/grants/oer.htm

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting, a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClinicalTrials.gov.

For this trial, the responsible party is DMID, which will register the trial and post results.

15 LITERATURE REFERENCES

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APPENDIX A: SCHEDULE OF EVENTS

Evaluation	Screening/Enrollment Visit 1
Signed consent/assent form	X
Confirmation of eligibility and enrollment	X
Demographics	X
Sexual health and reproductive history	X
Self-reported symptoms	X
Concomitant medications and supplements	X
Genital products	X
Self-collected vaginal swab	X
HCP-collected vaginal swabs	X
Adverse event during/post-vaginal swab collection	X