Clinical Validation of a Molecular Test for Ciprofloxacin-Susceptibility in *Neisseria gonorrhoeae*

DMID Protocol Number: 15-0090

DMID Funding Mechanism:
Contract Number: HHSN272201300014I
Task Order Number: HHSN27200006

Sponsor:
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
National Institutes of Health

Principal Investigator: Jeffrey D. Klausner, MD, MPH

DMID Clinical Project Manager: Peter Wolff, MHA

Version Number: 7.0

Date: 17 December 2018

STATEMENT OF COMPLIANCE

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

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 812)
- International Conference on Harmonisation: Good Clinical Practice (ICH) E6; 62 Federal Register 25691 (1997); and future revisions
- NIH Clinical Terms of Award

The study is a nonsignificant risk device study and must comply with the abbreviated Investigational Device Exemption (IDE) requirements under 21 CFR §812.2 (b).

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

TABLE OF CONTENTS

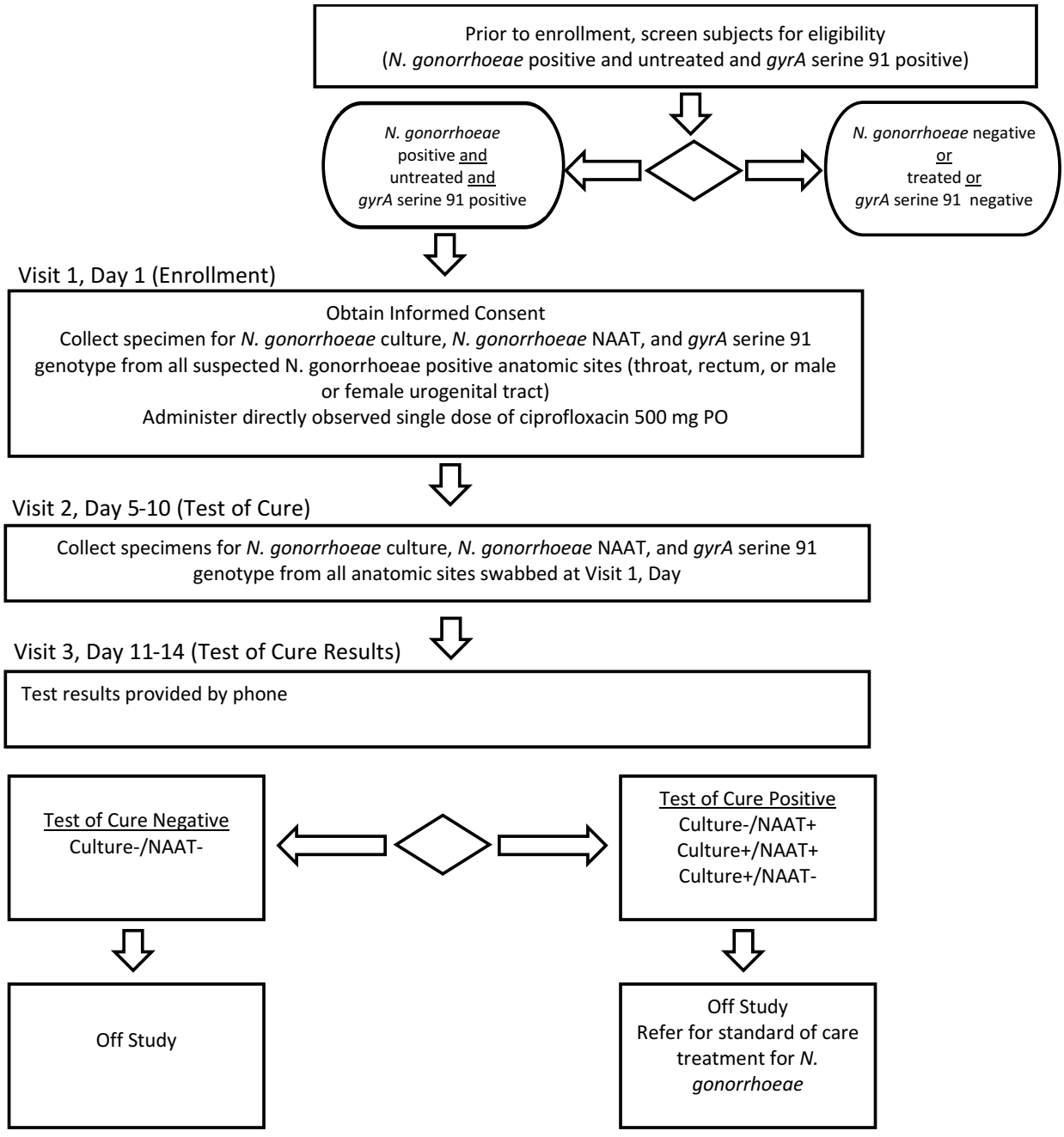
LIST OF ABBREVIATIONS

BV	Bacterial Vaginosis
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CRF	Case Report Form
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DNA	Deoxyribonucleic Acid
eCRF	Electronic Case Report Form
*gyr*A	Gyrase A Genotype
ICH	International Council for Harmonisation
ICMJE	International Committee of Medical Journal Editors
IEC	Independent or Institutional Ethics Committee
IRB	Institutional Review Board
ITT	Intent-to-Treat
MIC	Minimum Inhibitory Concentration
micro-ITT	Microbiological Intent-to-Treat
NAAT	Nucleic Acid Amplification Test
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PCR	Polymerase Chain Reaction
PP	Per-Protocol
QA	Quality Assurance
QC	Quality Control
SDCC	Statistical Data and Coordinating Center
SOP	Standard Operating Procedure
STD	Sexually Transmitted Disease

PROTOCOL SUMMARY

Title:	Clinical Validation of a Molecular Test for Ciprofloxacin-Susceptibility in *Neisseria gonorrhoeae*
Population:	Approximately 381 subjects will be enrolled to identify approximately 257 per protocol eligible subjects diagnosed with untreated serine 91 gyrase A genotype *Neisseria gonorrhoeae* of the rectum, or male or female urogenital tract
Number of Sites:	Up to eight sites in the United States
Study Duration:	Approximately 30 months after enrollment of the first subject
Subject Participation Duration:	11-14 days per subject
Description of Agent or Intervention:	Directly observed treatment with a single dose of ciprofloxacin 500 mg orally for *Neisseria gonorrhoeae* infections deemed serine 91 gyrase A genotype by real-time polymerase chain reaction with fluorescent hybridization probe detection of sequence
Objectives:	Primary: To determine the efficacy of ciprofloxacin for treatment of uncomplicated *Neisseria gonorrhoeae* infections with *gyrA* serine 91 genotype Secondary: To investigate efficacy of ciprofloxacin for treatment of uncomplicated serine 91 gyrase A genotype *Neisseria gonorrhoeae* infection by anatomic site To determine the sensitivity of the gyrase A assay for detection of ciprofloxacin-susceptible *Neisseria gonorrhoeae* infections
Description of Study Design:	See Schematic
Estimated Time to Complete Enrollment:	Approximately 24 months

Schematic


Prior to enrollment, screen subjects for eligibility
(N. gonorrhoeae positive and untreated and gyrA serine 91 positive)
N. gonorrhoeae positive and untreated and gyrA serine 91 positive
N. gonorrhoeae negative or treated or gyrA serine 91 negative
Visit 1, Day 1 (Enrollment)
Obtain Informed Consent
Collect specimen for N. gonorrhoeae culture, N. gonorrhoeae NAAT, and gyrA serine 91 genotype from all suspected N. gonorrhoeae positive anatomic sites (throat, rectum, or male or female urogenital tract)
Administer directly observed single dose of ciprofloxacin 500 mg PO
Visit 2, Day 5-10 (Test of Cure)
Collect specimens for N. gonorrhoeae culture, N. gonorrhoeae NAAT, and gyrA serine 91 genotype from all anatomic sites swabbed at Visit 1, Day
Visit 3, Day 11-14 (Test of Cure Results)
Test results provided by phone
Test of Cure Negative
Culture-/NAAT-
Test of Cure Positive
Culture-/NAAT+
Culture+/NAAT+
Culture+/NAAT-
Off Study
Off Study
Refer for standard of care treatment for N. gonorrhoeae

1 KEY ROLES

Principal Investigator	Jeffrey D. Klausner, MD, MPH Principal Investigator, STAR STI CTG Professor of Medicine and Public Health Division of Infectious Diseases David Geffen School of Medicine and Jonathan and Karin Fielding School of Public Health University of California, Los Angeles Los Angeles, CA 90095-1772 Phone: XXXXXXXXXX Email: XXXXXXXXXX
Co-Investigator	Sheldon Morris, MD, MPH Antiviral Research Center University of California, San Diego 220 Dickinson Street, Suite A San Diego, CA 92103 Phone: XXXXXXXXXX Email: XXXXXXXXXX

Contact information for site investigators and key staff at DMID, the coordinating center, and the SDCC is located in the Manual of Procedures.

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Gonorrhea is the second most commonly reported notifiable disease in the United States with more than 330,000 cases reported in 2012, a rate of 107.5 per 100,000 population [1]. Over the past 75 years, *Neisseria gonorrhoeae* (*N. gonorrhoeae)* infections have become increasingly resistant to antimicrobial therapy, first to sulfa-based antibiotics, then penicillins, tetracyclines, fluoroquinolones, and now extended-spectrum cephalosporins [2]. Prior to the advent of ciprofloxacin-resistant *N. gonorrhoeae*, ciprofloxacin was the primary first-line recommended therapy for *N. gonorrhoeae* infections of the throat, rectum, urethra, and cervix. From 1998 to 2006, ciprofloxacin was the Centers for Disease Control and Prevention (CDC) recommended treatment for *N. gonorrhoeae* in all anatomic sites. Prior to widespread ciprofloxacin resistance, the efficacy of ciprofloxacin treatment in susceptible *N. gonorrhoeae* infections was estimated at more than 99% [3-4]. In 2007, due to high rates of ciprofloxacin resistance in *N. gonorrhoeae* nationally, the CDC recommended the discontinuation of ciprofloxacin treatment for gonorrhea, which was followed by a decline in ciprofloxacin *N. gonorrhoeae* resistance nationally [5]. At this time, the only recommended treatment for *N. gonorrhoeae* infection is dual antibiotic therapy with injectable ceftriaxone and oral azithromycin or doxycycline [6].

For decades, bacterial culture was the standard diagnostic modality for *N. gonorrhoeae* infections. Culture is specific, but studies have demonstrated that the molecular-based enzyme immunoassay or nucleic acid amplification test (NAAT) has a higher clinical sensitivity than culture [7]. However, there has not been a widely used and reliable technology that allows for antibiotic susceptibility testing from non-culture specimens.

It has been recently demonstrated that *N. gonorrhoeae*-deoxyribonucleic acid (DNA) mutations can be detected using modern molecular techniques and real-time polymerase chain reaction (PCR) assays can be used to detect reproducible, clinically significant resistance [8-10]. A protocol for the detection of alterations in the gyrase A gene (*gyrA),* the target of ciprofloxacin anti-gonococcal activity, has been validated [8]. While molecular markers of susceptibility have high concordance, sensitivity and a specificity of 93% and 100%, respectively, compared with standard antimicrobial susceptibility testing, the clinical performance of the test and the response to therapy based on a molecular susceptibility result in *N. gonorrhoeae* is unknown.

2.2 Rationale

Resistance to extended-spectrum cephalosporins was first identified in Japan and is increasing in the western United States and among men who have sex with men [11]. Antimicrobial resistance mechanisms have been fairly well described as mutations in target proteins, alterations in cell wall components, or increases in efflux pumps [2].

Currently, the nationwide frequency of *N. gonorrhoeae* isolates with decreased ciprofloxacin susceptibility, minimum inhibitory concentration (MIC) ≥ 0.125 µg/mL, or resistance (MIC ≥ 0.5 µg/mL) is relatively modest at 13.5% [12]. Prior experience with changing antibiotic treatment guidelines has demonstrated that major shifts in antibiotic use may modify the ecology of drug resistance. For example, restricting erythromycin use in Group A streptococcal infections in Finland led to a population-level decrease in erythromycin-resistance in that organism [13].

While molecular methods to diagnose *N. gonorrhoeae* have greatly improved the detection of infection [14], they have historically precluded the ability to perform antibiotic susceptibility testing. Although regional surveillance data critically inform treatment recommendations, those data cannot be used for real-time individualized clinical management. The clinical validation of an assay to determine the ciprofloxacin susceptibility of *N. gonorrhoeae* infections could result in increased use of ciprofloxacin for ciprofloxacin-susceptible *N. gonorrhoeae* infections and resultant decreased use of extended-spectrum cephalosporins, thereby reducing the selection pressure for cephalosporin resistance in *N. gonorrhoeae*. An immediate benefit to taking ciprofloxacin would be for those with cephalosporin allergies who would have to take combination therapy that is more difficult to administer.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

Performance of the assay

Clinical specimens will be tested for a molecular marker of ciprofloxacin susceptibility. Treatment decisions will be made by the subject's clinician, per standard protocols, based on interpretation of laboratory results and selection of the best course of treatment based on available information. There is a small risk of a misclassification of susceptibility if the test were to show that the *N. gonorrhoeae* infection is ciprofloxacin-susceptible when in fact, it is resistant, and a subject with ciprofloxacin-resistant *N. gonorrhoeae* infection would not receive the appropriate treatment. The risk would be ameliorated if the test of cure visit (Visit 2, Day 5-10) displayed a treatment failure and the subject received standard treatment. The risk would therefore be that this would result in a delay of effective treatment.

The risk of a *N. gonorrhoeae* false negative test of cure result is estimated to be less than 0.5% and would result in inappropriate treatment for a subject with ciprofloxacin-resistant *N. gonorrhoeae* infection [9]. The risks of treatment failure include continued symptoms and potential transmission of *N. gonorrhoeae* to partners. Without effective re-treatment, treatment failure might result in complications including pelvic inflammatory disease, ectopic pregnancy,

infertility, chronic pelvic pain, and increased risk of transmission and HIV infection.

Risks from study procedures
Subjects may become embarrassed, worried, or anxious when discussing their sexual practices or ways for protecting against HIV and other infections passed during sex. They may also experience anxiety while they await test results. Women may expect to feel a little discomfort during the speculum exam. Subjects may experience gagging, discomfort, fainting, or bleeding when swabs are collected from the throat, rectum, urethra or cervix.

Risks of ciprofloxacin
Ciprofloxacin may cause dizziness, nervousness, difficulty sleeping, or increased sensitivity to sunlight, possibly resulting in sunburn. Like many antibiotics used to treat infections, ciprofloxacin may cause nausea, vomiting, stomach pain, headaches, tiredness, pale skin or a mild skin rash. Less often, hearing loss, kidney, liver or nerve problems, tendon pain, an irregular heartbeat, or fainting may occur**.** It is not known if ciprofloxacin will harm a fetus. Pregnancy tests can miss the first 14 days of pregnancy; therefore, fetuses may be exposed to ciprofloxacin if a subject's pregnancy is undetected at enrollment.

Significant side effects of ciprofloxacin
Significant side effects are rare, estimated to be less than 2%, and may include severe skin rash; hives; itching; difficulty breathing or swallowing; swelling of the face, throat, tongue, lips, eyes, hands, feet, ankles, or lower legs; hoarseness; yellowing of the skin or eyes; and rapid, pounding, or irregular heartbeat.

Unknown risks
There may be unknown risks that cannot be foreseen**.**

2.3.2 Known Potential Benefits

There are no known benefits to individual subjects. A potential benefit to future *N. gonorrhoeae*-susceptible infected individuals is treatment with a less invasive regimen (orally administered vs. intramuscular injection), which is less painful. Results from this study may help gonorrhea-infected individuals by reducing drug resistance as a result of the use of the most appropriate treatment, which is guided by pretreatment determination of ciprofloxacin-susceptible gonococcal infection.

The study will help to assess the potential impact of a strategy to address the critical problem of emerging drug resistance in *N. gonorrhoeae*. Specifically, the information gained from this study can be used to influence national sexually transmitted disease (STD) treatment policies and, therefore, result in a marked

decrease in extended-spectrum cephalosporin-resistant *N. gonorrhoeae* infection, improving the availability of treatment options for individuals infected with *N. gonorrhoeae*.

This study may provide an alternative regimen for the treatment of gonorrhea in cases of cephalosporin allergy or antimicrobial resistance to cephalosporins. It may also provide an oral regimen for settings in which injections are not feasible. In addition, it may provide a convenient and easy-to-administer patient-delivered therapy for the partners of index cases.

3 OBJECTIVES

3.1 Study Objectives

3.1.1 Primary Objective

To determine the efficacy of ciprofloxacin for treatment of uncomplicated *N. gonorrhoeae* infections with *gyrA* serine 91 genotype

3.1.2 Secondary Objectives

1) To investigate the efficacy of ciprofloxacin for treatment of uncomplicated serine 91 *gyrA N. gonorrhoeae* infection by anatomic site

2) To determine the sensitivity of the *gyrA* assay for detection of ciprofloxacin-susceptible *N. gonorrhoeae* infections

3.1.3 Exploratory Objectives

1) To characterize *N. gonorrhoeae* isolates and non-*gyrA* gene-dependent mechanisms of ciprofloxacin resistance post-treatment of uncomplicated *N. gonorrhoeae* infection by anatomic site using traditional and molecular methods like genotyping and sequencing

2) To investigate the efficacy of ciprofloxacin for treatment of uncomplicated serine 91 *gyrA N. gonorrhoeae* infections by demographic and clinical characteristics

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measure

The proportion of subjects infected with *gyrA* serine 91 genotype *N. gonorrhoeae* with microbiological cure at Visit 2 (Day 5-10)

3.2.2 Secondary Outcome Measures

1) The proportion of subjects infected with *gyrA* serine 91 genotype *N. gonorrhoeae* with microbiological cure at each anatomic site (throat, rectum, male or female urogenital tract) at Visit 2 (Day 5-10)

2) The proportion of subjects infected with *gyrA* serine 91 genotype *N. gonorrhoeae* identified by *gyrA* gene PCR who have ciprofloxacin-susceptible *N. gonorrhoeae* as identified by culture-based antimicrobial susceptibility testing at Visit 1 (Day 1)

3.2.3 Exploratory Outcome Measures

1) Identification and characterization of non-*gyrA* gene-dependent mechanisms of ciprofloxacin resistance by genotyping and whole genome sequencing (e.g., Illumina) of nucleic acid isolated from uncured subjects at Visit 2 (Day

5-10). Genotyping and sequencing may be performed on nucleic acid isolated from different anatomic sites.

2) The proportion of subjects infected with *gyrA* serine 91 genotype *N. gonorrhoeae* with microbiological cure at Visit 2 (Day 5-10) by demographic and clinical characteristics

4 STUDY DESIGN

This study is a multi-center, single-arm, open-label clinical study to assess the efficacy of one dose of ciprofloxacin given orally in subjects infected with untreated *gyrA* serine 91 genotype *N. gonorrhoeae* as determined by a real-time PCR assay. The study will enroll approximately 381 subjects to obtain a per protocol eligible target of 257 subjects age 18 years or older regardless of gender identification who are seeking care in STD clinics at participating sites in the United States. Subjects who have untreated *gyrA* serine 91 genotype *N. gonorrhoeae* of the rectum, or male or female urogenital tract identified by a positive culture or NAAT conducted at a prior visit will be offered enrollment in the study. Subjects not consenting to participate in the study will receive treatment per local standard of care.

At enrollment, Visit 1 (Day 1), a brief physical exam and medical and sexual history will be done. Subjects will have samples collected for *N. gonorrhoeae* culture and *N. gonorrhoeae* NAAT (to include *gyrA* serine 91 genotype) from each infected anatomic site (throat, rectum, male or female urogenital tract) to verify infection status.

Subjects with *gyrA* serine 91 genotype rectal or urogenital *N. gonorrhoeae* will receive one dose of directly observed ciprofloxacin 500 mg. Subjects will be observed in the clinic for at least 5 minutes after receiving ciprofloxacin and will be instructed to return to the clinic or to contact the study site coordinator immediately if they vomit within 1 hour of receiving ciprofloxacin. Subjects who vomit within 1 hour of study drug administration should be retreated for *N. gonorrhoeae* infection with an alternative recommended regimen per local standard of care.

Subjects will return for clinical and laboratory assessments at Visit 2 (Day 5-10), test of cure visit. Subjects will have repeat samples collected for *N. gonorrhoeae* culture and *N. gonorrhoeae* NAAT from all anatomic sites swabbed at Visit 1 (Day 1).

For Visit 3 (Day 11-14), test results will be provided to subjects by phone call. No further follow-up is required for subjects who test culture negative and NAAT negative. Subjects who test culture positive or NAAT positive will be referred for standard of care treatment for *N. gonorrhoeae*.

The duration of the study for each subject will be approximately 11-14 days. Study enrollment is expected to be completed in 24 months.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Subject Inclusion Criteria

Subjects must meet all of the inclusion criteria in order to be eligible to participate in the study. Subjects who present with a new *N. gonorrhoeae* infection may be rescreened for eligibility. No exemptions are granted on Subject Inclusion/Exclusion Criteria in DMID-sponsored studies.

1. Willing and able to give voluntary written informed consent before any study related procedure is performed.
2. 18 years or older on the day of enrollment.
3. Untreated *gyrA* serine 91 genotype *N. gonorrhoeae* of the rectum, or male or female urogenital tract*, as determined by the *gyrA* test assay on a specimen collected within 30 days of enrollment.
   *Subjects must have *gyrA* serine 91 genotype detected for *N. gonorrhoeae* from at least one non-pharyngeal site.
4. Willing to abstain from sexual intercourse or use condoms during any sexual contact until the Test of Cure Visit (Visit 2, Day 5-10) is complete.
5. Women of childbearing potential* must have no vaginal intercourse 14 days before enrollment or use an effective birth control method ** for 30 days before enrollment and agree to continue through visit 2.
   *A woman is considered of childbearing potential unless post-menopausal (>2 years) or surgically sterilized (tubal ligation >1 year, bilateral oophorectomy, or hysterectomy).
   **Acceptable birth control methods for the purposes of this study may include abstinence from intercourse with a male partner, monogamous relationship with vasectomized partner, male condoms with the use of applied spermicide, intrauterine devices, and licensed hormonal methods. A highly effective method of contraception is defined as one that results in a low failure rate (i.e., less than 1% per year) when used consistently and correctly.
6. Able to swallow pills.
7. Willing to comply with protocol requirements, including availability for follow-up for the duration of the study.
8. Agree to avoid systemic or intravaginal antibiotics* with activity against *N. gonorrhoeae* from enrollment through Visit 2 (Day 5-10). *Topical and intravaginal antifungals are permitted.
9. Agree to avoid magnesium/aluminum antacids, sucralfate, didanosine, or highly buffered drugs, up to 2 hours after receipt of study drug.

5.2 Subject Exclusion Criteria

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

1. Known renal insufficiency from clinical history.
2. Use of systemic or intravaginal antibiotics* with potential activity against *N. gonorrhoeae* within 30 days prior to study drug administration. *Topical and intravaginal antifungals are permitted.
3. Use of systemic corticosteroid drugs or other immunosuppressive therapy within 30 days prior to enrollment.
4. Receipt or planned receipt of an investigational product in a clinical trial within 30 days prior to or 7 days after treatment administration.
5. Pregnant or breastfeeding.
6. Clinical diagnosis of pelvic inflammatory disease or genital ulcer.
7. Confirmed or suspected complicated or systemic gonococcal infection, such as abdominal pain, testicular pain, epididymitis, orchitis, arthritis, or endocarditis.
8. Receipt of magnesium/aluminum antacids, sucralfate, didanosine, or highly buffered drugs, within 6 hours before receipt of study drug.
9. Mutant *N. gonorrhoeae gyrA* serine 91 genotype from any anatomic site, as determined by the *gyr*A test assay on a specimen collected within 30 days of enrollment.
10. Known allergy or history of adverse reaction to ciprofloxacin.
11. Known allergy to quinolones.
12. Previous enrollment in this study.
13. Medical condition or other factor that in the judgment of the investigator might affect ability to comply with procedures.

5.3 Subject Withdrawal

5.3.1 Reasons for Withdrawal

Subjects may voluntarily withdraw their consent for further study participation at any time and for any reason, without penalty.

Refer to section 11 for how subjects who withdraw from study participation will be handled in study analyses.

Subjects may withdraw or be withdrawn from the study for any of the reasons given below. The reason for withdrawal will be recorded in the data collection form.

- Subject vomits within 1 hour of study medication administration.
- Subject receives any systemic antibiotic other than the study medication prior to Visit 2 (Day 5-10).
- Subject is pregnant or breastfeeding.
- Discretionary decision by the site investigator.
- At the discretion of the IRB, NIH, or other government agency as part of their duties to ensure that research subjects are protected.

5.3.2 Handling of Withdrawals

Subjects who withdraw voluntarily prior to receiving ciprofloxacin will be offered local standard of care treatment for *N. gonorrhoeae* and followed per clinic guidelines.

Subjects who withdraw voluntarily or never return after receiving ciprofloxacin and tolerated medication without difficulty will not be contacted for repeat gonorrhea testing or treatment.

Subjects who withdraw voluntarily or never return after being found to be positive for *N. gonorrhoeae* at Visit 2 (Day 5-10) will be contacted by phone and by certified mail with instructions that they should follow-up for treatment per local standard of care and should be followed per clinic guidelines.

5.3.2.1 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.

6 GYRASE A ASSAY/STUDY TREATMENT

6.1 Gyrase A Assay Description

Real-time PCR testing protocol: The laboratories participating in this study will follow the testing protocol as described in Siedner et al [15] with modifications. For real-time PCR analysis of the *gyrA* gene, remnant specimens will be subject to nucleic acid (DNA) extraction by using automated nucleic acid extraction utilizing either the bioMerieux easyMag, or the Qiagen EZ1, or Qiacube platforms, as determined in the optimization studies. Extracted samples will be used for real-time PCR. Amplification and melting-curve analysis of specimens will be performed by using a Roche LightCycler 480 or Light Cycler 2.0 platforms (Roche Diagnostics, Pleasanton, CA). Amplified regions of the *gyrA* gene will be assessed for mutations in the Ser 91 codon through use of melting-curve analysis with probes specific to that region of the *gyrA* gene. The probes for the serine 91 amplicon (*gyrA*-ser-Flu and *gyrA*-ser-LC) are a paired set of FRET probes, including a LightCycler Red640 probe and a fluorescein-labeled probe, separated by 1 bp. The Red640 oligonucleotide has a maximal absorption and emissions of 622 and 638 nm, respectively. Amplification of PCR product will be followed by melt-curve analysis to confirm specificity of amplification and genotype. Melting-curve analysis will be performed by using LightCycler software (v.4.0). The software will be used to plot the negative value of the first derivative of fluorescence per unit time to distinguish peak melting temperatures from melting-curve plots. Designation of predicted susceptibility is based upon the observed melting curve parameters as previously described [15].

6.2 Ciprofloxacin Description

Ciprofloxacin is a quinolone medication approved by the FDA for the treatment of gonorrhea. Ciprofloxacin hydrochloride, USP, a fluoroquinolone, is the monohydrochloride monohydrate salt of 1- cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1piperazinyl)-3-quinolinecarboxylic acid. The molecular weight of the ciprofloxacin hydrochloride monohydrate is 385.82. It is a faintly yellowish to light yellow crystalline substance.

6.2.1 Ciprofloxacin Formulation

Ciprofloxacin 500 mg tablets

6.2.2 Ciprofloxacin Acquisition

Ciprofloxacin will be purchased centrally, and upon request by DMID, will be transferred to the following address:

DMID Clinical Agents Repository (CAR)
XXXXXXXXXX

Ciprofloxacin will be shipped from the DMID CAR to the study site upon request and approval by DMID.

6.2.3 Storage and Stability

Ciprofloxacin is stable at room temperature. Stability of this medication will be maintained by storing the drug below 30°C (86°F). The storage room temperature will be monitored on business days.

6.2.4 Dosage Preparation and Administration

Ciprofloxacin tablets will be administered orally and can be taken with or without food. Subjects will receive 500mg and will be directly observed.

6.2.5 Accountability Procedures

Fisher repository will ship packaged ciprofloxacin to the investigational sites. At each site, medication packages will be kept in a locked storage cabinet at the clinic and administered by the study personnel. Accountability records will be maintained by designated site staff (i.e., study personnel or pharmacy staff). Unused study treatment will be destroyed or returned to Fisher BioServices at the end of the study. Unused study treatment will not be destroyed without DMID approval.

6.2.6 Assessment of Subject Compliance

Medication will be given and directly observed by the study staff and recorded in the subject's case report form (CRF). Since medication will be directly observed, there will be no need for return of study medication packaging or a medication diary at the follow-up visit.

6.2.7 Concomitant Medications

Administration of any contraindicated medications or new antibiotics after enrollment will be recorded on the appropriate data collection form. Contraindicated medications will include all medications contraindicated with ciprofloxacin (described in section 5.2) taken 30 days prior to signing the informed consent form through Visit 2 or early termination, whichever occurs first.

Subjects who have received study medication and are subsequently diagnosed with a concomitant infection, which requires systemic antibiotics will receive treatment according to the local clinic's standard of care.

Use of new medication should prompt evaluation for the presence of a new diagnosis of chronic medical disease or condition.

Oral contraceptives are allowed, as they do not alter the metabolism of ciprofloxacin.

7 STUDY SCHEDULE

Complete study schedule details listed by study visit day are described below. Refer also to Appendix A.

7.1 Visit 1, Day 1 (Screening/Enrollment)

Screening and enrollment will occur at the same clinic visit. Potential subjects will be informed about the study and invited to participate. Subjects who are interested in the study will be asked to sign a consent form that will describe the purpose of the study, the procedures to be followed, the risks and benefits of participating, and will include a separate section for consent to store samples for future use. A copy of the consent form will be given to the subject and this fact will be documented in the CRF.

The following will be performed for subjects who give informed consent:

1. Demographic information including age, sex, and gender will be collected from the subject
2. Eligibility criteria will be reviewed with the subject
3. Discuss pertinent medical history for assessment of specific symptoms that are potentially related to fluoroquinolone use as described in section 9, Assessment of Safety, and sexual history will be obtained by interview of subjects to assure eligibility. Concomitant infections will be reviewed.
4. Review of signs and symptoms within the past 7 days
5. All new antibiotics or concomitant medications contraindicated with ciprofloxacin taken in the 30 days prior to signing the informed consent will be recorded on the appropriate data collection form
6. Targeted physical exam
7. Speculum exam for females
   Note: Women diagnosed with BV by local standard test at Visit 1 will receive treatment for BV at Visit 2 according to local standard of care.
8. Urine pregnancy test for females of childbearing potential
9. One swab for *N. gonorrhoeae* culture and one swab for NAAT and *gyrA* serine 91 genotype from the throat*
10. One swab for *N. gonorrhoeae* culture and one swab for NAAT and *gyrA* serine 91 genotype from the rectum*
11. One swab for *N. gonorrhoeae* culture from the urethra (males)*
12. Urine specimen for NAAT and *gyrA* serine 91 genotype (males)*
13. Cervical swab for *N. gonorrhoeae* culture (females)*
14. Cervical or vaginal swab for NAAT and *gyrA* serine 91 genotype (females)*
    *Note 1: Only at the suspected infected anatomical site based on prior test results or other sites as clinically indicated
    *Note 2: G*yrA* assay is required only if NAAT is positive

15. Once swabs have been collected, and all eligibility criteria have been assessed, subjects will receive one dose of ciprofloxacin 500 mg by mouth under direct observation with the date and time of administration documented.
    - Subjects will be observed for at least 5 minutes after administration of ciprofloxacin.
    - Subjects will be instructed to return to the clinic or to contact the study site coordinator immediately if they vomit within 1 hour of receiving study medication.
    - Subjects who vomit within 1 hour of study drug administration will be retreated for *N. gonorrhoeae* infection with an alternative recommended regimen per the local standard of care.
16. Reminder to abstain from sexual intercourse or use condoms during any sexual contact until the Test of Cure Visit (Visit 2, Day 5-10) is complete
17. Reminder to bring sex partners in for testing and treatment, if needed
18. Reminder to return for Visit 2 (Day 5-10)

7.2 Visit 2, Day 5-10 (Test of Cure Visit)

Subjects will have the following:

1. Interim medical history for assessment of specific symptoms that are potentially related to fluoroquinolone use as described in section 9, Assessment of Safety, and interim sexual history
2. Review of signs and symptoms at site of infection since the previous visit
3. Review of new antibiotics or concomitant medications contraindicated with ciprofloxacin
4. Targeted physical exam
5. One swab for *N. gonorrhoeae* culture and one swab for NAAT and *gyrA* serine 91 genotype from the throat*
6. One swab for *N. gonorrhoeae* culture and one swab for NAAT and *gyrA* serine 91 genotype from the rectum*
7. One swab for *N. gonorrhoeae* culture from the urethra (males)*
8. Urine specimen for NAAT and *gyrA* serine 91 genotype (males)*
9. Cervical swab for *N. gonorrhoeae* culture (females)*
10. Cervical or vaginal swab for NAAT and *gyrA* serine 91 genotype (females)*
    *Note 1: Repeat samples to be collected only from those anatomical sites swabbed for *N. gonorrhoeae* culture or NAAT at Visit 1
    *Note 2: G*yrA* assay is required only if NAAT is positive
11. Reminder to bring sex partners in for testing and treatment, if needed
12. Reminder that test results will be provided to subjects by phone call (Visit 3, Day 11-14)

Women diagnosed with bacterial vaginosis (BV) will receive treatment according to local standard of care.

7.3 Visit 3, Day 11 to 14 (Test of Cure Results)

1. Test results will be provided to subjects by phone call.
2. Subjects whose Visit 2 (Day 5-10) *N. gonorrhoeae* culture and NAAT results are negative will have completed participation in the study; no further follow-up is required.
3. Subjects who are *N. gonorrhoeae* culture positive or NAAT positive will be referred for standard of care treatment for *N. gonorrhoeae*.
4. Reminder to bring sex partners in for testing and treatment, if needed

7.4 Unscheduled/Early Termination Visit

For an unscheduled visit at the subject's request or the site investigator's recommendation, subjects will have the following:

1. Interim medical history for assessment of specific symptoms that are potentially related to fluoroquinolone use as described in section 9, Assessment of Safety, and interim sexual history
2. Review of signs and symptoms at site of infection since the previous visit
3. Review of new antibiotics or concomitant medications contraindicated with ciprofloxacin
4. Targeted physical exam
5. Reminder to abstain from sexual intercourse or use condoms during any sexual contact until the Test of Cure Visit (Visit 2, Day 5-10) is complete
6. Reminder to bring sex partners in for testing and treatment, if needed
7. Reminder to return for follow-up visit

For an early termination visit, subjects will have the following:

1. Interim medical history for assessment of specific symptoms that are potentially related to fluoroquinolone use as described in section 9, Assessment of Safety, and interim sexual history
2. Review of signs and symptoms at site of infection since the previous visit
3. Review of new antibiotics or concomitant medications contraindicated with ciprofloxacin
4. Targeted physical exam
5. Reminder to bring sex partners in for testing and treatment, if needed

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

Discuss pertinent medical history for assessment of specific symptoms that are potentially related to fluoroquinolone use as described in section 9, Assessment of Safety, and sexual history (including assessment of signs and symptoms) will be obtained by interview of the subjects at Visit 1 prior to study treatment. Subjects will be asked if they can swallow pills.

- For female cervicovaginal infections, subjects will be classified as symptomatic if they report any of the following symptoms: abnormal vaginal discharge, abnormal vaginal bleeding, or pain during intercourse.
- For male urethral infections, subjects will be classified as symptomatic if they report any of the following symptoms: urethral discharge or pain with urination.
- For testing of rectal swab specimens, subjects will be classified as symptomatic if they report any of the following symptoms: anorectal pain, anorectal discharge, or anorectal bleeding.
- For testing of pharyngeal swab specimens, subjects will be classified as symptomatic if they report having a sore throat.
- For all specimen types, subjects will be considered asymptomatic if they do not endorse these symptoms.

At Visit 2 (Day 5-10) and unscheduled visits, an interim medical history will be obtained by interview if the subject notes any changes since the previous visit in specific symptoms that are potentially related to fluoroquinolone use as described in section 9, Assessment of Safety. The interim medical history should include an assessment for new medical conditions.

Sexual history including sexual orientation will be obtained by interview of the subjects at Visit 1. At Visit 2 (Day 5-10) and unscheduled visits, subjects will be queried regarding sexual activity and condom use since the previous clinic visit.

Medications history (concomitant medications contraindicated with ciprofloxacin) will include a review of all current medications and medications taken 30 days prior to study enrollment. Assessment of eligibility will also include a review of all permitted and prohibited medications per the Subject Inclusion and Exclusion Criteria (see section 5).

A targeted physical exam will be performed based on subject's clinical history. The exam will be performed by appropriate study personnel to assess general physical condition and will at a minimum include the following areas/systems: throat, genitals, and rectum. A speculum exam will be performed on females. All physical examinations will be performed by a study clinician licensed to make medical diagnoses. At visits following

Visit 1, a targeted physical examination may be conducted based on interim medical history.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

Prior to the study implementation period, each laboratory will verify the performance of the *gyrA* serine 91 genotype real-time polymerase chain reaction with fluorescent hybridization probe detection assay, to meet the CLIA requirements, including accuracy, precision, limit of detection, interfering substances, and normal values. Full details of the verification protocol and *gyrA* serine 91 genotype assay standard operating procedure (SOP) are in the study's Manual of Procedures.

Specimens positive for gonococcal DNA or RNA from the participating clinical sites will be tested by PCR for detection of wild-type *gyrA* serine 91 genotype. Subjects who have been treated empirically (i.e., symptomatic subjects) may opt out of this testing, if indicated by the clinical site on the laboratory requisition.

The presence or absence of mutations in the *gyrA* gene will be determined by real-time PCR. For real-time PCR analysis of the *gyrA* gene, remnant NAAT specimens will be extracted, using either a Roche MagNAPure instrument or Qiacube, which will then be used for the *gyrA* real-time PCR, using a Roche LightCycler platform. The *gyrA* assay combines amplification of the *gyrA* gene in the serine 91 coding region and melting-curve analysis of to determine wild-type vs. mutation in this region. This is done through use of Fluorescence Resonance Energy Transfer (FRET) probes for the serine 91 amplicon.

8.2.2 Special Assays or Procedures for Enrolled Subjects

Urine collected at Visit 1 will be analyzed for detecting pregnancies by use of a standard rapid urine βhCG assay.

Swabs (non-cotton) will be used to collect specimens at each site (throat, rectal, urethral or cervical) for *N. gonorrhoeae* culture. The swab will be immediately smeared onto a modified Thayer Martin plate, which is placed into a candle jar or other CO_2-rich transport media, at the clinic. These plates will be transported in the CO_2-rich transport media to the local laboratory for culture for *N. gonorrhoeae*. The local laboratory will culture and isolate the gonococcal strain, according to their SOP for *N. gonorrhoeae* culture. *N. gonorrhoeae* culture specimens will be obtained first; this sequence maximizes the load collected, which increases the likelihood of a successful culture. A second swab or urine will be collected for NAAT for *N. gonorrhoeae* detection, according to the

laboratory SOP, using a collection kit with liquid transport specific to the local protocols (e.g. unisex swab specimen collection kit).

For each positive culture (regardless of anatomical site or visit number), a subculture of each gonococcal isolate will be subject to antimicrobial susceptibility testing using the agar dilution method for azithromycin, cefixime, ceftriaxone, ciprofloxacin, gentamicin, penicillin, spectinomycin, and tetracycline. M100 Clinical and Laboratory Standards Institute (CLSI) interpretive criteria will be used to evaluate susceptibility to each agent, with the exception of azithromycin, where CDC criteria will be used [16].

For each positive NAAT, or negative NAAT with a positive culture for the same visit, up to two 500 uL aliquots of the specimen will be frozen in screw-top 2.0 mL tubes, at -70℃ for discordant resolution, if needed. These frozen remnants will be stocked at the local laboratory until they are sent to the central laboratories approximately halfway through the study and at study completion.

For subjects who demonstrate culture-based antibiotic resistance to ciprofloxacin but are deemed ciprofloxacin-susceptible by the *gyrA* gene-dependent mechanism, or have culture positivity at Visit 2, whole genome sequencing will be performed on their *N. gonorrhoeae* isolates from all visits. DNA samples will be extracted from the cultures and multiplexed libraries will be created. Sequencing will be performed according to the laboratory SOP using a platform such as the MiSeq (Illumina, San Diego, CA). Genomic assembly and annotation will be performed using sequenced *N. gonorrhoeae* strains as reference scaffolding and phylogenetic analyses will be conducted based on nucleotide polymorphisms [17].

Specific instructions and numbering schemes for study isolates and specimens are described in the study's Manual of Procedures.

8.2.3 Specimen Preparation, Handling, and Shipping

8.2.3.1 Instructions for Specimen Preparation, Handling, and Storage

Stocked clinical isolates of *N. gonorrhoeae* and remnant specimens will be stored at the performing laboratory site until they are sent to the central laboratories approximately halfway through the study and at the end of the study.

Specific instructions for specimen preparation, handling, and storage are described in the study's Manual of Procedures.

8.2.3.2 Specimen Shipment

Bacterial isolates will be shipped frozen on dry ice in accordance with IATA shipping regulations. *N. gonorrhoeae* DNA remnant specimens will be shipped to the reference laboratory frozen on dry ice at specified intervals in accordance with IATA shipping regulations. Specimens will be shipped as category B (infectious) substances to meet the requirements outlined in 49 CFR 178.609.

Specimens and isolates for use/long-term storage will be shipped to:

ATTN: DeeDee Pacheco, Laboratory Manager
University of California San Diego AVRC
XXXXXXXXXX

Specific instructions for specimen shipment will be described in the study's Manual of Procedures.

9 ASSESSMENT OF SAFETY

Ciprofloxacin has a well-established safety profile and has been prescribed to several millions of people in the US and worldwide as it was the CDC's recommended treatment regimen for uncomplicated gonorrhea from 1993 to 2007. Therefore, adverse event assessment is not required for this study.

In order to address the FDA Drug Safety Communication dated May 12, 2016, regarding fluoroquinolone use for certain uncomplicated infections, subjects will be asked at Visit 1, Visit 2, and any Unscheduled or Early Termination Visit if they have experienced any serious side effects related to fluoroquinolone use. These include tendon pain, joint pain, muscle pain, tingling or pricking sensation, confusion, and hallucinations. This information will be recorded in the study source documents as a change in health status. Any subject who experiences such an event will be given instructions on how to submit a MedWatch Consumer Voluntary Reporting Form to the FDA if they desire.

10 CLINICAL MONITORING

10.1 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 Manual of Procedures 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 eCRFs, 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 principal investigators to discuss any problems and actions to be taken and will document visit findings and discussions.

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

The primary objective of the study is to clinically validate a *gyrA* assay used to determine ciprofloxacin susceptibility in the treatment of uncomplicated gonococcal infection by assessing the efficacy of ciprofloxacin for treatment of uncomplicated *N. gonorrhoeae* infections with serine 91 *gyrA* genotype as identified by the *gyrA* assay.

The study is designed to test the following hypothesis in the primary efficacy analysis conducted in the per-protocol (PP) analysis population:

Null hypothesis: The proportion of ciprofloxacin-susceptible (as identified by the *gyrA* assay) *N. gonorrhoeae*-infected subjects with microbiological cure 5-10 days after receiving ciprofloxacin is less than 0.95.

Alternative hypothesis: The proportion of ciprofloxacin-susceptible (as identified by the *gyr*A assay) *N. gonorrhoeae*-infected subjects with microbiological cure 5-10 days after receiving ciprofloxacin is at least 0.95.

The cut-off of 0.95 was chosen based on the CDC criteria for treatments of uncomplicated *N. gonorrhoeae* infections. The criterion for recommended treatments is a cure rate of greater than 95% with a lower bound of the 95% confidence interval of least 95%. The criterion for alternative treatments is a cure rate of greater than 95% with a lower bound of the 95% confidence interval of at least 90%.

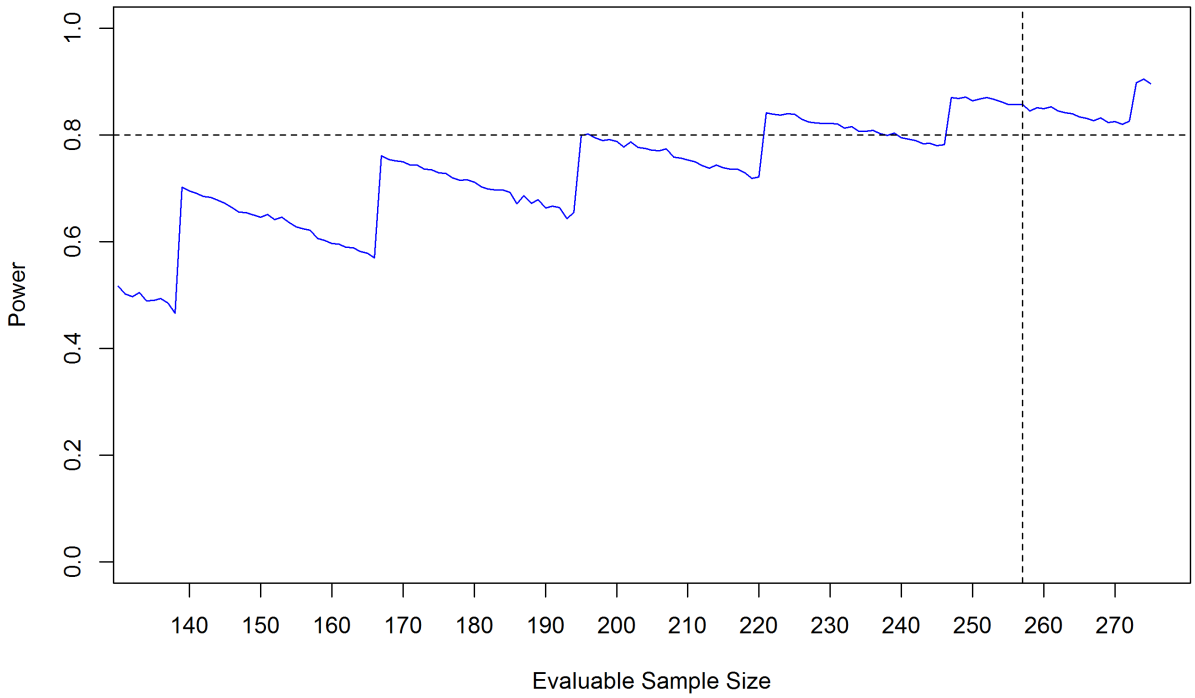
11.2 Sample Size Considerations

The primary analysis will compute the proportion of ciprofloxacin-susceptible *N. gonorrhoeae*-infected subjects with microbiological cure 5-10 days after receiving ciprofloxacin in the PP analysis population. The confidence interval for the microbiological cure proportion will be computed using a one-sided modified Jeffreys interval using a significance level of 0.05. Power calculations were performed via a simulation study based on 10,000 replications assuming a 98% microbiological cure rate.

Figure 11.2 below presents the power available to test that the microbiological cure rate is at least 95% (i.e., lower bound of the one-sided confidence interval is at least 95%) across a range of evaluable sample sizes. Here, evaluable denotes eligible for the PP analysis population. The power reaches 80% at a sample size of 195 evaluable subjects; however, since the power curve is not monotonic, sample sizes directly on either side of 195 are below 80%. Since the exact number of evaluable subjects at the end of the study is difficult to predict, an evaluable target of 257 subjects is chosen to ensure at least 80% even if the exact number of evaluable subjects at the end of the study differs

slightly from 257. With a target of 257, the power available is approximately 85% and we can fall at most 10 subjects short (sample size of 247 evaluable subjects) and still ensure 80% power. Any sample size greater than 257 will ensure at least 80% power.

Figure 11.2: Power Available to Test That the Microbiological Cure Rate Is at Least 95%



To calculate the number of subjects required to enroll to reach 257 subjects in the PP analysis population, the following assumptions are made:

- 25% of enrolled subjects will be culture negative at baseline.
- 10% of subjects will be excluded from the PP analysis population (reasons for exclusion are stated in section 11.4.1)

Under these assumptions, enrolling 381 subjects will provide 257 subjects in the PP analysis population. Since the proportion of subjects who will be culture negative at baseline can vary depending on a number of factors, 25% is a rough estimate. The exact number of subjects needed to provide 257 subjects in the PP analysis population may vary from 381, so 381 is an approximate enrollment target.

11.3 Planned Analyses

11.3.1 Planned Futility Review

The study will be monitored for futility. The number of treatment failures among subjects eligible for the PP analysis population will be monitored by a statistician independent from the study team. If, at any point in the trial, 30 subjects eligible for the PP analysis population are classified as treatment failures, then the statistician will notify DMID to consider termination of the trial.

The cut-off was chosen because 29 or fewer treatment failures among 257 subjects eligible for the PP analysis population would result in a lower bound of the one-sided 95% Jeffreys confidence interval greater than or equal to 85%. Under the protocol's assumptions of the cure rate, a simulation study based on 50,000 replications found that the operating characteristics (power and type-I error) of this futility review do not vary from those described in section 11.2.

11.4 Final Analysis Plan

A separate statistical analysis plan document will be generated that will contain the details of the analyses. This section outlines the major components of the analyses.

11.4.1 Analysis Populations

Per-Protocol (PP) Population: This analysis population includes enrolled subjects who met all inclusion/exclusion criteria, have *N. gonorrhoeae* isolated on Day 1 culture from the rectum or urogenital site, have an evaluable microbiological outcome status (see section 11.4.3.1), complied with study treatment, did not receive any contraindicated medication or systemic antibiotic other than the study medication for any concomitant infection prior to Visit 2 (Day 5-10), and returned for their Visit 2 (Test of Cure) visit within window.

To be compliant with study treatment, a subject must be able to swallow the pill and not vomit within 1 hour of administration.

For the primary analysis, subjects need only have *N. gonorrhoeae* isolated on Day 1 culture from the rectum or urethra (male) or cervix/vagina (female). For the analysis of secondary objective 1, subjects need to have *N. gonorrhoeae* isolated on Day 1 culture at the particular site being assessed.

In addition, alternate per-protocol analyses which exclude subjects with reported concomitant infection/disease/procedure that may interfere with study product, use of concomitant medications or products that may interfere with study product, significant protocol deviations, and other events that may impact study product

effectiveness or study analyses will be explored. Details will be described in the separate statistical analysis plan.

Microbiological Intent-to-Treat (micro-ITT) Population: This analysis population includes all enrolled subjects who have *N. gonorrhoeae* isolated from the rectum or urogenital site on Day 1 culture.

For the primary analysis, subjects need only have *N. gonorrhoeae* isolated on Day 1 culture from the rectum or urethra (male) or cervix/vagina (female). For the analysis of secondary objective 1, subjects need to have *N. gonorrhoeae* isolated on Day 1 culture at the particular site being assessed.

Intent-to-Treat (ITT) Population: This analysis population includes all enrolled subjects.

Subjects who receive any treatment other than the study medication for their *N. gonorrhoeae* infection will be included in all efficacy analyses as treatment failures.

11.4.2 Baseline Characteristics

Baseline and demographic characteristics will be summarized. 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.

11.4.3 Efficacy and Other Analysis Plan

In this section, the primary and secondary analyses are described. The exploratory analysis will be addressed in the statistical analysis plan and is not described here.

11.4.3.1 Primary Analysis

The primary analysis will test whether the microbiological cure rate of ciprofloxacin in subjects infected with *gyrA* serine 91 genotype *N. gonorrhoeae* is greater than or equal to 95%. The cure rate will be estimated by the proportion of subjects infected with *gyrA* serine 91 genotype *N. gonorrhoeae* with microbiological cure at Visit 2 (Day 5-10).

Microbiological outcome status for the primary analysis is evaluated at Visit 2 (Day 5-10) and is defined as follows:

- Microbiological Cure: *N. gonorrhoeae* not detectable by culture from all anatomical sites that had detectable *N. gonorrhoeae* at Visit 1.

- Microbiological Failure: *N. gonorrhoeae* detectable by culture from at least one anatomical site that had detectable *N. gonorrhoeae* at Visit 1.
- Non-evaluable: At least one of the following: Subject did not return for Visit 2 (Day 5-10), or culture results at Visit 2 (Day 5-10) were not available for any reason, or subject could not be evaluated for microbiological outcome status for any other reason.

For the PP analysis population, subjects with non-evaluable outcome status will be excluded from the analysis. For the micro-ITT and ITT analysis populations**,** subjects with non-evaluable outcome status will be included in analyses and classified as treatment failures. Subjects who discontinued early from the study due to a lack of treatment effect are included in all analysis populations as treatment failures.

The test will be performed by calculating the one-sided 95% modified Jeffreys confidence interval and comparing it to the value 0.95. The modification to the Jeffreys confidence interval was introduced in Brown, Cai, and DasGupta [18]. The lower bound of the confidence interval will be calculated as follows:

If the number of cures, denoted X, is less than the number of subjects included in the analysis, denoted N, then the lower bound of the one-sided 95% confidence interval is:

$$B(0.95; X + 0.5; N - X + 0.5)$$

where $B(\alpha; m_1; m_2)$ denotes the α quantile of a Beta(m_1, m_2) distribution.

If the number of cures is equal to the number of subjects included in the analysis, denoted N, then the lower bound of the one-sided 95% confidence interval is:

$$(0.05)^{1/N}$$

The primary analysis will be performed in the PP analysis population. Secondary analyses of the primary outcome measure will be performed in the micro-ITT and ITT analysis populations. Sensitivity analyses may be performed to assess the impact of the choice of confidence interval. These analyses will be specified in the statistical analysis plan.

11.4.3.2 Secondary Analyses

The analysis of the efficacy of by anatomic site (secondary objective 1) will proceed as described in section 11.4.3.1 where the modification to

the definition of microbiological outcome status is provided below. Summaries of the cure rate estimate and associated confidence interval and hypothesis test will be generated for each individual anatomic site.

For the analysis of secondary objective 1, microbiological outcome status at each anatomical site is evaluated at Visit 2 (Day 5-10) and is defined for a particular anatomical site as follows:

- Microbiological Cure: *N. gonorrhoeae* not detectable by culture at the particular anatomical site that had detectable *N. gonorrhoeae* at Visit 1.
- Microbiological Failure: *N. gonorrhoeae* detectable by culture at the particular anatomical site that had detectable *N. gonorrhoeae* at Visit 1.
- Non-evaluable: At least one of the following: Subject did not return for Visit 2 (Day 5-10), or culture results at Visit 2 (Day 5-10) were not available for any reason, or subject could not be evaluated for microbiological outcome status for any other reason.

For the analysis of the sensitivity of the *gyrA* assay for the detection of ciprofloxacin-susceptible *N. gonorrhoeae* infections (secondary objective 2), the sensitivity will be estimated by calculating the proportion of enrolled subjects who have ciprofloxacin-susceptible *N. gonorrhoeae* as identified by culture-based antimicrobial susceptibility testing at Visit 1. The proportion of enrolled subjects who do not have ciprofloxacin-susceptible *N. gonorrhoeae* will also be estimated. This analysis will be performed in the micro-ITT analysis population. Secondary and sensitivity analyses, which will assess the impact of analysis population and missing antimicrobial susceptibility testing, may be performed. These analyses will be specified in the separate statistical analysis plan document.

11.4.3.3 Exploratory Analysis

The exploratory analysis will be described in the separate statistical analysis plan.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate research records for this trial, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. As part of participating in a DMID-sponsored study, each site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) study records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all study source data. Study source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, the participating site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site principal investigator will provide direct access to all trial-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 principal investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The Statistical Data and Coordinating Center (SDCC) will implement quality control procedures beginning with the data entry system and generate data quality control checks on the database. Any missing data or data anomalies will be communicated to the site for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

This study will be conducted in full conformity with the Declaration of Helsinki, or with the ICH-GCP regulations and guidelines, whichever affords the greater protection to the subject.

The investigator will also ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

14.2 Institutional Review Board

Each participating institution will provide review and approval of this protocol and the associated informed consent documents and recruitment material by an appropriate independent ethics committee (IEC) or IRB registered with the Office for Human Research Protections (OHRP). Any amendments to the protocol or consent materials will also be approved before they are placed into use. In the United States and in other countries, only institutions holding a current US Federalwide Assurance issued by OHRP may participate.

14.3 Informed Consent Process

Informed consent is a process that is initiated prior to the subject agreeing to participate in the study and continuing throughout the subject's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the subjects and their families. Consent forms describing in detail the study treatment, procedures, and risks are given to the subject and written documentation of informed consent is required prior to administering study treatment. 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 subject will sign the informed consent document prior to any procedures being done specifically for the study. The subject should have the opportunity to discuss the study with his/her surrogates or think about it prior to agreeing to participate. The subject may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subject. The rights and welfare of 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.

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

This trial will be inclusive of all adults who meet the subject inclusion/exclusion criteria, regardless of religion, sex, or ethnic background. Should the outcome of this trial be deemed acceptable, additional trials may be initiated in other populations. The demographic and ethnic distribution of the study population will be generally reflective of the distribution of subjects with *N. gonorrhoeae* within each STD clinic. The following populations will be excluded from study participation:

- *Pregnant women*
  Pregnant women are excluded from this study, as it is not known if ciprofloxacin will harm a fetus.

- *Children*
  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 18 to 20 who are able to give informed consent. This study meets "Justifications for Exclusion" criteria for younger children as set forth by the NIH. Specifically, "insufficient data are available in adults to judge potential risk in children" and "children should not be the initial group to be involved in research studies."

14.5 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 and genetic tests in addition to the clinical information relating to participating subjects.

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

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 assure confidentiality and privacy to subjects.

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

14.6 Study Discontinuation

If the study is discontinued, subjects will be given appropriate care and treatment for gonorrhea under medical supervision until the infection has resolved.

14.7 Future Use of Stored Specimens

All gonococcal strains will be stored in a secured location and may be subject to additional future testing by authorized staff to better understand resistant gonorrhea. The strains will not be stored with any personal identifying information but will include a new unique subject ID number so that specimens from the same individual are linked but the subject's identify will remain unknown. The samples will not contain human genetic material. Future tests will be limited to those related to the study of *N. gonorrhoeae* and not of any human material. The informed consent process will include appropriate consent to obtain and store these samples. 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.

15 DATA HANDLING AND RECORD KEEPING

The site principal investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms will be derived from the eCRF and provided by the SDCC to record and maintain data for each subject enrolled in the study. All data collection forms should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, cross out the original entry with a single line and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Data reported in the eCRF derived from the data collection forms should be consistent with the data collection forms or the discrepancies should be explained.
The sponsor and/or its designee will provide guidance to site principal investigators and other study personnel on making corrections to the data collection forms and eCRF.

15.1 Data Management Responsibilities

All data collection forms and laboratory reports must be reviewed by the clinical team and data entry personnel, who will ensure that they are accurate and complete.

Data collection is the responsibility of the study personnel at the participating sites under the supervision of the respective site principal investigator. During the study, the site principal investigator must maintain complete and accurate documentation for the study.

The Emmes Corporation will serve as the SDCC for this study and will be responsible for data management, quality review, analysis, and reporting of the study data**.**

15.2 Data Capture Methods

Clinical (including, but not limited to, concomitant medications, medical history, physical assessments, clinical laboratory values) 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 completed by the study personnel.

15.3 Types of Data

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

15.4 Timing/Reports

A final report will be prepared following the availability of all the safety and laboratory data. Interim statistical reports may be generated as deemed necessary and appropriate by DMID.

15.5 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 medication 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.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or Manual of Procedures requirements. The noncompliance may be either 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
5.1 Quality Assurance and Quality Control, section 5.1.1
5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site principal investigator 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 AdvantageEDC℠.

All deviations from the protocol must be addressed in study subject source documents. A completed copy of the DMID Protocol Deviation Form must be maintained in the regulatory file, as well as in the subject's source document. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site principal investigator and other study personnel are responsible for knowing and adhering to their IRB/IEC requirements.

16 PUBLICATION POLICY

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 (http://www.ncbi.nlm.nih.gov/pmc/) no later than 12 months after publication.

Refer to:
• NIH Public Access Policy, http://publicaccess.nih.gov/
• NIH OER Grants and Funding, http://grants.nih.gov/grants/oer.htm

Following completion of the study, the lead principal investigator is expected to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine [19]. Other biomedical journals are considering adopting similar policies.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase I trials), would be exempt from this policy. Results of any exploratory analysis will not be published prior to publication of the primary results for this study.

All clinical trials supported by the NIH must be registered on ClinicalTrials.gov. As part of the result posting, a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be included in the post on ClincialTrials.gov.

For trials in which DMID is not the investigational new drug/investigational new device exemption (IND/IDE) sponsor, or there is no IND/IDE, and DMID does not provide data management services, it is the responsibility of the investigator to register the trial and post results in compliance with Public Law 110-85, the Food and Drug Administration Amendments Act of 2007 (FDAAA).

17 LITERATURE REFERENCES

1. Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance 2012. http://wwwcdcgov/std/stats12/Surv2012pdf 2012; accessed Aug 13, 2014.
2. Barry PM, Klausner JD. The use of cephalosporins for gonorrhea: the impending problem of resistance. Expert Opinion on Pharmacotherapy 2009 Mar; 10(4):555-77. [PMID: 19284360]
3. Echols RM, Heyd A, O'Keeffe BJ, Schacht P. Single-dose ciprofloxacin for the treatment of uncomplicated gonorrhea: a worldwide summary. Sex Transmitted Diseases. 1994 Nov-Dec;21(6):345-52. [PMID: 7871449]
4. Hook EW 3rd, Jones RB, Martin DH, Bolan GA, Mroczkowski TF, Neumann TM, Haag JJ, Echols R. Comparison of ciprofloxacin and ceftriaxone as single-dose therapy for uncomplicated gonorrhea in women. Antimicrobial Agents and Chemotherapy. 1993 Aug;37(8):1670-3. [PMID:8215281]
5. Centers for Disease Control and Prevention. Cephalosporin susceptibility among *Neisseria gonorrhoeae* isolates, United States, 2000-2010. Morbidity and Mortality Weekly Report: 2011 Jul 8;60(26):873-7. [PMID: 21734634]
6. Centers for Disease Control and Prevention. Update to CDC's Sexually transmitted diseases treatment guidelines, 2010: oral cephalosporins no longer a recommended treatment for gonococcal infections. Morbidity and Mortality Weekly Report 2012; Aug 10; 61(31):590-4. [PMID: 22874837]
7. Centers for Disease Control and Prevention. Recommendations for the laboratory-based detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*—2014. Morbidity and Mortality Weekly Report (MMWR). Recommendations and Reports. March 14, 2014/63(RR02);63:1-19. http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6302a1.htm
8. Siedner MJ, Pandori M, et al. Real-time PCR assay for detection of quinolone-resistant *Neisseria gonorrhoeae* in urine samples. Journal of Clinical Microbiology 2007 Apr; 45(4):1250-4. [PMID: 17267635]
9. Pandori M, Barry PM, et al. Mosaic penicillin-binding protein 2 in *Neisseria gonorrhoeae* isolates collected in 2008 in San Francisco, California. Antimicrobial Agents and Chemotherapy 2009 Sept; 53(9):4032-4. [PMID: 19546370]
10. Buono S, Wu A, Hess DC, Carlson JS, Rauch L, Philip SS, et al. Using the *Neisseria gonorrhoeae* multiantigen sequence-typing method to assess strain diversity and antibiotic resistance in San Francisco, California. Microbial Drug Resistance 2012 Oct; 18(5): 510-7. [PMID: 22686196]
11. Bolan GA, Sparling PF, Wasserheit JN. The emerging threat of untreatable gonococcal infection. New England Journal of Medicine 2012 Feb 9; 366(6):485-7. [PMID: 22316442].
12. Kirkcaldy RD, Kidd S, Weinstock HS et al. Trends in antimicrobial resistance in *Neisseria gonorrhoeae* in the USA: the Gonococcal Isolate Surveillance Project (GISP), January 2006–June 2012. Sexually Transmitted Infections. 2013;89:suppl4:iv5–10.

13. Seppälä H, Klaukka T, Vuopio-Varkila J, Muotiala A, Helenius H, Lager K, Huovinen P. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland: Finnish Study Group for Antimicrobial Resistance. New England Journal of Medicine 1997 Aug 14; 337(7):441-6. [PMID: 9250845]
14. Dicker LW, Mosure DJ, Steece R, Stone KM. Testing for sexually transmitted diseases in U.S. public health laboratories in 2004. Sexually Transmitted Diseases 2007 Jan; 34(1):41-6. [PMID: 16735955]
15. Siedner MJ, Pandori M, Castro L, Barry P, Whittington WL, Liska S, Klausner JD. Real-time PCR assay for detection of quinolone-resistant Neisseria gonorrhoeae in urine samples. Journal of Clinical Microbiology 2007 Apr;45(4):1250-4.
16. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: 12th Informational Supplement. M100-S1727], 124-25. 2007. Ref Type: Serial (Book, Monograph)
17. Demczuk w, Lynch T, Martin I, et al. Whole-genome phylogenomic heterogeneity of Neisseria gonorrhoeae isolates with decreased cephalosporin susceptibility collected in Canada between 1989 and 2013. Journal of Clinical Microbiology 2015; 53(1):191-200.
18. Brown L, Cai T, DasGupta A. Interval estimation for a binomial proportion. Statistical Science 2001 16(2):101–33. doi:10.1214/ss/1009213286
19. De Angelis C, Drazen JM, Frizelle FA, Haug C, Hoey J, et al. Clinical trial registration: a statement from the International Committee of Medical Journal Editors. New England Journal of Medicine. 2004 Sep 16;351(12):1250-1. Epub 2004 Sep 8

APPENDIX A: SCHEDULE OF EVENTS

Evaluation	Screening/ Enrollment Visit 1 Day 1	Visit 2 Day 5-10 (Test of Cure)	Visit 3 Day 11-14[3] (Test of Cure Results)	Unscheduled/ Early Termination Visit[5]
Signed consent form	X			
Confirmation of eligibility criteria	X			
Medical history for assessment of specific symptoms that are potentially related to fluoroquinolone use as described in section 9, Assessment of Safety	X	X		X
Signs/symptoms	X	X		X
Concomitant medications	X	X		X
Sexual history	X			
Interim sexual history		X		X
Targeted physical examination	X	X		X
Rapid urine βhCG pregnancy test (females)	X			
Speculum examination (females)	X			
Cervical swab for *N. gonorrhoeae* culture (females)	X[2]	X[2]		
Cervical or vaginal swab for NAAT and *gyrA* assay (females)[1]	X[2]	X[2]		
Urethral swab for *N. gonorrhoeae* culture (males)	X[2]	X[2]		
Urine specimen for NAAT and *gyrA* assay (males)[1]	X[2]	X[2]		
Throat swab for *N. gonorrhoeae* culture and NAAT and *gyrA* assay[1]	X[2]	X[2]		
Rectal swab for *N. gonorrhoeae* culture and NAAT and *gyrA* assay[1]	X[2]	X[2]		
Study intervention	X			
Reminder to abstain from sexual intercourse or use condoms	X			X[5]
Reminder to bring partners in for treatment	X	X	X	X
Follow-up for test of cure results			X[4]	

1. G*yrA* assay required only if NAAT is positive.
2. Collect specimen at anatomic site of *N. gonorrhoeae* infection.
3. Follow-up for test of cure results will be by phone call.
4. For subjects *N. gonorrhoeae* culture negative and NAAT negative, no further follow-up required. Subjects who are *N. gonorrhoeae* culture positive or NAAT positive will be referred for standard of care treatment for *N. gonorrhoeae*.
5. Not required at the Early Termination Visit.