

Experimental Human Infection with Isogenic Mutants of *Neisseria gonorrhoeae*

DMID Protocol Number: 09-0106

Other Identifying Numbers: UNC IRB Study # 12-0482

DMID Funding Mechanism: NIH grant U01AI114378

IND Sponsor: National Institute of Allergy and Infectious Diseases

Principal Investigator: Marcia M. Hobbs, PhD

Co-Principal Investigator: Joseph A. Duncan, MD, PhD

DMID Clinical Project Manager: Barbara Hahn, RN, BSN

DMID Medical Officer: Lori Newman, MD

DMID Medical Monitor: Mohamed Elsafy, MD

Version Number: 12.0

09 August 2019

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 312)
- ICH E6; 62 Federal Register 25691 (1997)
- NIH Clinical Terms of Award

All individuals responsible for the design and conduct of this study have completed Human Subjects Protection 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 Investigators:

Signed: _____ Date: _____

Name: Marcia M. Hobbs, PhD

Title: Professor of Medicine

Signed: _____ Date: _____

Name: Joseph A. Duncan, MD, PhD

Title: Associate Professor of Medicine

TABLE OF CONTENTS

Statement of Compliance.....	ii
Signature Page	iii
Table of Contents	iv
List of Tables	vii
List of Abbreviations	viii
Protocol Summary	x
1 Key Roles	14
2 Background Information and Scientific Rationale	17
2.1 Background Information	17
2.2 Rationale	18
2.3 Potential Risks and Benefits	19
2.3.1 Potential Risks	19
2.3.2 Known Potential Benefits	21
3 Objectives	22
3.1 Study Objectives	22
3.2 Study Outcome Measures	22
3.2.1 Primary Outcome Measures	22
3.2.2 Secondary Outcome Measures	23
3.2.3 Exploratory Outcome Measures	23
4 Study Design	24
4.1 Substudies	25
5 Study Enrollment and Withdrawal.....	26
5.1 Subject Inclusion Criteria	26
5.2 Subject Exclusion Criteria	27
5.3 Treatment Assignment Procedures	28
5.3.1 Randomization Procedures	28
5.3.2 Masking Procedures	28
5.3.3 Reasons for Withdrawal.....	28
5.3.4 Handling of Withdrawals.....	29
5.3.5 Termination of Study	29
6 Study Intervention/Investigational Product	30
6.1 Study Product Description	30
6.1.1 Acquisition.....	30
6.1.2 Formulation, Packaging, and Labeling.....	30
6.1.3 Product Storage and Stability.....	31
6.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product	32

6.3	Modification of Study Intervention/Investigational Product for a Participant	33
6.4	Accountability Procedures for the Study Intervention/Investigational Product(s)...	33
6.5	Assessment of Subject Compliance with Study Intervention/Investigational Product	34
6.6	Concomitant Medications/Treatments	34
7	Study Schedule.....	36
7.1	Screening.....	36
7.2	Enrollment/Baseline.....	37
7.3	Follow-up.....	37
7.4	Final Study Visit	39
7.5	Early Termination Visit	39
7.6	Unscheduled Visit.....	39
8	Study Procedures/Evaluations	40
8.1	Clinical Evaluations	40
8.2	Laboratory Evaluations	41
8.2.1	Clinical Laboratory Evaluations	41
8.2.2	Special Assays or Procedures	41
8.2.3	Specimen Preparation, Handling, and Shipping	42
9	Assessment of Safety	44
9.1	Specification of Safety Parameters	44
9.2	Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters	45
9.2.1	Adverse Events	45
9.2.2	Reactogenicity (for Vaccine Studies and Some Therapeutic Trials).....	46
9.2.3	Serious Adverse Events	46
9.2.4	Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings	49
9.3	Reporting Procedures.....	49
9.3.1	Serious Adverse Events	49
9.3.2	Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND	49
9.3.3	Regulatory Reporting for Studies Not Conducted Under DMID-Sponsored IND.....	50
9.3.4	Other Adverse Events (if applicable).....	50
9.3.5	Reporting of Pregnancy	51
9.4	Type and Duration of Follow-up of Subjects after Adverse Events.....	51
9.5	Halting Rules	51
9.6	Safety Oversight (ISM plus SMC).....	52
10	Clinical Monitoring.....	53
10.1	Site Monitoring Plan.....	53

11	Statistical Considerations.....	54
11.1	Study Hypotheses.....	54
11.2	Sample Size Considerations.....	55
11.3	Planned Interim Analyses (if applicable).....	56
11.3.1	Infectivity Outcomes Review	56
11.3.2	Safety Review	57
11.3.3	Immunogenicity or Efficacy Review	57
11.4	Final Analysis Plan	57
12	Source Documents and Access to Source Data/Documents	59
13	Quality Control and Quality Assurance.....	60
14	Ethics/Protection of Human Subjects	61
14.1	Ethical Standard	61
14.2	Institutional Review Board	61
14.3	Informed Consent Process	61
14.3.1	Informed Consent/Assent Process (in Case of a Minor).....	62
14.4	Exclusion of Women, Minorities, and Children (Special Populations).....	62
14.5	Subject Confidentiality	62
14.6	Study Discontinuation.....	63
14.7	Future Use of Stored Specimens.....	63
15	Data Handling and Record Keeping	64
15.1	Data Management Responsibilities.....	64
15.2	Data Capture Methods	64
15.3	Types of Data.....	64
15.4	Timing/Reports	65
15.5	Study Records Retention.....	65
15.6	Protocol Deviations.....	65
16	Publication Policy	66
17	Literature References	68
18	Supplements/Appendices.....	70
18.1	Appendix A: Schedule of Events.....	70
18.2	Appendix B: Subject Comprehension.....	72
18.3	Appendix C: Toxicity Table	73

LIST OF TABLES

Table 1. Abnormal vital signs.....	38
Table 2. Sample size considerations for noncompetitive infection studies	55

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
ANC	Absolute Neutrophil Count
BSE	Bovine spongiform encephalopathy
CFAR	Center for AIDS Research
CFR	Code of Federal Regulations
CFU	Colony forming unit
CH50	Total Complement
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CFR	Code of Federal Regulations
CRF	Case Report Form
CRO	Contract Research Organization
CT	<i>Chlamydia trachomatis</i> , <i>C. trachomatis</i>
CTRC	Clinical and Translational Research Center
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
GCB	Gonococcal culture broth
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
ID _{xx}	Infectious dose (xx% of inoculated subjects are expected to become infected)
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
inoc.	inoculum
IRB	Institutional Review Board
ISM	Independent Safety Monitor
JAMA	Journal of the American Medical Association
LLN	Lower limit of normal

MedDRA®	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
mRNA	messenger RNA encoding expressed proteins
mut	mutant
N	Number (typically refers to subjects)
NAAT	Nucleic Acid Amplification Test
NCI	National Cancer Institute, NIH, DHHS
NDA	New Drug Application
NEJM	New England Journal of Medicine
NG	<i>Neisseria gonorrhoeae</i> , <i>N. gonorrhoeae</i> , the causative agent of gonorrhea
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
OHSR	Office for Human Subjects Research
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PCR	Polymerase Chain Reaction
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
PMN	Polymorphonuclear cell
prop'n	proportion
QA	Quality Assurance
QC	Quality Control
RNASeq	Sequence data derived from human or bacterial mRNA
RT PCR	Reverse transcriptase PCR
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
STI	Sexually Transmitted Infection
TV	<i>Trichomonas vaginalis</i> , <i>T. vaginalis</i>
UNC	University of North Carolina at Chapel Hill
US	United States
ULN	Upper limit of normal
VIM	Virology, Immunology & Microbiology Core Laboratory
WBC	White blood cell
WHO	World Health Organization
WNL	Within normal limits
WT	Wild type

PROTOCOL SUMMARY

Title:	Experimental Human Infection with Isogenic Mutants of <i>Neisseria gonorrhoeae</i>
Phase:	Phase I
Population:	Up to 32 male subjects, ≥ 18 and < 36 years old, living in central North Carolina, in general good health with no history of STIs.
Number of Sites:	Single site: University of North Carolina at Chapel Hill
Study Duration:	18 months
Subject Participation Duration:	3 weeks including 6 days in the CTIC, a follow-up visit 1 week after discharge from the CTIC and a follow-up telephone call 2 weeks after discharge from the CTIC.
Description of Agent or Intervention:	Subjects will receive up to 0.4 mL of a suspension containing 10^5 - 10^6 CFU of <i>Neisseria gonorrhoeae</i> , in phosphate-buffered saline, delivered to the anterior urethra through a No.8 pediatric French catheter.
Objectives:	<p>Primary:</p> <ul style="list-style-type: none">• Compare infectivity of different isogenic mutants with wild-type (WT) <i>N. gonorrhoeae</i> in noncompetitive infections.• For mutants with WT infectivity, assess relative fitness of the mutant in competitive infections initiated by inocula containing equivalent numbers of both WT and mutant strains. Note, competitive infections will not be performed if non-competitive infections are substantially different. If this is the case, there will be no analysis for this primary objective. <p>Secondary:</p> <ul style="list-style-type: none">• Compare the clinical course of infection with mutant and WT <i>N. gonorrhoeae</i> in noncompetitive infections. <p>Exploratory:</p>

- Characterize host immune responses during experimental gonococcal infection.
- Characterize bacterial gene expression during experimental gonococcal infection.

Description of Study Design:

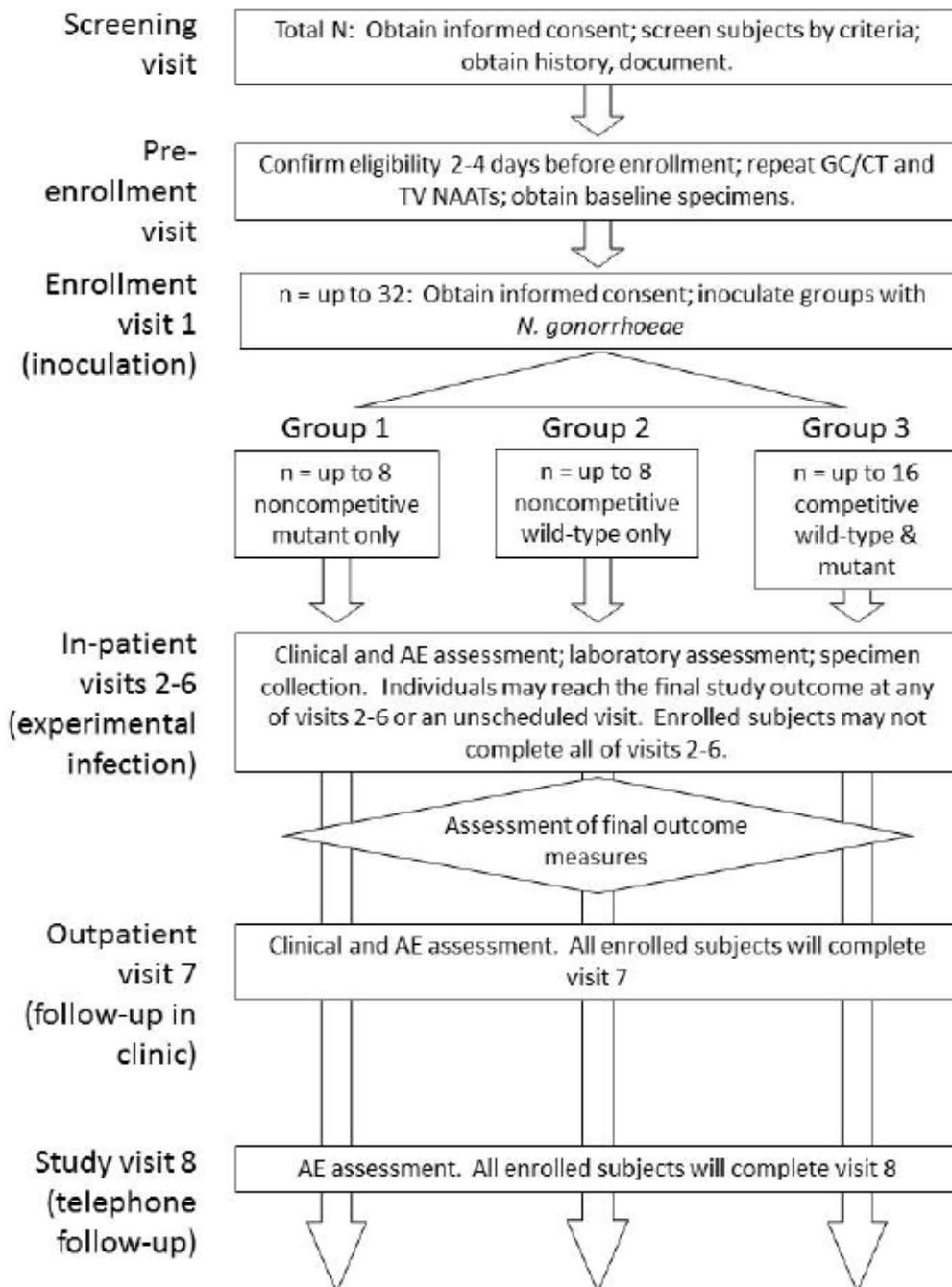
This is an experimental infection model study designed to test the requirements of predicted *N. gonorrhoeae* virulence determinants for gonococcal infection in the male urethra. Subjects will receive a suspension containing $10^5 - 10^6$ CFU of *N. gonorrhoeae* delivered to the anterior urethra through a small diameter catheter. Inoculation takes place in the inpatient unit of the UNC Clinical Translational Research Center (CTRC). During the inpatient portion of the trial, subjects may leave the clinical research ward provided they sign out, identify their whereabouts when not on the ward, carry a cell phone, and return by 9pm each evening to stay overnight on the ward. All subjects will be examined daily for signs or symptoms of infection, provide daily first-void urine specimens for gonococcal culture to monitor infection and receive 100% effective antibiotic treatment (cefixime or ceftriaxone) at the end of the inpatient portion of the trial, whether infected or not. Within 7 days of antibiotic treatment, all subjects return for a test of cure and follow up examination. A final follow-up interview is conducted with all subjects by phone within 2 weeks of antibiotic treatment.

For each mutant to be investigated under this protocol, groups of subjects will be enrolled first in noncompetitive infection studies: Group 1 (n = up to 8 evaluable subjects) will receive a bacterial inoculum containing only the isogenic mutant *N. gonorrhoeae* strain, and Group 2 (n = up to 8 evaluable subjects) will receive a bacterial inoculum containing only the WT *N. gonorrhoeae* strain. If primary and secondary outcomes are not different for the isogenic mutant and WT strains, a group of subjects will be enrolled in competitive infection studies: Group 3 (up to 16 evaluable subjects) will receive a bacterial inoculum containing a mixture of equivalent numbers the isogenic mutant and WT strain. A competitive advantage for one strain during urethral infection will be manifest by recovery of that strain in a statistically significantly higher proportion of isolates recovered from infected subjects than in the inoculum.

This protocol describes (1) the overall experimental infection program with fixed details that do not change with the specific gonococcal strains to be studied, including study recruitment, screening, enrollment and experimental infection and monitoring procedures, and (2) the MtrD study including specific details of the experimental inocula and outcome measures to test the requirement for the MtrD component of the gonococcal Mtr efflux pump during experimental urethral infection. For all future studies designed to test other *N. gonorrhoeae* strains, a protocol amendment will be submitted including the rationale, details of the experimental inoculum, outcome measures and consent forms specific for each study. The study population of up to 32 subjects reflects the sample size required for the MtrD study and includes the total number of subjects to be enrolled in noncompetitive and competitive infection groups for this mutant.

**Estimated Time to
Complete Enrollment:**

18 months

Schematic of Study Design:

Note, the total number of subjects in Groups 1, 2 and 3 combined will not exceed 32. Outcomes for Groups 1 and 2 will determine whether Group 3 will be enrolled for each isogenic mutant.

1 KEY ROLES

Individuals:

Clinical Project Manager:

Barbara Hahn, RN, BSN
Nurse Consultant/Clinical Project Manager
Sexually Transmitted Infections Branch
DMID, NIAID, NIH
5601 Fishers Lane, Room E49
Bethesda, MD 20892-9825
(if using FedEx or UPS, use zip code 20891)
Tel: (240) 627-3267
Email: bhahn@niaid.nih.gov

Medical Officer:

Lori M. Newman, MD
DMID/NIAID/NIH
5601 Fishers Lane, Room 8E45, Rockville, MD 20852
Phone: (301) 761-6958
Email: lori.newman@nih.gov

Principal Investigator:

Marcia M. Hobbs, PhD
Associate Professor of Medicine, Microbiology & Immunology
University of North Carolina at Chapel Hill
Infectious Diseases Research
111 Mason Farm Road, 8309 MBRB CB# 7031
Chapel Hill, NC 27599
Phone: (919) 843-6893
Fax: (919) 843-1015
Email: mmhobbs@med.unc.edu

Co-Principal Investigator:

Joseph A. Duncan, MD, PhD
Associate Professor of Medicine and Pharmacology
University of North Carolina at Chapel Hill
Infectious Diseases Research
111 Mason Farm Road, 8335 MBRB CB# 7031
Chapel Hill, NC 27599
Phone: (919) 843-0715
Fax: (919) 843-1015
Email: joseph_duncan@med.unc.edu

Medical Monitor:

Mohamed M. Elsafy, MD
Medical Officer
NIH/NIAID/DMID/OCRA
5601 Fishers Lane, Room 7E56
Bethesda, MD 20892-9825
Phone: (240) 292-4241
Email: mohamed.elsafy@nih.gov

Institutions:**Clinical site of volunteer screening, and follow-up visits:**

NC TraCS Institute – CTSC Outpatient Unit
University of North Carolina at Chapel Hill
160 Dental Circle
Burnett-Womack Bldg, 1st Floor
Room 1036, CB# 7064
Chapel Hill, NC 27599-7064
Contact: Janette Goins, RN, BSN, Director of Nursing
Phone: (919) 843-1070
Fax: (919) 966-1576
Email: janette_goins@med.unc.edu

Clinical site of subject enrollment, experimental procedures and follow-up visits:

NC TraCS Institute – CTSC Inpatient Unit
University of North Carolina at Chapel Hill
101 Manning Drive, CB# 7600
Chapel Hill, NC 27599-7600
Contact: Janette Goins, RN, BSN, Director of Nursing
Phone: (919) 843-1070
Fax: (919) 966-1576
Email: janette_goins@med.unc.edu

Clinical Laboratory:

McLendon Clinical Laboratories
University of North Carolina Hospitals
Chapel Hill, NC 27514
Contact: Herbert C. Whinna, MD, PhD, Director
Phone: (919) 966-2318
Fax: (919) 966-6407
Email: herb_whinna@med.unc.edu

Optional:**Co-Investigators intervening with subjects:**

Susan Blevins, ANP-C
Joseph Eron, Jr., MD; David Margolis, MD; David Wohl, MD;
Michelle Floris-Moore, MD, MS; Cynthia Gay, MD, MPH;
Christopher Hurt, MD; Claire Farel, MD

Co-Investigators not intervening with subjects:

William M. Shafer, PhD (Emory University)

UNC Institutional Review Board:

CB 7097
720 Martin Luther King Jr. Blvd.
Bldg # 385, Second Floor
Chapel Hill, NC 27599-7097
Contact: Cassandra Myers, Director
Phone: (919) 966-3113
Fax: (919) 966-7879
Email: irb_questions@unc.edu

Independent Safety Monitor:

David J. Weber, MD
130 Mason Farm Road, CB#7030
2153 Bioinformatics
Chapel Hill, NC 27599
Phone: (919) 966-2536
Fax: (919) 966-1451
pager: (919) 347-0639
Email: dweber@unch.unc.edu

Statistical Data Coordinating Center (SDCC)

The Emmes Corporation
SDCC Project Manager: Amy Price, MS
SDCC Data Manager: Logan Richlak
SDCC Statistician: Amy Tan, MS
401 N. Washington St., Suite 700
Rockville, MD 20850
Phone: (301) 251-1161
Email: sti@emmes.com

2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Gonorrhea remains a major global health concern. With over 106 million cases estimated by WHO in 2008, world-wide incidence of gonorrhea surpassed that of chlamydia as the most common bacterial sexually transmitted infection (STI).(1) The CDC reported over 350,000 cases of *Neisseria gonorrhoeae* infection in the US in 2014, a 5% increase compared to 2013.(2) Uncomplicated gonorrhea can be asymptomatic or manifest as urethritis in men and cervicitis in women. Complications of untreated gonococcal infections include orchitis, epididymitis and pelvic inflammatory disease. Furthermore, like other inflammatory mucosal infections, gonorrhea also facilitates HIV transmission and acquisition.(3-5) Appropriate treatment with effective antimicrobials is essential for control of gonorrhea, and *N. gonorrhoeae* (the gonococcus or GC) has a long history of emerging antimicrobial resistance, reducing the effectiveness of antibiotics used to treat infection. In 2012, the CDC responded to recent dramatic decreases in susceptibility to cefixime among circulating GC strains by recommending against the use of oral cephalosporins as first-line treatment for gonorrhea.(6) Currently, increasing GC resistance to the extended-spectrum injectable cephalosporin, ceftriaxone, threatens the last remaining first-line treatment option.(7) With few new antimicrobial therapies in the pipeline and no effective GC vaccine, fears of incurable gonorrhea are all too real. The potential adverse reproductive health consequences of untreatable GC and attendant increases in HIV transmission in areas where both infections are prevalent is alarming.(8) New therapeutic and vaccine targets for gonorrhea are urgently needed.

Limited in nature to infection of humans, *N. gonorrhoeae* are equipped with multiple mechanisms to subvert our immune responses, and natural GC infection does not result in protective immunity. We hypothesize that mutations that alter or eliminate GC mediators of resistance to human innate immune responses will reduce infectivity, decrease inflammation or increase clearance compared to wild-type (WT) GC. In this clinical trial, we will test the requirement for gonococcal structures that mediate resistance to innate host.

The MtrC-MtrD-MtrE efflux pump endows gonococci with a mechanism to export hydrophobic antibiotics(9) and host-derived antimicrobials (antimicrobial peptides, bile salts and progesterone) that bathe mucosal surfaces.(10, 11) The proteins that form the pump are encoded by the *mtrCDE* operon, which is transcriptionally regulated by *cis*- and *trans*-acting regulatory control processes.(12, 13) Loss-of-function mutations in *mtrCDE* significantly reduce the ability of gonococci to infect the lower genital tract of female mice.¹⁴ Moreover, a null mutation in the *mtrR* gene, which encodes a transcriptional repressor of *mtrCDE*, or mutations in the DNA sequence upstream of *mtrCDE* enhance both antimicrobial resistance¹³ and *in vivo* fitness in the mouse infection model.(14) First with an isogenic *mtrD* mutant and then with an isogenic *mtrE* mutant, we will examine the role of the *mtr* system in the experimental human infection model. Only the *mtrD* mutant will be tested under this version of the protocol; modifications will be submitted as an amendment for future mutants to be tested.

A major limitation in the development of gonococcal vaccines has been the lack of an animal model. We and others have developed a model of experimental human gonococcal infection (reviewed by Hobbs *et al.*(15)) The model takes advantage of the self-limited nature of gonococcal urethral infection in male subjects. Asymptomatic infection can be treated very efficiently and quickly. Urethral infection can be initiated in male volunteer subjects through urethral inoculation, and the experimental infection model has proven to be a safe and efficient means of studying gonococcal infection in men.(15) To date, over 200 subjects have been studied in experimental gonococcal infection trials at UNC without serious complications.(15)

The experimental infection model has been used in several different ways to study the roles of putative *N. gonorrhoeae* virulence determinants in male urethral infection. Early observational studies with wild-type strain FA1090 established infectious doses for this well-described strain(15) and characterized antigenic variation of outer membrane proteins during infection.(16, 17) In later studies to compare the infectivity of WT gonococci with that of isogenic mutants lacking gonococcal virulence factors, individual subjects were inoculated with similar doses of wild-type or mutant bacteria in noncompetitive infection studies. These studies demonstrated that substantial differences in infectivity between strains could be detected with statistical significance using this model with reasonable numbers of subjects. Competitive infection with mixed inocula increases the sensitivity of the model allowing detection of more subtle differences in gonococcal virulence than can be demonstrated in noncompetitive infections with a single test strain.(18, 19)

2.2 Rationale

Despite the robust inflammation that characterizes symptomatic gonorrhea, natural GC infection does not confer protection from subsequent infection. Limited to human hosts in nature, GC are highly adapted to evade our innate defenses. Results of experimental infection studies in the female mouse model have informed the selection of isogenic mutants to be tested in this clinical trial. However, the natural restriction of *N. gonorrhoeae* infection to human hosts limits the extent to which animal models can predict results that are clinically translatable. The experimental human infection model is essential for testing hypotheses regarding the roles of putative virulence factors. The rationale for this study is that the human challenge model so closely resembles natural infection that the results will provide a compelling agenda for scientific research and greatly facilitate vaccine development. ***We hypothesize that key virulence determinants involved in N. gonorrhoeae resistance to innate immunity are essential for infection in the male urethra.*** We predict that mutations abolishing expression of these virulence determinants will eliminate or significantly reduce gonococcal infectivity, thus identifying potential vaccine candidates.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

Subjects will provide blood samples via venipuncture. Risks of venipuncture include:

- acute pain and discomfort
- development of a bruise
- formation of a blood clot in the vein that has been stuck
- infection
- Rarely, blood donors become faint while blood is being obtained.

Subjects may experience some discomfort associated with the urethral catheterization during inoculation including mild irritation or itching for several minutes after the procedure. Risks of gonococcal urethritis include urethral inflammation and the possibility of epididymitis, orchitis, gonococcal bacteremia with attendant meningitis and endocarditis, and immunological responses to infection. The risks of serious complications from urethral gonococcal infection are extremely rare, even in natural infection. Careful screening and observation during participation, habitation in the Clinical and Translational Research Center (CTRC), immediate treatment of signs or symptoms of urethritis and treatment prior to discharge from the CTRC are all designed to minimize the possibility of complications. Among the 219 subjects previously studied, one subject was discovered to have asymptomatic hematuria and one subject experienced dysuria relieved with pyridium. No other complications have been noted.

The research laboratory follow rigorous quality control procedures, nevertheless, the plates on which gonococcal inocula are grown may become contaminated with other bacteria. In 58 different experimental trials including 219 individual subjects, contaminants were found in the inoculum once (1.7% of the time). In one trial, five subjects received contaminated bacterial suspensions. One day after inoculation, we realized that the subjects had been inoculated with contaminants, identified as *Staphylococcus species*. The trial was stopped, and subjects were treated with ceftriaxone for possible gonorrhea. To treat possible staphylococcal infection, four subjects received a 3-day course of Trimethoprim/Sulfamethoxazole (Septra). The fifth subject reported a sulfa allergy and was treated with a 3-day course of Nitrofurantoin (Macrochantin). Subjects returned for follow-up examinations one week after the trial and presented without complaints, signs or symptoms of urethritis; urinalysis results for all subjects were normal.

In response to the adverse event described above, protocol procedures were carefully reviewed and the following corrective and preventive measures have been instituted to reduce the likelihood of similar contamination in future trials. First, all agar plates containing gonococcal growth for use in human challenge experiments shall be thoroughly examined using a dissecting microscope; plates will be examined for at least one full minute each. Plates containing any contaminating colonies will not be used to prepare the inoculum suspension. Second, in addition to quantitative cultures of the inoculum suspension plated on GCB agar to enumerate the dose of gonococci delivered, we will plate the inoculum suspension, undiluted, on LB agar, which does not support the growth of *N. gonorrhoeae*, but does support the growth of common bacterial contaminants (including *Staphylococcus* and *Streptococcus* species). In the event that

contaminating bacteria are detected by growth on LB agar, urine specimens will be obtained from subjects as soon as they return to the research unit for the first overnight stay. Specimens will be cultured and growth identified to guide appropriate antibiotic treatment. As soon as this information is available, subjects will be treated appropriately for the contaminating bacteria and for potential gonococcal infection.

For experimental gonococcal infections, subjects are treated with a third-generation cephalosporin: cefixime (single 400 mg oral dose) or ceftriaxone (single 250 mg dose delivered intramuscularly) when infection is clinically apparent, defined by reported symptoms of urethritis including urethral discharge or dysuria, plus the recovery of viable *N. gonorrhoeae* from urine or urethral swab cultures, or at the end of the inpatient portion of the trial, whichever comes first. In the unlikely event of a treatment failure, alternative treatment would be with a fluoroquinolone (ciprofloxacin, single 500 mg oral dose). Although monotherapy with oral cephalosporins or fluoroquinolones including ciprofloxacin is no longer recommended by the CDC for treatment of naturally-acquired gonorrhea, all *N. gonorrhoeae* strains included in this protocol have documented susceptibility to all antibiotics listed in the protocol that could potentially be used to treat experimental gonococcal infection. Coagulopathy and bleeding have been reported with third-generation cephalosporins, including cefixime and ceftriaxone. Potential side effects including nausea, diarrhea, anorexia can occur, particularly in children. Hematologic reactions are rare, and none of the third-generation cephalosporins is significantly nephrotoxic.(20) Although an increased risk of allergic reaction among patients with penicillin allergy has been observed for some cephalosporins, this risk is quite low for third-generation cephalosporins including cefixime and ceftriaxone.(21) The most frequent reactions to cephalosporins are non-pruritic, non-urticarial rashes, which occur in less than 3% of patients. Peripheral neuropathy is an identified risk of fluoroquinolones, including ciprofloxacin; rapid onset and permanent damage are possible. In addition, fluoroquinolones are rarely associated with tendinopathy, such as Achilles tendon rupture.(22) Allergic reactions, including serious anaphylactic reactions, and central nervous system events, including convulsions, confusion, tremors, hallucinations, and depression, have been reported rarely in patients receiving fluoroquinolone antibiotics including ciprofloxacin. Common side effects of ciprofloxacin, which are usually mild, include nausea, headache, dyspepsia, dizziness, vaginal yeast infection and diarrhea. It is also possible for fluoroquinolones to cause vomiting, rash, and abdominal discomfort/pain. Some patients taking fluoroquinolones may become more sensitive to sunlight or ultraviolet light. On 12 May 2016, the FDA advised that the serious side effects associated with fluoroquinolone antibacterial drugs generally outweigh the benefits for patients with uncomplicated urinary tract infections who have other treatment options. However, if needed in case of cephalosporin treatment failure, a single dose of ciprofloxacin would be used, which minimizes risks other than allergic reactions.

Transmission of *N. gonorrhoeae* to another individual is also a risk of the study, but this has not occurred in previous experimental infection studies at UNC in which 120 subjects were determined to be infected. Subjects sleep in the inpatient unit of the CTRC, and signed consent forms specifically instruct participants to abstain from all sexual activity until the completion of the study and the follow-up test for gonorrhea is negative.

2.3.2 Known Potential Benefits

Direct benefits to subjects are limited to the possible discovery of a previously unrecognized medical condition. In addition, the information generated from the study will allow us to examine hypotheses that individual gonococcal gene products play a role in the ability of *N. gonorrhoeae* to establish infection in the human host. These studies will establish whether specific protein expression is required for urethral infection and may provide a rationale for a gonococcal vaccine.

3 OBJECTIVES

3.1 Study Objectives

Primary:

- Compare infectivity of different isogenic mutants with wild-type (WT) *N. gonorrhoeae* in noncompetitive infections.
- For mutants with WT infectivity, assess relative fitness of the mutant in competitive infections initiated by inocula containing equivalent numbers of both WT and mutant strains. Note, competitive infections will not be performed if non-competitive infections are substantially different. If this is the case, there will be no analysis for this primary objective.

Secondary:

- Compare the clinical course of infection with mutant and WT *N. gonorrhoeae* in noncompetitive infections.

Exploratory:

- Characterize host immune responses during experimental gonococcal infection.
- Characterize bacterial gene expression during experimental gonococcal infection.

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

To compare the ability of mutant gonococci to infect the male urethra, we will first conduct noncompetitive infection studies with inocula containing mutant or wild-type *N. gonorrhoeae*. For infections initiated with individual *N. gonorrhoeae* strains, the primary outcome measure is:

- The proportion of subjects in each group that become infected by day 6 as defined by a positive urine or urethral swab culture.

For isogenic mutants that do not demonstrate significantly lower infectivity compared to WT *N. gonorrhoeae*, we will assess relative fitness of the mutant in competitive infections initiated by inocula containing equivalent numbers of both WT and mutant strains. Competitive infections allow demonstration of a difference in fitness between two strains with statistical confidence

using the smallest possible number of subjects. For infections initiated with mixed inocula, the outcome measures are:

- The proportion of subjects that become infected by day 6 as defined by a positive urine or urethral swab culture.
- The competitive index (CI) of the mutant compared to WT proportion of organisms with the predicted competitive advantage recovered from urine and/or urethral swab specimens from among individual infected subjects, defined by the ratio of colony forming units (cfu) of the two strains recovered from urine cultures on the day of treatment (output) compared to the ratio of strains in the inoculum (input). $CI = \frac{\text{mutant cfu(output)}}{\text{wild-type cfu(output)}} \div \frac{\text{mutant cfu(input)}}{\text{wild-type cfu(input)}}$.

3.2.2 Secondary Outcome Measures

To compare the clinical course of infection with mutant and WT *N. gonorrhoeae* in non-competitive infections, the outcome measures are:

- The occurrence of signs and symptoms of urethritis attributable to gonococcal infection by day 6
- The occurrence of bacteriuria (Log_{10} cfu *N. gonorrhoeae*/mL urine sediment) on day of treatment
- The occurrence of urethritis ($> 5.8 \text{ Log}_{10}$ WBC/mL urine sediment) on day of treatment
- Median time from inoculation to treatment among infected subjects by day 6, by strain

3.2.3 Exploratory Outcome Measures

To characterize host immune responses during experimental gonococcal infection, the outcome measures are:

- The detection of local cytokines in urine and systemic cytokines in blood
- Expression of human genes identified by RNASeq in urine sediment and whole blood

To characterize bacterial gene expression during experimental gonococcal infection, the outcome measure is:

- Expression levels of specific bacterial genes in urine sediment

4 STUDY DESIGN

Subject Recruitment.

Healthy adult men, ≥ 18 and < 36 years old, will be recruited into the study by flyer, newspaper, radio or electronic advertisements for participation in a bacterial infection study. Potential subjects are given preliminary information about the study by phone. Interested subjects then undergo a screening examination and lab tests to provide further information. Written informed consent is obtained both at the screening visit and upon admission to the Clinical and Translational Research Center (CTRC) before inoculation, following thorough discussion with study investigators. Recruitment, screening, and follow-up visits will be conducted in the outpatient unit of the CTRC; trial procedures will be conducted in the inpatient unit, with provisions for subjects to leave the unit during the day. Inclusion and exclusion criteria are described in detail in sections 5.1 and 5.2.

Experimental procedures.

During the screening visit, the consent form is reviewed in detail, and all aspects of the study are discussed. The subject is given ample time to address questions and concerns. If the subject agrees to participate, written consent is obtained for the main study and subjects initial a statement to indicate permission for specimen storage, and a medical history and complete physical are performed. Up to 30 mL of blood and a urine sample are obtained. Screening lab work includes: a complete blood count (CBC) with differential (for WBC count, ANC, and hemoglobin level), serum ALT, serum creatinine, HIV, syphilis, HBV and HCV serologies, CH50 test for complement deficiency, urinalysis and urine nucleic acid amplification testing for chlamydia, gonorrhea and trichomoniasis. Screening precludes enrollment of subjects with health problems, urethral inflammation, STIs, and factors such as complement deficiency that might lead to complications of a local infection. If the results of CBC, serum ALT, creatinine or urinalysis obtained at initial screening are outside acceptable limits, and the clinician judges the deviation unlikely to be clinically relevant, one time repeat screening is permitted. If the results of initial or repeat screening tests are within acceptable limits, the subject may be enrolled in the next scheduled trial within 30 days of screening. Within one week prior to the inoculation visit, subjects are sent a confirmation letter or email with directions to return to the outpatient unit of the CTRC to provide a urine specimen for repeat STI testing and blood for determination of baseline host immune responses 2-4 days before the start date and for presentation at UNC Hospital on the start date.

For inoculation, subjects recline on a hospital bed. The pelvic area is covered with a waterproof plastic drape, and the penis is extended through a hole in the drape. The physician dons sterile gloves immediately before the procedure, as does the subject. The skin around the urethra is cleansed with 70% isopropanol and allowed to dry. Bacterial suspensions in phosphate-buffered saline will be delivered to the anterior urethra through a No.8 pediatric French catheter, inserted 5 cm into the urethra. The PI or designee will load the inoculum into a dedicated pipette, and a licensed physician wearing sterile gloves, will moisten the catheter using sterile saline, insert the catheter and deliver the inoculum using aseptic technique. Inoculation takes only a few seconds

and produces minimal discomfort. Subjects are instructed to apply gentle pressure to the end of the penis for 30 minutes to minimize leakage of the inoculum.

Each day after inoculation, subjects are examined between 6:30am and 7:30am for signs or symptoms of urethritis, and a first-void urine specimen of 50 mL is collected for gonococcal culture, urine dipstick analysis, and microscopic examination of urine sediment for PMNs as an indicator of urethral inflammation. On the day on which a subject is treated, a urethral swab will be collected from each subject for gonococcal culture. All subjects receive antibiotic treatment before discharge from the study. Subjects are treated during the inpatient portion of the study when (1) requested by an individual subject, regardless of signs, symptoms or positive cultures (2) purulent urethral discharge is observed by the examining clinician or reported by the subject or (3) prior to discharge from the CTRC, whether infected or not. Antibiotics are administered by a CTRC nurse in an inpatient room of the research unit; subjects will be observed for approximately 20 minutes following administration of study antibiotics. Subjects return to the CTRC in one week for reexamination and urine testing by nucleic acid amplification and for compensation.

Results from urine cultures are available 24-48h after specimens are plated, however, a positive urine culture in the absence of signs or symptoms does not necessitate immediate treatment. Among previous experimentally infected subjects, 68% (71/104) had positive urine cultures on day 2. A minority of infected subjects (8/107, 7.5%) reported symptoms (ranging from a slight tingling sensation to dysuria) on day 2. Most positive urine cultures (239/269, 89%) were from days without reported symptoms. Bacterial counts from positive cultures generally increased from day 2-6, and the proportion of subjects with positive cultures who were symptomatic increased from 6% to 22% on days 2-5 ($\chi^2 P = 0.035$). However, by day 6, 95% of remaining infected subjects were asymptomatic.

4.1 Substudies

None

5 STUDY ENROLLMENT AND WITHDRAWAL

The study population includes healthy men ≥ 18 and < 36 years old. Due to potential complications from ascendant gonococcal infection, women cannot safely be included. Children under the age of 18 are inappropriate for inclusion in a study of a sexually transmitted infection. The ethnic and racial composition of the study population is expected to reflect those features of the UNC undergraduate, graduate and medical student populations, from which most subjects will be recruited.

For the MtrD Study, we will enroll up to 32 subjects. Potential male subjects will be recruited from the UNC undergraduate, graduate and medical student populations and from neighboring communities by flyer, newspaper and/or electronic advertisements for participation in a bacterial infection study.

5.1 Subject Inclusion Criteria

Subjects must meet all the inclusion criteria to participate in the study.

1. Healthy man ≥ 18 and < 36 years old
2. Able and willing to be located easily by providing street address and telephone number (land line and/or cell phone number)
3. Willingness to provide written informed consent
4. Able and willing to attend all study visits including 6-day stay in the CTRC during the trial (with ability to leave the unit during the day) and follow-up visit during the week after treatment
5. Able and willing to abstain from all sexual activity until completion of the study and the follow-up test for gonorrhea is negative
6. Acceptable medical history by screening evaluation
7. No clinically significant abnormalities on physical exam
8. Urinalysis: leukocyte esterase and WBC values within normal limits
9. CH50 WNL
10. Urine negative for chlamydia, gonorrhea, and trichomonas
11. Negative HIV, syphilis, and HCV test results
12. Negative HBV core and surface antibodies or results consistent with immunization (negative HBV core antibody/positive HBV surface antibody)
13. Denies history of STIs including gonorrhea, chlamydia, syphilis, HIV, HBV, and HCV
14. Denies history of bleeding diathesis
15. Denies history of seizures (due to reports of seizures with ciprofloxacin)
16. Denies history of cancer, except basal cell carcinoma of the skin >5 years ago
17. Denies history of drug abuse
18. Denies history of genitourinary surgery

5.2 Subject Exclusion Criteria

1. Subjects meeting any of the exclusion criteria at screening will be excluded from study participation. Laboratory values are based on the Toxicity Table in [Appendix C](#). Student or employee under the direct supervision of any of the study investigators
2. Any known immunodeficiencies including complement deficiency, antibody deficiency, chronic granulomatous disease or HIV infection
3. Psychiatric disorders that, in the opinion of the physician, would interfere with the integrity of the data or volunteer safety
4. Unstable depression (defined as receiving either <3 months of the same medication (and dose) or a decompensating event during the previous 3 months) or depression that, in the opinion of the investigator, will compromise the subject's ability to comply with protocol requirements
5. Heart murmur or heart disease
6. Anatomic abnormality of the urinary tract
7. Any antibiotic treatment in the past 30 days, or azithromycin in the past 60 days
8. Self-reported chemotherapy within the past year
9. Current steroid use, except for topical application
10. Allergy to penicillin, cephalosporins or ciprofloxacin or to lidocaine
11. Treatment with medications in the previous month that are contraindicated with cefixime, ceftriaxone or ciprofloxacin and that cannot be withheld for the single doses given in this study
12. Serum creatinine level < 0.7 or > 1.75 mg/dL and deemed clinically significant by the study physician
13. Serum ALT level < LLN or > 105 U/L and deemed clinically significant by the study physician
14. WBC count < 2.5 or > 15.0 x10⁹/L and deemed clinically significant by the study physician
15. Absolute neutrophil count (ANC) < 1.5 or > 7.5 x 10⁹/L and deemed clinically significant by the study physician
Exception: For African Americans, ANC values as low as 1.3 x 10⁹/L will be allowed (25)
16. Hemoglobin level < 12.0 g/dL or above ULN and deemed clinically significant by the study physician
17. Urinalysis: Qualitative protein level > 1+ or RBC count > 10/hpf

Medications not permitted with cefixime or ceftriaxone:

- Warfarin
- Probenecid
- Aspirin
- Diuretics such as furosemide
- Aminoglycoside antibiotics
- Chloramphenicol

Medications not permitted with ciprofloxacin:

-
- Tizanidine
 - Theophylline
 - Warfarin
 - Glyburide
 - Cyclosporine
 - Probenecid
 - Phenytoin
 - Methotrexate
 - Antacids, multivitamins, and other dietary supplements containing magnesium, calcium, aluminum, iron or zinc
 - Caffeine-containing medications
 - Sucralfate or didanosine chewable or buffered tablets

5.3 Treatment Assignment Procedures

5.3.1 Randomization Procedures

This is not a randomized trial. In cohorts (up to 8 subjects enrolled on the same date) for noncompetitive infection groups (up to 8 subjects per group as shown in Schematic of Study Design on p. xii), all subjects enrolled on a given date will receive the same inoculum suspension containing either mutant or WT *N. gonorrhoeae*. In cohorts for competitive infection groups (up to 16 subjects as shown in Schematic of Study Design on p. xii), all subjects will receive the same inoculum suspension containing approximately equal numbers of the two bacterial strains under investigation. The identity of the suspension to be used for each cohort is determined by the results of previous studies. Subjects will not know which bacterial strain is being used for the cohort in which they are enrolled.

5.3.2 Masking Procedures

Because all subjects in a given cohort will receive the same inoculum suspension, masking procedures during the trial are not applicable. Laboratory personnel performing assays to monitor host responses will be blinded to the subjects' infection status at the time of specimen collection.

5.3.3 Reasons for Withdrawal

A study subject will be discontinued from participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, intercurrent illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.

-
- A subject signs the consent and changes his mind about participating in the study before inoculation; he will be withdrawn from study participation.
 - A urethral abnormality that prevents normal insertion of the catheter is found during the inoculation procedure. The subject will be treated to prevent infection from potential bacterial exposure during attempted inoculation and will be withdrawn from study participation. A follow-up visit will be scheduled for reexamination and urine testing for *N. gonorrhoeae* infection.
 - Following inoculation, subjects will be withdrawn if they experience trauma from insertion of the catheter as determined by 5 or more red blood cells per high power field observed in urine sediment, or if they develop an infection unrelated to the study that requires antibiotic treatment. Subjects who are withdrawn for urethral trauma will be treated whether known to be infected or not, and a follow-up visit will be scheduled for urine testing for *N. gonorrhoeae* infection, and the subject will be contacted for the 2-week final follow-up call.

Subjects are free to withdraw from participating in the study at any time upon request.

5.3.4 Handling of Withdrawals

Occasionally, subjects fail to appear for inoculation after initially signing the informed consent form at screening. For subjects who fail to appear on the day of inoculation, previously obtained blood and urine specimens will be destroyed. All subjects who are inoculated, or for whom inoculation is attempted but unsuccessful due to a previously unrecognized anatomical abnormality, will be treated and followed for safety even if they decide to withdraw from the study or are withdrawn for any of the reasons listed above in section 5.3.3. Subjects who are withdrawn after inoculation or attempted inoculation will be scheduled to return for the 1 week follow-up visit including the test of cure and will be contacted 2 weeks after the subject received treatment for the final follow-up phone call. Withdrawn subjects may be replaced. If the results of the withdrawn subject(s) would have impacted the decision to continue to enroll up to 8 subjects in the mutant group, then the subject(s) will be replaced. Otherwise, the withdrawn subject(s) will not be replaced. Similarly, for competitive infections, the withdrawn subject(s) will only be replaced if their results would have impacted the decision to continue to enroll up to 16 subjects.

5.3.5 Termination of Study

Although the study Sponsor has every intention of completing the study, it 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 due to SMC review and recommendation; and/or at the discretion of DMID.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

N. gonorrhoeae strain FA1090, the parent strain for isogenic mutants in this study, is serum and streptomycin resistant and is sensitive to cefixime, ciprofloxacin and ceftriaxone. This strain expresses porin serovar PIB3 and is a proline auxotroph. All FA1090 derivatives used for experimental infection studies retain the antibiotic susceptibility profile of the parent without additional resistance markers. The genome of strain FA1090 was sequenced with support from USPHS/NIH grant #AI38399, and B.A. Roe, L. Song, S. P. Lin, X. Yuan, S. Clifton, Tom Ducey, Lisa Lewis and D.W. Dyer at the University of Oklahoma. The GenBank accession number for the completed *Neisseria gonorrhoeae* genome is AE004868. Strain FA1090 has been submitted to the ATCC and given accession number ATCC 700825.

6.1 Study Product Description

6.1.1 Acquisition

FA1090 has been passaged repeatedly in the research laboratories at UNC since its isolation from a woman with cervicitis in the 1970s at UNC Hospitals. The original inoculum stock used at UNC in experimental human infection studies, prepared in 1991, was an Opa-negative, piliated variant designated FA1090 A21.(17) Two independent second generation stocks, A22 and A23 (also Opa-negative and piliated) were prepared from FA1090 A21 in 1994 and 1997, respectively. In December 2015, the isogenic mutant FA7537 (FA1090 \square *mtrD*) that does not express MtrD and the fourth generation wild type, Opa-negative, piliated variant, FA1090 A26 were derived directly from FA1090 A25. The isogenic mutant strain to be used in the MtrD Study was constructed in the laboratory of Dr. William Shafer at Emory University and characterized for experimental human infections in the laboratory of Dr. Marcia Hobbs at the University of North Carolina at Chapel Hill. FA7537 contains a deletion of the *mtrD* gene, made without leaving behind a selectable marker as previously described.(23) Briefly, a two-gene cassette containing both a selectable marker (chloramphenicol acetyl transferase [CAT] conferring chloramphenicol resistance) and a counter selectable marker (*rpsL*, conferring streptomycin susceptibility on the naturally resistant FA1090) was cloned into the gene of interest and used to replace the wild-type gene on the chromosome by allelic exchange. A second transformation replaced the cassette-containing version of the gene with an engineered version with an unmarked deletion or other mutation. Importantly, susceptibility to cefixime, ceftriaxone and ciprofloxacin are documented for FA7537 and all *N. gonorrhoeae* strains for use in experimental infection studies. All strains will be reviewed and approved for use in the experimental human infection model by the Institutional Biosafety Committee (IBC) of the University of North Carolina at Chapel Hill.

6.1.2 Formulation, Packaging, and Labeling

All components used to make the media and buffers used to prepare the bacterial inocula are made from sequestered lots whose certificates of analysis are on file. GC agar base (used to

prepare solid microbiological media [GCB agar] on which bacterial inoculum strains are grown) and Trypticase soy broth powder (used to prepare freezer storage medium [FSM] in which master/working bank stocks are stored) are derived from animal sources. We use GC agar base and trypticase soy broth powder only made from certified BSE-free herds from BSE-free countries. Tests of sterility are also done on all media and buffers used to prepare the inoculum. These media and buffers are clearly marked as use only for experimental human infection and are stored in a cabinet separate from other laboratory reagents.

A master/working bank of each individual *N. gonorrhoeae* strain to be used in experimental human infections will be prepared from individually characterized colonies grown on GCB agar, selected to be phenotypically Opa-negative and expressing full-length lipooligosaccharide to match the characteristics of previous inocula.^{24, 29, 30} For each strain used to prepare a master/working bank, susceptibility to cefixime, ciprofloxacin and ceftriaxone will be confirmed in the University of North Carolina Hospitals McLendon Clinical Laboratories. Master/working banks will be amplified in one passage from selected colonies, suspended in freezing medium, and stored as multiple identical aliquots at $\leq -70^{\circ}\text{C}$ in a separate freezer compartment containing only *N. gonorrhoeae* for use in experimental human infection trials. No other bacteria or laboratory *N. gonorrhoeae* strains are stored in this freezer compartment. For master/working banks to be used more than 1 year after initial preparation, cfu/mL will be enumerated from a single vial every 18 months; if the concentration of *N. gonorrhoeae* in the vial is $<10^5$ cfu/mL, a new master/working bank shall be generated. For mixed infections, material from a single master/working bank vial for each inoculum strain will be grown overnight on GCB agar plates. Individual suspensions will be made in sterile phosphate-buffered saline (PBS) and bacterial concentrations will be estimated using a spectrophotometer. To prepare the desired mixture, approximately equal concentrations of the two strains will be combined in freezing medium. Mixed inocula will be aliquoted into single use vials labeled with the strain names and date of preparation and stored at $\leq -70^{\circ}\text{C}$ in the specified restricted freezer compartment until use. To confirm the ratio of the 2 strains in the mixture and to test for the presence of contaminants, the contents of one vial of the working stock will be grown on GCB agar overnight, resuspended in PBS and enumerated by CFU. Growth on plates will be inspected visually and using a dissecting microscope to confirm the presence of only *N. gonorrhoeae* colonies. Gonococcal colonies will be analyzed by culture on selective media to distinguish the individual strains or by PCR with specific primers to determine the proportion of each strain in the mixture. Inoculum mixtures prepared and stored as described are stable, and the proportions of individual strains recovered from single use vials are consistent in material analyzed up to 6 months after the initial working stock is prepared. A previous 50:50 mixture sampled 5 times over a 6 month period yielded a mean proportion of 53:47 with a standard error of 1.36. For working stock mixtures to be used more than 1 year after initial preparation, the composition of the stock will be verified before use to prepare an inoculum for experimental use.

6.1.3 Product Storage and Stability

Please refer to Sections 6.1.1 and 6.1.2 for storage and stability information regarding bacterial suspensions. The main supply of vials containing frozen stocks of *N. gonorrhoeae* is kept in a single compartment of a freezer maintained at $\leq -70^{\circ}\text{C}$. No other bacteria or laboratory *N. gonorrhoeae* strains that have not been approved by the Institutional Biosafety Committee for

prepare solid microbiological media [GCB agar] on which bacterial inoculum strains are grown) and Trypticase soy broth powder (used to prepare freezer storage medium [FSM] in which master/working bank stocks are stored) are derived from animal sources. We use GC agar base and trypticase soy broth powder only made from certified BSE-free herds from BSE-free countries. Tests of sterility are also done on all media and buffers used to prepare the inoculum. These media and buffers are clearly marked as use only for experimental human infection and are stored in a cabinet separate from other laboratory reagents.

A master/working bank of each individual *N. gonorrhoeae* strain to be used in experimental human infections will be prepared from individually characterized colonies grown on GCB agar, selected to be phenotypically Opa-negative and expressing full-length lipooligosaccharide to match the characteristics of previous inocula.^{24, 29, 30} For each strain used to prepare a master/working bank, susceptibility to cefixime, ciprofloxacin and ceftriaxone will be confirmed in the University of North Carolina Hospitals McLendon Clinical Laboratories. Master/working banks will be amplified in one passage from selected colonies, suspended in freezing medium, and stored as multiple identical aliquots at $\leq -70^{\circ}\text{C}$ in a separate freezer compartment containing only *N. gonorrhoeae* for use in experimental human infection trials. No other bacteria or laboratory *N. gonorrhoeae* strains are stored in this freezer compartment. For master/working banks to be used more than 1 year after initial preparation, cfu/mL will be enumerated from a single vial every 18 months; if the concentration of *N. gonorrhoeae* in the vial is $<10^5$ cfu/mL, a new master/working bank shall be generated. For mixed infections, material from a single master/working bank vial for each inoculum strain will be grown overnight on GCB agar plates. Individual suspensions will be made in sterile phosphate-buffered saline (PBS) and bacterial concentrations will be estimated using a spectrophotometer. To prepare the desired mixture, approximately equal concentrations of the two strains will be combined in freezing medium. Mixed inocula will be aliquoted into single use vials labeled with the strain names and date of preparation and stored at $\leq -70^{\circ}\text{C}$ in the specified restricted freezer compartment until use. To confirm the ratio of the 2 strains in the mixture and to test for the presence of contaminants, the contents of one vial of the working stock will be grown on GCB agar overnight, resuspended in PBS and enumerated by CFU. Growth on plates will be inspected visually and using a dissecting microscope to confirm the presence of only *N. gonorrhoeae* colonies. Gonococcal colonies will be analyzed by culture on selective media to distinguish the individual strains or by PCR with specific primers to determine the proportion of each strain in the mixture. Inoculum mixtures prepared and stored as described are stable, and the proportions of individual strains recovered from single use vials are consistent in material analyzed up to 6 months after the initial working stock is prepared. A previous 50:50 mixture sampled 5 times over a 6 month period yielded a mean proportion of 53:47 with a standard error of 1.36. For working stock mixtures to be used more than 1 year after initial preparation, the composition of the stock will be verified before use to prepare an inoculum for experimental use.

6.1.3 Product Storage and Stability

Please refer to Sections 6.1.1 and 6.1.2 for storage and stability information regarding bacterial suspensions. The main supply of vials containing frozen stocks of *N. gonorrhoeae* is kept in a single compartment of a freezer maintained at $\leq -70^{\circ}\text{C}$. No other bacteria or laboratory *N. gonorrhoeae* strains that have not been approved by the Institutional Biosafety Committee for

use in experimental human infection studies are stored in this freezer compartment. A subset of 1-3 vials of each stock is stored in a separate freezer maintained at $\leq -70^{\circ}\text{C}$ in a laboratory in a separate building on the UNC campus as a backup in case of extended power failure in the primary storage location.

Cefixime, ciprofloxacin, ceftriaxone, lidocaine and epinephrine are obtained from commercial sources and stored according to the instructions in the package inserts.

6.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product

For preparation of an inoculum for an experimental infection trial, the contents of one single-use vial from the appropriate working stock will be dispensed onto 5 individual GCB agar plates the day before scheduled inoculation. Inoculum plates will be prepared in a calibrated laminar-flow hood in the research laboratory. The hood will be thoroughly disinfected before and after use. Plates will be streaked with a sterile microbiological loop in a pattern to yield areas of dense growth as well as areas of isolated colony growth on the plate after incubation. On the day of inoculation, The PI or designee will examine growth on the plates by eye and with a dissecting microscope for purity and correct colony phenotype. Any plates containing contaminating bacterial growth will be discarded. From an area with isolated colony growth, several colonies from each plate will be tested with the oxidase reagent; colonies must test positive (turn dark purple within 30 seconds) to use growth from that plate for the inoculum suspension. Plates passing all pre-inoculation tests will be loaded into a candle canister for transport to the CTRC laboratory.

When all subjects have been prepared for inoculation in the CTRC inpatient unit, working in a disinfected dead air box providing a circulation free environment with UV light sterilization to protect the product from contamination, in the CTRC Investigators' laboratory, The PI or designee will prepare a bacterial suspension in sterile PBS from growth harvested with a sterile cotton swab from agar plates. Plates are retained for later examination for contamination. The suspension will be passed through a 1.2 μm sterile syringe filter to remove bacterial clumps leaving a uniform suspension of gonococci. The optical density of the filtered suspension will be determined as absorbance at 550 nm wavelength (A550). This value will be compared to previously determined A550 readings of filtered gonococcal suspensions for which the number of colony forming units (CFU)/mL are known. The A550 reading is adjusted by adding sterile PBS or more filtered bacteria to achieve the desired absorbance range corresponding to the target concentration of CFU/mL in the tube. The tube containing the final suspension will be delivered to the trial physician for inoculation. Inoculations will be completed within 30-minutes of the preparation of the bacterial suspension. A portion of the inoculum suspension is plated before the first and after the final subject is inoculated to rule out contamination and confirm the bacterial dose delivered.

For inoculation, subjects recline on a hospital bed and are required to don sterile gloves immediately before the procedure. The pelvic area is covered with a waterproof plastic drape,

and the penis is extended through a hole in the drape. The skin around the urethra is cleansed with 70% isopropanol and allowed to dry. Allergic reactions and other side effects of topical alcohol antiseptics are rare. The individually wrapped, premoistened gauze pads are the same as those routinely used in the hospital to cleanse skin before venipuncture. Up to 0.4 mL of bacterial suspension in phosphate-buffered saline will be delivered to the anterior urethra through a No.8 pediatric French catheter, inserted 5 cm into the urethra. The PI or designee will load the inoculum into a dedicated pipette, and a licensed physician wearing sterile gloves, will moisten the catheter using sterile saline, insert the catheter and deliver the inoculum using aseptic technique. Inoculation takes only a few seconds and produces minimal discomfort. Subjects are instructed to apply gentle pressure to the end of the penis for 30 minutes after inoculation to prevent leakage of the bacterial suspension. Subjects will be examined for signs or symptoms of urethritis and first-void urine will be collected daily. Subjects will be treated with cefixime (single 400 mg oral dose) or ceftriaxone (250 mg IM single dose) when infection is clinically apparent or at the end of the inpatient portion of the trial. On the day a subject is treated, a urethral swab will be collected from each subject for gram stain and gonococcal culture. Subjects will return within 7 days of the inpatient portion of the trial for reexamination and urine testing by nucleic acid amplification. In the unlikely event of antibiotic treatment failure, defined by a positive test of cure, subjects will be treated with ciprofloxacin (single 500 mg oral dose).

For noncompetitive infections in the MtrD Study, 10^6 CFU of FA1090 A26 (WT) or FA7537 (*ΔmtrD*), representing estimated ID₈₀, will be used for urethral inoculation. For competitive infections, equivalent numbers of WT FA1090 and the isogenic mutant, in a total dose ranging from 10^5 - 10^6 CFU, representing estimated ID₅₀₋₈₀, will be used for urethral inoculation. During experimental trials, output bacteria will be recovered from infected subjects daily from first void urine and at the end of the inpatient portion of the trial from a urethral swab, and the numbers of gonococci in each urine specimen will be enumerated by CFU. Strains from mixed infections will be distinguished by their ability to grow on culture medium without polymixin B or colistin (both FA1090 A26 and FA7537 will grow) and on culture medium containing polymixin B or colistin (only FA1090 A26 will grow).

6.3 Modification of Study Intervention/Investigational Product for a Participant

All participants in noncompetitive infection cohorts will receive the same inoculum containing WT or isogenic mutant. All participants in individual competitive infection cohorts will receive the same mixed inoculum at the dose indicated for the specific experimental infection trial.

6.4 Accountability Procedures for the Study Intervention/Investigational Product(s)

Preparation and characterization of master/working banks of individual strains and mixtures will be performed by Dr. William Shafer, Ms. Lorraine Balletta or Dr. Andreea Waltmann and

overseen by Dr. Marcia Hobbs. Please refer to section 6.2 for accountability of inoculum preparation and delivery; these details remain constant for all experimental infection trials to be conducted under this protocol, regardless of the *N. gonorrhoeae* strain(s) to be included in the inoculum.

6.5 Assessment of Subject Compliance with Study Intervention/Investigational Product

The administration of the investigational product to subjects will be under the direct control of the Principal Investigator, Dr. Marcia Hobbs and the trial physician, Dr. Joseph A. Duncan.

6.6 Concomitant Medications/Treatments

The study population will consist of healthy 18-35 year old men. Subjects will be excluded if there has been use of any antibiotics in the month prior to enrollment. During the study, all subjects will be treated with cefixime (single 400 mg oral dose) or ceftriaxone (250 mg IM single dose) when (1) he requests treatment, regardless of signs, symptoms or positive cultures (2) purulent urethral discharge is observed by the examining clinician or reported by the subject or (3) 5 days after inoculation, regardless of infection status. In the unlikely event of antibiotic treatment failure defined by a positive test of cure (which has never happened among more than 200 infected subjects to date), subjects will be treated with ciprofloxacin (single 500 mg oral dose).

Medications *not* permitted with cefixime or ceftriaxone:

- Warfarin
- Probenecid
- Aspirin
- Diuretics such as furosemide
- Aminoglycoside antibiotics
- Chloramphenicol

Medications *not* permitted with ciprofloxacin:

- Tizanidine
- Theophylline
- Warfarin
- Glyburide
- Cyclosporine
- Probenecid
- Phenytoin
- Methotrexate
- Antacids, multivitamins, and other dietary supplements containing magnesium, calcium, aluminum, iron or zinc
- Caffeine-containing medications

-
- Sucralfate or didanosine chewable or buffered tablets

In the event of an anaphylactic reaction to administration of the bacteria or to a study medication, the subject will be treated with 0.3 mg of epinephrine intramuscularly in the thigh using an EpiPen autoinjector. Emergency services (911) will be called, and the subject will be transported to the University Hospital Emergency Room.

7 STUDY SCHEDULE

7.1 Screening

- The study is explained in detail by the study coordinator, and subjects are given the opportunity to read the informed consent form and ask questions.
- If a subject agrees to participate, written informed consent is obtained for the main study and subjects initial a statement indicating permission for specimen storage. Subjects will complete a True/False test of understanding ([Appendix B](#)) following review of the consent form.
- Perform a complete physical exam.
- Obtain medical history and concomitant medications (prescription and over-the-counter drugs taken 30 days prior to enrollment will be reviewed and documented).
- Vital signs are obtained.
- Twenty-five mL of first catch urine is collected for urinalysis and nucleic acid amplification testing to rule out infection with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (CT/NG), and *Trichomonas vaginalis* (TV). Screening test results outside acceptable limits (as detailed in sections 5.1 and 5.2) will exclude subjects from the trial; they will be referred to appropriate caregivers to further investigate the reasons for results deemed clinically significant by the study physician.
- Up to 30mL of blood is collected for CBC with differential (for WBC, PMN and hemoglobin results), CH50 determination to rule out complement deficiency, ALT to rule out liver impairment, creatinine to rule out renal impairment, HIV, syphilis, HBV and HCV serologies. If the results of CBC, serum ALT, serum creatinine, or urinalysis obtained at initial screening are outside acceptable limits, and the clinician judges the deviation unlikely to be clinically relevant, one-time repeat screening is permitted. Positive HIV, abnormal CH50, repeated CBC, ALT or creatinine results outside acceptable limits will exclude subjects from the trial; they will be referred to appropriate caregivers to further investigate the reasons for results deemed clinically significant by the study physician.
- Subjects are contacted when screening test results are available. Subjects with positive results are excluded from participation in the study and referred to primary care.
- Screening is completed within 30 days prior to the inoculation visit.
- Subjects return to the Clinical Translational Research Center outpatient unit 2-4 days before enrollment and inoculation to provide a urine specimen for repeat testing for CT/NG and TV and for storage. In addition, up to 20mL of blood is obtained for studies of immune responses to gonorrhea. Results of the eligibility STI testing will be reviewed prior to

enrollment; all results must be negative to proceed with inoculation. Subjects with positive results are excluded from participation in the study and referred to primary care.

7.2 Enrollment/Baseline

- Each experimental trial begins with enrollment and inoculation for Visit 1 on Study Day 1. Subjects are instructed to report to the hospital for admission to the Clinical Translational Research Center inpatient unit at the earliest time permitted by the hospital schedule, and inoculation takes place between 9:00am and noon.
- In an individual inpatient room in the CTTC, the trial physician reviews screening medical history, concomitant medications, physical exam and laboratory results to verify that the subject meets inclusion criteria for study entry. Results of STI testing from urine obtained 2-4 days previously will be reviewed prior to enrollment; all results must be negative to proceed with inoculation. Subjects with positive results are excluded from participation in the study and referred to primary care. Informed consent statements are reviewed explaining procedures and risks. Subjects are given the opportunity to withdraw if they do not accept the risks of the study. Subjects who agree to proceed with participation in the study will sign and date the study consent form. Subjects are offered copies of the signed consent form.
- Vital signs are obtained, and the study physician's assistant, research nurse or trial physician conduct a targeted physical exam including brief reexamination of the genitals to exclude any new or previously unrecognized urethral abnormalities.
- For inoculation, subject reclines on a hospital bed and is required to don sterile gloves immediately before the procedure. The pelvic area is covered with a waterproof plastic drape, and the penis is extended through a hole in the drape. The skin around the urethra is cleansed with 70% isopropanol and allowed to dry. Bacterial suspensions in phosphate-buffered saline are delivered to the anterior urethra through a No.8 pediatric French catheter, inserted 5 cm into the urethra. A licensed physician wearing sterile gloves, will moisten the catheter using sterile saline, insert the catheter and deliver the inoculum using aseptic technique. Inoculation takes only a few seconds and produces minimal discomfort. The subject is instructed to apply gentle pressure to the end of the penis for 30 minutes to prevent leakage of the inoculum. Subjects may sign out of the research unit on the day of inoculation, no sooner than 45 minutes after the procedure. Any AE will be assessed prior to discharge from the clinic.

7.3 Follow-up

- Each day, up to 5 days after inoculation (Visits 2-6 on Study Days 2-6), the subject undergoes a targeted physical exam in the inpatient ward of the clinical research center by a study physician, research nurse or physician's assistant, usually between 6:30am and 7:30am for signs or symptoms of urethritis, review of adverse events, and a first-void urine specimen

of approximately 50 mL is collected for gonococcal culture, urine dipstick analysis, microscopic examination of urine sediment for PMNs as an indicator of urethral inflammation, and for storage.

- Study personnel will review subject's medical history and medications.
- Daily vital signs are obtained in the morning, typically between 6:00am and 8:00am, by CTRC nursing staff and include blood pressure, heart rate, respiration rate and temperature. In the event abnormal values (indicated in Table 1 below) are obtained, the trial physician will be called and s/he will use good clinical judgment to ensure appropriate treatment.

Table 1. Abnormal vital signs

Vital sign	Value resulting in call to physician
Blood pressure	Diastolic > 90
Heart rate	Pulse > 130
Respiration rate	> 25
Temperature	> 100.6 °F (> 38.1 °C)

- The subject is treated if (1) he requests treatment regardless of signs, symptoms or positive cultures (2) purulent urethral discharge is observed by the examining clinician or reported by the subject or (3) 5 days after inoculation, regardless of infection status. Treatment is a single observed 400 mg oral dose of cefixime. If he cannot swallow the oral pill, he will be treated with a single intramuscular dose of ceftriaxone (250 mg). Before antibiotic treatment on Day 6, or the day on which a subject is treated during the trial, a urethral swab and urine will be collected, and up to 20 mL of blood will be collected for studies of host immune responses during infection. Subjects will be observed for approximately 20 minutes after administration of study antibiotics. Any AE will be assessed prior to discharge from the clinic.
- Subjects who do not require treatment based on the criteria above may leave the CTRC ward as needed during the study on Days 1-6, provided they return to give specimens, identify their location when they are not on the ward, and stay overnight on the ward. In most instances, subjects will be able to leave the ward by 7:15am and return in the evening with a 9:00pm curfew to arrive back at the ward. Subjects will carry a cell phone so we can reach them at all times, and especially if they violate the agreed curfew. If a subject is not on the ward by 9:00pm, CTRC research staff will contact him and instruct him to return immediately.

7.4 Final Study Visit

- Subjects return to the outpatient unit of the CTRC 3 to 7 days following antibiotic treatment (Visit 7, Study Day 5-13, depending on how soon after inoculation an individual subject is treated and released from the clinical research unit) for re-examination by the trial clinician and urine testing for *N. gonorrhoeae* by nucleic acid amplification, and for compensation. At this visit, the subject will receive a targeted physical examination, review of medical history and medications, review of symptoms and Adverse Events, and vital signs will be obtained. Specimens obtained at this visit will include up to 20 mL of blood and 50 mL of first-void urine for urinalysis, CT/NG testing, and storage for studies of host immune responses post-infection.
- A final follow-up call will be conducted 7-10 days after the follow-up visit in the outpatient unit (Visit 8, Study Day 12-23). Subjects will be queried about symptoms or other problems potentially related to participation in the trial.

7.5 Early Termination Visit

- If a subject elects to terminate the study early following inoculation, he will receive treatment as described above, and follow-up Visit 7 for re-examination, test of cure and compensation will be scheduled, and the final follow-up call for Visit 8 will be conducted.

7.6 Unscheduled Visit

- During the trial, subjects receive a scheduled examination first thing in the morning, typically between 6:30am and 7:30am. Subjects are instructed to call and return to the research unit if they experience symptoms at anytime they are away from the CTRC. Nursing staff are available 24 hours/day in the inpatient unit and will page the on-call study clinician, who will call the subject to discuss the severity of symptoms and meet the subject in his inpatient room on the research ward if needed for evaluation, specimen collection and treatment as described above in section 7.3. Subjects who choose to remain in the unit can directly contact the nurse on duty. For any such unscheduled, but not unanticipated event, the nurse on duty pages the study clinician, who contacts the subject as soon as possible and sees the subject in the CTRC as needed.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

At screening, a standard medical history and complete physical examination will be performed and documented by the study physician's assistant in an outpatient room at the CTCRC.

Specifically, information will be obtained to exclude individuals with any of the following:

- History of STIs including gonorrhea, chlamydia, syphilis, HIV, hepatitis B & C
- History of bleeding diathesis
- History of seizures (due to reports of seizures with ciprofloxacin)
- History of cancer, except basal cell carcinoma of the skin more than 5 years ago
- History of drug abuse
- History of psychiatric disorders, except depression controlled by medication
- History of genitourinary surgery
- Any known immunodeficiencies including complement deficiency, antibody deficiency, chronic granulomatous disease or HIV infection
- Heart murmur or heart disease
- Clinically relevant abnormalities on physical exam
- Abnormal urine leukocyte esterase or WBC
- Protein or RBC in urine outside allowable limits
- WBC count, ANC, or hemoglobin level outside allowable limits
- Serum creatinine or ALT levels outside allowable limits
- Positive screening test for CT, NG or TV
- Positive serology for HIV, syphilis, HBV or HCV
- Anatomic abnormality of the urinary tract
- Antibiotic treatment in the past 30 days or azithromycin in the past 60 days
- Chemotherapy within the past year
- Current steroid use, except for topical application
- Allergy to penicillin, cefixime, ceftriaxone, ciprofloxacin or to lidocaine
- Treatment with medications that are contraindicated with cefixime, ceftriaxone or ciprofloxacin and that cannot be withheld for the single doses given in this study.

Two to four days before enrollment, subjects will provide a urine specimen for CT/NG and TV testing to confirm eligibility. At the enrollment visit and before inoculation, results from the eligibility testing will be reviewed; subjects with any positive test will be excluded. Provided all STI test results are negative, a brief urethral examination will be performed before inoculation.

For five days after inoculation, subjects will be examined daily. The following will be solicited during the targeted genital examination:

Subjective symptoms:

- Tingling
- Irritation or itching
- Dysuria

-
- Urinary frequency
 - Testicular pain
 - Feverish
 - Chills
 - Malaise
 - Diarrhea
 - Rash

Objective signs:

- Urethral discharge

A first-void urine specimen will be collected. All subjects will receive antibiotic treatment before discharge from the inpatient portion of the study. Subjects are treated if (1) he requests treatment regardless of signs, symptoms or positive cultures (2) purulent urethral discharge is observed by the examining clinician or reported by the subject or (3) 5 days after inoculation, regardless of infection status. On the last day of the inpatient portion of the trial, or the day on which a subject receives treatment for gonococcal infection, a urethral swab will be obtained. Subjects will be observed for approximately 20 minutes following administration of study antibiotics.

Subjects return to the CTRC within one week for re-examination by a physician or physician's assistant licensed to treat gonococcal infections and their complications, and a urine CT/NG NAAT test of cure. The test of cure is a nucleic acid amplification test. In the unlikely event of a positive test of cure, we will obtain a urethral specimen for culture to enable antibiotic susceptibility testing.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

- Serum CH50 test for complement deficiency, creatinine, ALT and HIV, syphilis, HBV and HCV serologies and CBC at screening
- Urine-based nucleic acid amplification testing for CT/NG and TV and urinalysis at screening; urine CT/NG and TV tests 2-4 days prior to enrollment; urine CT/NG test and urinalysis at 1 week follow-up (Day 5-13).

8.2.2 Special Assays or Procedures

- Daily urine sediment culture for *N. gonorrhoeae* to monitor infection (Study Days 2-6). Bacteria isolated from urine cultures will be enumerated, and individual colonies will be stored for research testing to compare numbers and the genotypes and phenotypes of output compared to input organisms.

-
- Daily urine dipstick analysis for hematuria (Study Days 2-6).
 - Daily microscopic examination of urine sediment and quantitation of WBCs to monitor inflammation (Study Days 2-6).
 - Stored urine (collected 2-4 days prior to enrollment, on Study Days 2-6, and at 1 week follow-up [Study Days 5-13]) and blood (collected 2-4 days prior to enrollment, on day of antibiotic treatment, and at 1 week follow-up [Study Days 5-13]) from subjects who provide permission for stored specimens will be used to measure cytokines, chemokines and other molecules that may mediate host immune responses to gonococcal infection. Levels of individual cytokines will be assessed using commercially available ELISAs. Multiplexed cytokine analysis will be measured using slide-based anticytokine antibody arrays or microparticle based arrays. Activation of inflammatory cell death programs will be assessed by staining with a fluorescent stain excluded by viable cells followed by quantitation of staining by flow cytometry. Assays of immunological parameters are purely for pursuit of research objectives, and results will not be used for clinical purposes.

8.2.3 Specimen Preparation, Handling, and Shipping

- Approximately 30 mL of blood will be obtained at screening. Screening labs will be sent to the NC Hospitals McLendon Clinical Laboratories for CH50, CBC, ALT, creatinine and HIV, syphilis, HBV and HCV serology tests. Blood and urine obtained at the eligibility testing visit and at followup will be sent to Dr. Hobbs' Laboratory where specimens will be processed and stored at $\leq -70^{\circ}\text{C}$ according to laboratory SOPs.
- Urine obtained at screening, before enrollment and at followup will be sent to the NC Hospitals McLendon Clinical or UNC CFAR VIM Core Laboratories for nucleic acid amplification testing for *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis*. Excess urine will be transferred to Dr. Hobbs' Lab, aliquoted and stored frozen at $\leq -70^{\circ}\text{C}$.
- Daily urine specimens will be processed by Dr. Hobbs or other study personnel in the investigators' laboratory at the CTRC. Urine dipstick analysis will be performed on whole urine (8-10 mL). This volume will then be centrifuged in the investigators' lab at the CTRC, and sediment will be examined microscopically to enumerate WBCs. The remaining urine from the original 50-mL specimen will be centrifuged separately. The supernatant from the spun urine will be aliquoted and stored frozen at $\leq -70^{\circ}\text{C}$ in Dr. Hobbs' Lab. The urine sediment will be inoculated onto GCB agar plates for *N. gonorrhoeae* culture; inoculated plates will be loaded into candle jars for transport from the CTRC to Dr. Hobbs' Lab. Individual *N. gonorrhoeae* colonies isolated from urine cultures (up to 100 per subject per day) will be stored frozen at $\leq -70^{\circ}\text{C}$ in Dr. Hobbs' Lab.

8.2.3.1 Instructions for Specimen Preparation, Handling, and Storage

- Specimens obtained from subjects who deny permission for long-term storage will be indicated with an easily visible sticker marked NO STORAGE. Such specimens will be destroyed at the conclusion of the study.

SOPs for specimen preparation, handling and storage according to the information in Section 8.2.3 will be submitted to the Clinical Project Manager following DMID protocol approval.

8.2.3.2 Specimen Shipment

No shipping to external facilities is required for this study. Specimens will be transported by hospital or study personnel in appropriate biosafety containers/bags.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

To minimize risks, the study is designed to enroll healthy male subjects and to exclude individuals with a complement deficiency, renal or liver impairment, or allergy to study medications and women, who cannot safely be infected due to potential complications from ascendant gonococcal infection. The bacterial doses range from 10^5 to 10^6 CFU of *N. gonorrhoeae*, corresponding to the approximate ID_{50} and ID_{80} , respectively, for the parent *N. gonorrhoeae* strain FA1090. These doses are similar to or smaller than typical exposures in naturally-acquired gonococcal infection.

Delivery of 10^6 CFU of wild type FA1090 to the anterior urethra results in infection approximately 80% of the time with symptoms of urethritis manifesting 3 to 4 days after inoculation.

Careful screening and observation during participation, overnight stays in the CTRC, prompt treatment of symptoms of gonococcal infection and treatment prior to discharge from the study are all designed to minimize the possibility of complications. Subjects will be examined daily by the study physician or the study physician's assistant first thing in the morning (typically between 6:30am and 7:30am), when signs or symptoms of urethral discharge or dysuria are most likely to be observed. In addition, study personnel are available for evaluation, specimen collection and treatment if subjects experience symptoms and require treatment any time during the day or night after the regularly scheduled morning examination. **A study physician is on call at all times during experimental infection trials.**

Infection will be terminated by treating the subject with a single observed 400 mg oral dose of cefixime. If he cannot swallow the oral pill, he will be treated with a single intramuscular dose of ceftriaxone (250 mg). Treatment will be given after one of the following endpoints has been achieved: (1) subject requests treatment regardless of signs, symptoms or positive cultures (2) purulent urethral discharge is observed by the examining clinician or reported by the subject or (3) 5 days after inoculation, regardless of infection status. Subjects will return within 7 days of antibiotic treatment for reexamination and a test of cure. In the unlikely event of antibiotic treatment failure, defined by a positive test of cure (which has never happened among more than 200 infected subjects to date), subjects will be treated with a single observed 500 mg oral dose of ciprofloxacin. In the event of an anaphylactic reaction to administration of the bacteria or to a study medication, the subject will be treated with 0.3 mg of epinephrine intramuscularly in the thigh using an EpiPen autoinjector. Emergency services (911) will be called, and the subject will be transported to the University Hospital Emergency Room.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

All AEs that occur from inoculation through the 2 week final follow-up phone call (Study Days 12-23) will be reported. At each scheduled visit, AEs will be assessed and any AEs identified during this period will be reported.

The Investigator is responsible for reporting all AEs that are observed or reported during the study, regardless of their relationship to study product.

Adverse Event: ICH E6 Good Clinical Practice Guidelines defines an Adverse Event (AE) as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an adverse event may come to the attention of study personnel during study visits and interviews or by a study recipient presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for “serious adverse events” should be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician’s assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include MD, PA, Nurse Practitioner, DO, or DDS), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the patient is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

All adverse events will be graded for severity and relationship to study product.

Severity of Event: All adverse events will be assessed by the investigator according to the DMID Toxicity Table (May 2015) as follows:

- Mild: events do not interfere with the patient’s daily activities.
- Moderate: events may cause some interference with activity not requiring medical intervention.
- Severe: events prevent daily activity and require medical intervention.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Relationship to study products: The investigator's assessment of an AE's relationship to study procedure or drug is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All adverse events must have their relationship to study product assessed using the following terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- **Related** – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- **Not Related** – There is not a reasonable possibility that the administration of the study product caused the event.

Relatedness to study product must specify whether to *N. gonorrhoeae* inoculum, cefixime, ceftriaxone or ciprofloxacin.

Expected AEs are those that occur during or following the study procedures (i.e. urethral inoculation, discomfort from collecting specimens, bruising from blood draw) or study medication administration which are outlined in the potential risks of the study medication package inserts and have no other definitely related explanation. Unexpected AEs are those events that occur and are not listed in the package insert for the study medication or events that are not the usual side effects associated with the study procedures.

9.2.2 Reactogenicity (for Vaccine Studies and Some Therapeutic Trials)

Not applicable

9.2.3 Serious Adverse Events

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse event or unexpected suspected adverse reaction. An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator

brochure or is not listed at the specificity or severity that has been observed or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. “Unexpected” as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

21 CFR 312.64 Investigator reports

Safety reports: An investigator must immediately report to the sponsor any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. Study endpoints that are serious adverse events (e.g., all cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the drug and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor. The investigator must record non serious adverse events and report them to the sponsor according to the timetable for reporting specified in the protocol.

Some protocols may list events specific to the protocol that they want reported as serious with expedited reporting. An example might be for a maternal immunization protocol before eclampsia or preterm delivery.

Serious Adverse Event (SAE):

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death,
- a life-threatening adverse event*,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or

-
- a congenital anomaly/birth defect.
 - Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

* Life-threatening adverse event. An adverse event is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event, had it occurred in a more severe form, might have caused death.

Relationship to study products: SAEs will be assessed regarding relationship (Related or Not Related, expected/unexpected) to study procedures or antibiotics used in the study. To help assess, the following guidelines are used.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

Relatedness to study product will specify whether to *N. gonorrhoeae* inoculum, cefixime, ceftriaxone or ciprofloxacin.

Expected SAEs are those that occur during or following the study procedures (i.e. Urethral inoculation, discomfort from collecting specimens, bruising from blood draw) or study medication administration, which are outlined in the potential risks of the study medication package inserts and have no other definitely related explanation. Unexpected SAEs are those events that occur that are not listed in the package insert for the study medication or events that are not the usual side effects associated with the study procedures.

9.2.4 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

Abnormal urine dipstick values will be reported to the study physician who will follow through with appropriate clinical testing, care and treatment.

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

Adverse Events

Adverse Events including local and systemic reactions not meeting the criteria for “serious adverse events” will be captured in the investigator’s records for the subject. Information to be collected includes event description, time of onset, investigator assessment of severity, relationship to study product, time of resolution of the event, seriousness, and outcome. All adverse events occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the patient is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study it should be recorded as an AE.

Serious Adverse Events

All serious adverse events will be:

- recorded on the appropriate serious adverse event case report form
- followed through resolution by a study physician
- reviewed by a study physician

9.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

All SAEs will be submitted within 24 hours of site awareness on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20814, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The DMID medical monitor and clinical protocol manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID medical monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the DMID Pharmacovigilance Group.

9.3.3 Regulatory Reporting for Studies Not Conducted Under DMID-Sponsored IND

Not applicable

9.3.4 Other Adverse Events (if applicable)

The occurrence of transmission of *N. gonorrhoeae* to a non-study subject will be reported. This has not occurred in prior studies. If an enrolled subject were to report having had sex during the timeframe relevant to study activities, the partner(s) would be referred to the UNC infectious diseases clinic, evaluated and given empiric treatment. Specimens would be obtained from the partner(s) to determine whether the infecting organism matched the inoculated subject's infection. The partner would be asked to provide informed consent to undergo non-standard of care procedures to evaluate the infection.

The occurrence of *N. gonorrhoeae* inoculum contamination and, if applicable, clinical events resulting from contamination, microbiological failure of cefixime or ceftriaxone treatment post infection, and response to ciprofloxacin in treatment failures will be reported to DMID Pharmacovigilance to be forwarded to DMID and the SMC and to the on-site ISM within 2 weeks of occurrence. If these events meet criteria for serious adverse event, they will be reported on an SAE form according to SAE reporting procedures.

- Site SAEs will be reported to DMID Pharmacovigilance according to the protocol and, separately, to the on-site ISM. DMID Pharmacovigilance will forward SAEs to DMID and, separately, to the DMID SMC Coordination staff for forwarding to the SMC.
- Any serious and unexpected (not listed in the package insert) adverse events considered to be related to licensed products that are administered in the course of the study (cefixime, ceftriaxone, ciprofloxacin, lidocaine, epinephrine) will be reported via DMID Pharmacovigilance to the FDA and separately to the on-site ISM. DMID Pharmacovigilance will forward SAEs to DMID and, separately, to the DMID SMC Coordination staff for forwarding to the SMC.

9.3.5 Reporting of Pregnancy

Not applicable

9.4 Type and Duration of Follow-up of Subjects after Adverse Events

Subjects who experience an adverse event will be followed until symptoms resolve or stabilize. Follow-up will continue through the duration of scheduled study visits. If a subject has completed the required study visits and symptoms persist, the subject will be monitored by telephone or clinic visit until the adverse event resolves or stabilizes.

9.5 Halting Rules

The risk of major complications to subjects in the experimental setting is remote based on the natural history of gonorrhea and previous human experimentation. However, the study will be monitored by a Safety Monitoring Committee (SMC) to ensure safety and proper performance of the study. If concerns affecting subject safety or data integrity are identified by the SMC, no new subjects will be enrolled, but protocol procedures will continue, to ensure the safety of the subjects who have been inoculated. The trial will be halted (at least temporarily) if at any time during the study:

1. One SAE related to study procedure or study product(s)
2. Three or more severe AEs of the same type (described by the same MedDRA SOC and Preferred Term) related to study procedure or study product(s)
3. One antibiotic treatment failure (so that susceptibility of the isolate may be evaluated)
4. Documented contamination of inoculum
5. A late, or systemic complication of gonococcal infection including any of the following is reported:
 - a. recovery of the infecting *N. gonorrhoeae* strain(s) from a normally sterile extragenital site
 - b. petechial or pustular acral skin lesions
 - c. asymmetric polyarthralgia
 - d. tenosynovitis
 - e. oligoarticular septic arthritis

Any events that trigger a halt of the study will be reviewed by the SMC, and SMC approval will be required before restart.

9.6 Safety Oversight (ISM plus SMC)

Safety oversight will be under the direction of an Independent Safety Monitor (ISM) and a Safety Monitoring Committee (SMC). The ISM will evaluate individual and cumulative participant data when making recommendations regarding safe continuation of the study at the time of each SMC meeting. ISM safety concerns are noted in the SMC report. In addition, the ISM will provide an independent written assessment to DMID for each SAE.

The primary responsibility of the SMC is to monitor participant safety. The SMC will review the research protocol and plans for data and safety monitoring. The committee will evaluate the progress of trials, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, and other factors that can affect study outcome. Monitoring will also consider factors external to the study when interpreting the data, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study. The committee will protect the confidentiality of the trial data and the results of monitoring.

The SMC will convene at the end of testing all subjects for a given *N. gonorrhoeae* mutant and on an *ad hoc* basis should immediate safety concerns arise; a simple majority of the SMC will be considered a quorum. In addition, the SMC will formally meet or electronically review the safety data from up to 16 evaluable subjects enrolled in noncompetitive infection groups and recommend the site to proceed with testing subjects in a competitive group for that mutant or to stop. Evaluable subjects are those who (1) receive a dose of *N. gonorrhoeae* within 1 log₁₀ of the intended dose and (2) reach an objective study endpoint (urethral discharge or day 6). When the investigators determine that a stopping point has been reached for an individual mutant under investigation, the SMC will meet to review adverse events and interim analyses, as summarized in the table below. Additional subjects beyond the number proposed in the schematic for an individual mutant will not be inoculated without approval of the SMC.

Cohort Stage	Milestone/Trigger	Type of Review
Noncompetitive infections	4-8 mutant and up to 8 WT subjects	SMC safety review and interim analyses
Competitive infections (if necessary)	Up to 16 subjects	SMC safety review and final analysis
Either stage	Presence of safety concern	Ad hoc SMC safety review

10 CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitoring will be conducted to ensure human subject protection, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, GCP/ICH, and regulatory guidelines, and that the study is conducted in accordance with the protocol and sponsor SOPs. DMID, the sponsoring agency, or its designee will conduct site monitoring visits as detailed in the 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, case report forms, informed consent forms, medical and laboratory reports and protocol compliance. Study monitors will meet with investigators to discuss any problems and actions to be taken and document visit finding and discussions.

11 STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

We hypothesize that key virulence determinants involved in *N. gonorrhoeae* resistance to innate immunity are essential for infection in the male urethra. We predict that mutations abolishing expression of these virulence determinants will eliminate or significantly reduce gonococcal infectivity or the ability to induce inflammation in an infected individual, thus identifying potential vaccine candidates.

This is an experimental infection model study designed to test the hypothesis that the gonococcal MtrD protein is required to establish infection in the male urethra. To compare the ability of mutant gonococci to infect the male urethra, we will first inoculate subjects with WT or an isogenic mutant strain of *N. gonorrhoeae* in noncompetitive infection studies. The null hypothesis for this comparison is: the proportion of subjects that become infected by day 6 with the mutant strain is equal to the proportion of subjects that become infected by day 6 with the WT strain. The one-sided alternative hypothesis for this comparison is: the proportion of subjects that become infected by day 6 with the mutant strain is less than the proportion of subjects that become infected by day 6 with the WT strain.

For isogenic mutants that do not demonstrate statistically significant differences in infectivity, we will conduct competitive infection studies to determine whether the mutant exhibits a fitness defect compared to WT. Competitive infections, initiated with equivalent numbers of WT and an isogenic mutant, allow demonstration of a difference in fitness between two strains using fewer subjects.

For competitive infections initiated with inocula containing a mixture of WT and isogenic mutant strains, the competitive index (CI) of the mutant compared to WT is the measure of relative fitness to determine the primary outcome. The precise proportion of each strain in the inoculum is determined for each experimental cohort. The CI is defined as the ratio of colony-forming units (cfu) of the two strains recovered from urine cultures on the day of treatment (output) compared to the ratio of strains in the inoculum (input) using the equation: $CI = \frac{\text{mutant cfu(output)}}{\text{wild-type cfu(output)}} \div \frac{\text{mutant cfu(input)}}{\text{wild-type cfu(input)}}$.

The null hypothesis for this comparison is: The ratio of colony-forming units of the mutant strain to those of the WT strain on the day of treatment is equal to the ratio of colony-forming units of the mutant strain to those of the WT strain in the inoculum, indicating no difference in fitness between the two strains during urethral infection.

The one-sided alternative hypothesis for this comparison is: The ratio of colony-forming units of the mutant strain to those of the WT strain at the time of treatment is less than the ratio of colony-forming units of the mutant strain to those of the WT strain in the inoculum, indicating the mutant has a fitness defect compared to the wild-type.

The hypotheses are formally tested at the interim analysis and again at the final analysis. Thus a type I error rate of 0.025 is used for each hypothesis at the interim and final analyses to maintain an overall rate of 0.05 for each hypothesis. Because of the exploratory nature of the trial, no corrections for multiplicity will be performed.

11.2 Sample Size Considerations

Due to the experimental nature of the study, the small sample sizes of the individual cohorts were chosen based on logistical and feasibility considerations.

For noncompetitive infections initiated with inocula containing an individual GC strain, the proportion of subjects that become infected is the measure of infectivity. Sample size considerations for noncompetitive infections are summarized in Table 2 below. Subjects will be enrolled in cohorts of up to 8. We will compare infectivity of WT *N. gonorrhoeae* and the isogenic mutant using Fisher's Exact Test. The interim analysis (see also Section 11.3) will include results from up to 8 subjects inoculated with the WT *N. gonorrhoeae* strain only and at least 4 subjects inoculated with the isogenic mutant only. Assuming the true probabilities of infection are 80% with wild-type and 4% with mutant inocula, 8 men per group provides 89% power to detect a difference using a 1-sided Fisher exact test and significance level of 0.025.

Table 2. Sample size considerations for noncompetitive infection studies

Infection Status				P-value ¹	Number of Subjects Needed for analysis
Wild-Type		Mutant			
Yes	No	Yes	No		
8	0	0	4	0.002	12
		1	3	0.018	
		2	2	0.091	
		2	6	0.003	16
		3	5	0.013	
		4	4	0.038	
7	1	0	4	0.010	12
		1	3	0.066	16
		1	7	0.005	
		2	6	0.020	
		3	5	0.059	
6	2	0	4	0.030	16
		0	8	0.003	
		1	7	0.020	
		2	6	0.066	
5	3	0	4	0.071	16
		0	8	0.012	
		1	7	0.059	

¹P-value is from the one-sided Fisher's Exact Test with alpha = 0.025

For competitive infections with mixed inocula, sample sizes are determined by the expected standard deviation of the mean proportion of recovered WT gonococci. Because calculations are based on the numbers of bacteria, constituting larger sample sizes than infectious dose calculations based on the number of infected subjects, mixed infections can provide a more accurate indication of attenuation using fewer subjects than traditional comparisons of infectivity using separate, inocula with individual strains (24). Note that competitive infections will not necessarily be conducted for isogenic mutants that demonstrate statistically significant differences in the primary and secondary outcomes specified for noncompetitive infections. The total number of subjects specified in the current protocol ($n = 32$) refers to the maximum number of participants to be prospectively enrolled in noncompetitive and competitive studies for the MtrD mutant. The total of 32 prospectively enrolled subjects for the combined MtrD mutant studies provides sufficient sample sizes for both noncompetitive and competitive analyses as described.

11.3 Planned Interim Analyses (if applicable)

11.3.1 Infectivity Outcomes Review

Interim analyses for noncompetitive infection studies will include results from up to 8 evaluable subjects inoculated with the WT *N. gonorrhoeae* strain and at least 4 evaluable subjects inoculated with the isogenic mutant (See Table 2 above). For interim analyses of noncompetitive infections, data for WT may include results from the current working bank, regardless of the proximity in time of the specific isogenic mutant group. See Section 5.3.4 for handling of withdrawals.

At the interim analysis, the following decision rule for the noncompetitive analysis will be used:

- If the p-value from Fisher's Exact Test is less than 0.025, we will consider stopping the mutant group.
- If the p-value from Fisher's Exact Test is greater than 0.025 and the p-value will still be greater than 0.025 regardless of the results from additional subjects in the mutant group, we may consider closing enrollment for the noncompetitive infection study early for futility and proceeding with the competitive infection study.
- If the p-value from Fisher's Exact Test is greater than or equal to 0.025, we will continue to enroll until evaluable data are obtained from at least 7 subjects in the mutant group.

If additional subjects are enrolled in the mutant group, a second interim analysis will include results from all evaluable subjects in the mutant and WT groups. At the second interim analysis, the following decision rule for the noncompetitive analysis will be used:

- If the p-value from Fisher's Exact Test is less than 0.025, we will consider stopping studies for the isogenic mutant.

-
- If the p-value from Fisher's Exact Test is greater than or equal to 0.025, we will conclude that a difference in infectivity cannot be determined in noncompetitive experimental human infections.

If the infectivity of the two strains is not significantly different at that point, we will proceed with competitive infections to determine the relative fitness of the isogenic mutant. Interim analyses for competitive infections will be conducted after at least 5 subjects have been inoculated with a specific mixture of WT and mutant. The following decision rule will be used for the interim analysis:

- If the p-value from the sign test of the competitive index is less than 0.025, we will consider stopping the competitive infection group
- If the p-value from the sign test of the competitive index is greater than or equal to 0.025, we will continue to enroll up to 16 subjects.

11.3.2 Safety Review

The SMC will convene to review subject safety as described in section 9.6. Safety outcome measures include adverse events, protocol deviations and any unexpected events that occurred since the previous review. Primary and secondary study outcomes for subject groups enrolled to test an individual mutant and statistical rules for halting noncompetitive infections will be reviewed to determine whether or not to proceed to competitive infections.

11.3.3 Immunogenicity or Efficacy Review

Not applicable

11.4 Final Analysis Plan

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.

Baseline and demographic characteristics will be summarized. All subjects inoculated with a dose of *N. gonorrhoeae* within 1 log₁₀ of the intended dose and with necessary data elements available will be included in the analyses. Data from subjects who are enrolled in the trial but who receive an unintended inoculum dose or who withdraw from the study before objective study endpoints are reached will not be included in final analyses. For noncompetitive experimental infections with individual WT or isogenic mutant strains of *N. gonorrhoeae*, the primary outcome is the proportion of subjects that become infected after inoculation with each

strain. To assess the contribution of expression of the gene that was inactivated in the mutant, we will use a one-sided Fisher's Exact Test with significance level of 0.025 to assess the difference between the infectivity of WT and mutant strains.

For mutants that do not demonstrate significantly different infectivity compared to WT, we will determine relative fitness of the mutant in competitive infections. To assess whether the fitness of a given mutant is different than that of wild-type, we will compare the ratio of colony-forming units of the mutant strain to those of the WT strain at the time of treatment and in the inoculum using a Wilcoxon Signed-Rank Test with a significance level of 0.025.

For noncompetitive infections, multiple analyses are planned to assess the secondary outcome to compare the clinical course of infection with mutant and WT *N. gonorrhoeae*. We will summarize the following by strain:

- The occurrence of signs and symptoms of urethritis attributable to gonococcal infection by day 6
- The occurrence of bacteriuria (Log_{10} cfu/mL urine sediment) on day of antibiotic treatment
- The occurrence of urethritis ($\geq 5.8 \text{ Log}_{10}$ WBC/mL urine sediment) on day of treatment
- The time from inoculation to treatment

For noncompetitive infections, analyses of exploratory outcomes will include assessment of host responses to infection by summarizing and comparing the following by strain:

- The detection of local cytokines in urine and systemic cytokines in blood
- Expression of human genes identified by RNASeq in urine sediment and whole blood from infected subjects

In addition, we will assess bacterial gene expression during experimental infection by comparing expression levels of specific gonococcal genes detected by targeted reverse transcriptase PCR and at the genome level by bacterial RNASeq from urine from subjects infected with WT vs. mutant strains.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The investigators at the University of North Carolina at Chapel Hill will maintain appropriate research records for this study, in compliance with ICH E6 GCP and regulatory and institutional requirements for the protection of human subjects. As part of conducting this DMID-sponsored study, study documentation will be made available to authorized representatives of the sponsor and regulatory agencies to examine and when required by law to copy clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

Source documents for this study are maintained in the office of Dr. Marcia Hobbs and in the Regulatory Department of the Institute for Global Health and Infectious Diseases. Documents containing identifiable information are kept in locked storage and password-protected electronic files. Source documents include daily trial logs developed by Dr. Hobbs to record daily urinalysis and culture results; standard clinic forms for history, physical examinations, clinical data and laboratory results.

13 QUALITY CONTROL AND QUALITY ASSURANCE

University of North Carolina research personnel will review study materials produced during this study for compliance with the protocol and for accuracy in relation to source documents (Refer to Section 12). All personnel reviewing the study materials will be knowledgeable about the study and will have been trained in the ethical and regulatory requirements for protection of human research participants in compliance with NIH requirements and The University of North Carolina at Chapel Hill “Policy on Education and Certification of Investigators Involved in Human Subjects Research.” <http://ohre.unc.edu/irbtraining/>

Study records will be reviewed according to the following audit criteria:

1. Meets inclusion criteria (as described in detail in section 5.1)
2. No exclusion criteria present (as described in detail in section 5.2)
3. History and physical examination documented
4. Copies of laboratory reports in chart
5. Proper informed consent for study participation signed
6. Copy of informed consents provided to participant
7. Blood drawing at the time of enrollment does not exceed 45 cc
8. Correct documentation of daily examinations after inoculation (vital signs and examination for urethral discharge)
9. Treatment with antibiotics documented
10. Follow-up examination documented and test of cure within 7 days of antibiotic treatment
11. Follow-up telephone call and any AEs documented

The Statistical and Data Coordinating Center will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run 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

The investigator will ensure that this study is conducted in full conformity with the principals set forth in the Belmont Report: Ethical Principals 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 28, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997).

14.2 Institutional Review Board

The investigator will provide for review and approval of this protocol and the associated informed consent documents by the University of North Carolina at Chapel Hill Biomedical Institutional Review Board (IRB) holding US Federal Wide Assurance #4801 and Office of Human Research Protections (OHRP) Registration #: 00000538, 00000539, 00001648 and 00001649. Any amendments to the protocol or consent will be approved prior to their use.

The principal investigator is responsible for keeping the IRB apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case at least once a year. The investigator will also keep the IRB informed of adverse events that affect subject safety or protocol violations that affect data integrity.

14.3 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 this therapy will be provided to the subjects and their families. The consent form describing in detail the study interventions/products, study procedures, and risks is given to the subject and written documentation of informed consent is required prior to starting intervention/administering study product. For this study, written informed consent for all study procedures and permission for specimen storage will be obtained both at the screening visit and again at enrollment before the study begins. The consent form 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. At the screening visit, subjects will complete a True/False test of understanding ([Appendix B](#)) following review of the consent form. The investigator obtaining informed consent will review specific sections of the consent form related to questions that the subject does not answer correctly. Following this directed review of the consent form and prior to any procedures being done specifically for the study, subjects will sign the informed consent document and initial a statement indicating permission for specimen storage. Subjects who sign the consent form but deny permission for specimen storage will be allowed to participate in the trial. The subjects will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of

the trial. 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.

14.3.1 Informed Consent/Assent Process (in Case of a Minor)

Not applicable; individuals below the age of 18 will be excluded from the study.

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

Females will not be included in the study because of the risk of complications from ascendant gonococcal infection. Male subjects of any race will be included. Children under 18 years of age are deemed inappropriate for inclusion in a study of a sexually transmitted infection.

14.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. Results of study research may be published, but subject identities will not be revealed. After written informed consent is provided, consecutive study code numbers will be assigned to the subjects to ensure confidentiality. This study code number will be used for laboratory transport and processing, result reporting, and data recording. Additionally, the principal investigator will keep records locked in cabinets. Data entered into computerized files will be stored only in a password protected file on a computer in the PI's office, which is accessible only by authorized personnel directly involved with the study. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

This research is covered by a Certificate of Confidentiality from NIH. The researchers with this Certificate may not disclose or use information, documents, or biospecimens that may identify subjects in any federal, state, or local civil, criminal, administrative, legislative, or other action, suit, or proceeding, or be used as evidence, for example, if there is a court subpoena, unless subjects have consented for this use. Information, documents, or biospecimens protected by this Certificate cannot be disclosed to anyone else who is not connected with the research except, if there is a federal, state, or local law that requires disclosure (such as to report child abuse or communicable diseases but not for federal, state, or local civil, criminal, administrative, legislative, or other proceedings, see below); if subjects have consented to the disclosure, including for medical treatment; or if it is used for other scientific research, as allowed by federal regulations protecting research subjects.

The Certificate cannot be used to refuse a request for information from personnel of the US federal or state government agency sponsoring the project that is needed for auditing or program evaluation by NIAID, which is funding this project, or for information that must be disclosed in order to meet the requirements of the US FDA. The study monitor or other authorized representatives of the sponsor such as the investigator and her research associates, the UNC-CH

Biomedical IRB or its designees, OHRP, the FDA, and DMID may inspect all documents and records required to be maintained by the investigator including, but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records. The Certificate of Confidentiality does not prevent subjects from voluntarily releasing information about themselves or their involvement in this research. If subjects want research information released to an insurer, medical care provider, or any other person not connected with the research, they must provide consent to allow the researchers to release it.

The Certificate of Confidentiality will not be used to prevent disclosure as required by federal, state, or local law of reportable STIs *other than experimental gonococcal infection* if such infections are identified by tests performed for screening or follow up as described in this protocol.

14.6 Study Discontinuation

In case this study is discontinued, all subjects will receive treatment for termination of infection, follow-up examination and test of cure.

The risk of major complications to subjects in the experimental setting is remote based on the natural history of gonorrhea and previous human experimentation. However, the study will be monitored by a SMC to ensure safety and proper performance of the study. If concerns affecting subject safety or data integrity are identified by the SMC, enrollment into the study and study procedures will be halted until the identified issues are addressed.

14.7 Future Use of Stored Specimens

Bacteria isolated from urine cultures will be stored for future use in studies of gonococcal pathogenesis. Stored urine, serum and isolated peripheral white blood cells from subjects who provide permission for stored specimens will be used to measure cytokines, chemokines and other molecules that may mediate host immune responses to gonococcal infection. Specimens will be stored in the laboratories of the study investigators on the University of North Carolina campus in Chapel Hill, NC. Stored specimens will be used only for studies approved by the University of North Carolina IRB. All specimens obtained for the purpose of this study become the exclusive property of the University of North Carolina at Chapel Hill. The researchers may retain, preserve or dispose of these specimens and may use these specimens for research that may result in commercial applications. Subjects will not be compensated for any future commercial use of these specimens.

15 DATA HANDLING AND RECORD KEEPING

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data. All source documents should be neat and in a legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, 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.**

Study visit checklists, logs and flowsheets will be used as source documents and maintained for recording data for each subject enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained

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

15.1 Data Management Responsibilities

All source documents and laboratory reports must be reviewed by the clinical team and verified for completeness and accuracy prior to data entry. Adverse events must be graded, assessed for severity and causality, and reviewed by the Principal Investigator or designee.

Data collection is the responsibility of the clinical trial staff at University of North Carolina at Chapel Hill under the supervision of the Principal Investigator. During the study, the investigator must maintain complete and accurate documentation.

The Emmes Corporation will serve as the Statistical and Data Coordinating Center 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 data (including AEs) will be recorded on paper and entered from source documents into a 21 CFR Part 11-compliant Internet Data Entry System (IDES) provided by The Emmes Corporation. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

15.3 Types of Data

The database will include information under a subject's coded number about age, race, trial dates, inoculum received, whether the subject is infected, whether (and when) he develops signs or symptoms of urethritis, and quantitative laboratory data regarding bacteruria and pyuria.

15.4 Timing/Reports

The Emmes Corporation will conduct interim analyses as specified in sections 11.2 and 11.3. Results will be included in SMC reports prepared in advance of meetings scheduled as specified in section 9.6.

15.5 Study Records Retention

Study records will be retained a minimum of 10 years.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or protocol-specific Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. 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 investigator or study staff 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 IDES or via the TRI/ICON DMID-Clinical Research Operations and Management Support (CROMS) email (protocoldeviations@dmidcroms.com), web- (www.dmidctm.com) or fax-based system (1-215-699-6288). As appropriate, corrective actions are to be developed by the site and implemented promptly. Protocol deviations will be maintained in the study binder on a protocol deviation form. Protocol deviations will be reviewed as they occur by the study investigator and study coordinator to determine how improvements can be made to prevent future protocol deviations.

All deviations from the protocol must be addressed in study subject source documents. A completed copy of the DMID Protocol Deviation Form (DMID-CROMS 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 PI/study staff is responsible for knowing and adhering to their IRB/IEC requirements.

16 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. The responsible party does not plan to request certification of delayed posting.

Refer to:

- Public Law 110-85, Section 801, Clinical Trial Databases
- 42CFR11
- NIH NOT-OD-16-149

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* (<http://clinicaltrials.gov/>), which is sponsored by the National Library of Medicine. 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.

*Journal Citation: 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. *N Engl J Med.* 2004; 351:1250-1

17 LITERATURE REFERENCES

1. World Health Organization. Global incidence and prevalence of selected curable sexually transmitted infections - 2008. Geneva: WHO Press; 2012.
2. Centers for Disease Control and Prevention. Sexually Transmitted Disease Surveillance 2014. US Department of Health and Human Services. Atlanta, 2015.
3. Cohen MS. HIV and sexually transmitted diseases: lethal synergy. *Top HIV Med.* 2004;12(4):104-7.
4. Cohen MS, Miller WC. Sexually transmitted diseases and human immunodeficiency virus infection: cause, effect, or both? *Int'l J Infect Dis.* 1998;3(1):1-4.
5. Cohen MS. Sexually transmitted diseases enhance HIV transmission: no longer a hypothesis. *Lancet.* 1998;351 (suppl III):5-7.
6. CDC. Update to CDC's Sexually Transmitted Diseases Treatment Guidelines, 2010: Oral cephalosporins no longer a recommended treatment for gonococcal infections. *MMWR.* 2012;61(31):590-4.
7. Unemo M, Shafer WM. Antibiotic resistance in *Neisseria gonorrhoeae*: origin, evolution, and lessons learned for the future. *Ann N Y Acad Sci.* 2011;1230:E19-28.
8. Lewis DA. The Gonococcus fights back: is this time a knock out? *Sex Transm Infect.* 2010;86(6):415-21.
9. Hagman K, Pan W, Spratt BG, Balthazar JT, Judd RC, Shafer WM. Resistance of *Neisseria gonorrhoeae* to antimicrobial hydrophobic agents is modulated by the *mtrRCDE* efflux system. *Microbiol.* 1995;141:611-22.
10. Lucas CE, Hagman KE, Levin JC, Stein DC, Shafer WM. Importance of lipooligosaccharide structure in determining gonococcal resistance to hydrophobic antimicrobial agents due to the *mtr* efflux system. *Mol Microbiol.* 1995;16:1001-10.
11. Shafer WM, Balthazar JT, Hagman K, Morse SA. Missense mutations that alter the DNA-binding domain of the MtrR protein occur frequently in rectal isolates of *Neisseria gonorrhoeae* that are resistant to faecal lipids. *Microbiol.* 1995;141:907-11.
12. Hagman K, Shafer WM. Transcriptional control of the *mtr* efflux system of *Neisseria gonorrhoeae*. *J Bacteriol.* 1995;179:4123-8.
13. Lucas CE, Balthazar JT, Hagman K, Shafer WM. The MtrR repressor binds the DNA sequence between the *mtrR* and *mtrC* genes of *Neisseria gonorrhoeae*. *J Bacteriol.* 1997;179(13):4123-8.
14. Jerse AE, Sharma ND, Simms AN, Crow ET, Snyder LA, Shafer WM. A gonococcal efflux pump system enhances bacterial survival in a female mouse model of genital tract infection. *Infect Immun.* 2003;71(10):5576-82.
15. Hobbs MM, Sparling PF, Cohen MS, Shafer WM, Deal CD, Jerse AE. Experimental gonococcal infection in male volunteers: Cumulative experience with *Neisseria gonorrhoeae* strains FA1090 and MS11mkC. *Front Microbiol.* 2011;2:123.
16. Seifert HS, Wright CJ, Jerse AE, Cohen MS, Cannon JG. Multiple gonococcal pilin antigenic variants are produced during experimental human infections. *J Clin Invest.* 1994;93(6):2744-9.

17. Jerse AE, Cohen MS, Drown PM, Whicker LG, Isbey SF, Seifert HS, Cannon JG. Multiple gonococcal opacity proteins are expressed during experimental urethral infection in the male. *J Exp Med*. 1994;179:911-20.
18. Hobbs MM, Anderson JE, Balthazar JT, Kandler JL, Carlson RW, Ganguly J, Begum AA, Duncan JA, Lin JT, Sparling PF, Jerse AE, Shafer WM. Lipid A's structure mediates *Neisseria gonorrhoeae* fitness during experimental infection of mice and men. *MBio*. 2013;4(6):e00892-13.
19. Anderson JE, Hobbs MM, Biswas GD, Sparling PF. Opposing selective forces for expression of the gonococcal lactoferrin receptor. *Mol Microbiol*. 2003;48(5):1325-37.
20. Fekety FR. Safety of parenteral third-generation cephalosporins. *Am J Med*. 1990;88(4A):38S-44S.
21. Pichichero ME. Use of selected cephalosporins in penicillin-allergic patients: a paradigm shift. *Diagn Microbiol Infect Dis*. 2007;57(3 Suppl):13S-8S.
22. Khaliq Y, Zhanel GG. Fluoroquinolone-associated tendinopathy: a critical review of the literature. *Clin Infect Dis*. 2003;36(11):1404-10.
23. Johnston DM, Cannon JG. Construction of mutant strains of *Neisseria gonorrhoeae* lacking new antibiotic resistance markers using a two gene cassette with positive and negative selection. *Gene*. 1999;236(1):179-84.
24. Beuzon CR, Holden DW. Use of mixed infection with *Salmonella* strains to study virulence genes and their interactions *in vivo*. *Microbes Infect*. 2001;3:1345-52.
25. Lim EM, Cembrowski G, Cembrowski M, Clarke G. Race-specific WBC and neutrophil count reference intervals. *Int J Lab Hematol*. 2010;32:590-7.

18 SUPPLEMENTS/APPENDICES

18.1 Appendix A: Schedule of Events

Evaluation	Screening Visit	Eligibility Testing	Entry Visit	Daily follow-up for up to 5 days	Day of Antibiotic Treatment	1 week follow-up visit	2 week final follow-up phone call
Study Day	-30 to -2	-2 to -4	1	2 to 6	2 to 6	5 to 13	12 to 23
Study Visit Number	00A	00B	01	02,03,04,05,06	02,03,04,05,06	07	08
Review inclusion/ exclusion criteria and risks of participation; subject signs ICF.	X		X				
Subject takes ICF test of understanding	X						
Medical History	X						
Review Medical History			X	X		X	
Review of Medications	X		X	X		X	
Complete Physical Exam	X						
Targeted Physical Exam			X	X		X	
Vital signs	X		X	X		X	
Complete Blood Count with differential (for WBC, PMN and hemoglobin results)	X						
Serum CH50 testing	X						
Serum creatinine testing	X						
Serum ALT testing	X						

Evaluation	Screening Visit	Eligibility Testing	Entry Visit	Daily follow-up for up to 5 days	Day of Antibiotic Treatment	1 week follow-up visit	2 week final follow-up phone call
Study Day	-30 to -2	-2 to -4	1	2 to 6	2 to 6	5 to 13	12 to 23
Study Visit Number	00A	00B	01	02,03,04,05,06	02,03,04,05,06	07	08
HIV testing (combination Ag/Ab test)	X						
Syphilis, HBV and HCV serologies	X						
Urine CT/NG and TV NAAT	X	X					
Urine CT/NG NAAT (Test of cure)						X	
Urinalysis	X					X	
Intraurethral Inoculation			X				
Stored blood		X			X	X	
Urine culture for <i>N. gonorrhoeae</i>				X			
Urine storage		X		X	X	X	
Urethral swab					X		
Antibiotic treatment					X		
Review Symptoms/Adverse Events							→

18.2 Appendix B: Subject Comprehension

These questions will be given to potential study subjects after initial review of informed consent form during the screening visit. Incorrect answers will prompt re-review of the relevant section(s) of consent form to clarify the content [indicated in square brackets].

Instructions: Respond to the statements below answering T for TRUE or F for FALSE.

1.	As a volunteer, I do not have to participate in this research study if I do not want to. [p.1 paragraph 1; p.7 last paragraph]	T
2.	One goal of this study is to give me a gonorrhea infection with symptoms including urethral discharge and discomfort during urination. [p.2 paragraph 2; p.4 paragraph 4]	T
3.	Screening tests to determine if I can participate in this study will not include an HIV test. [p.4 paragraph 2]	F
4.	The investigators have assured me that there are no risks to me or others associated with my participation in this research study. [p.1 paragraph 2; pp.6-7; p.8 paragraph 3]	F
5.	A solution containing live bacteria capable of causing gonorrhea will be delivered through a small tube into my penis. [p.4 paragraph 4; p.6 paragraph 2]	T
6.	Gonorrhea is difficult to spread to others and there will be no restrictions on my sexual activity during my participation. [p.3 paragraph 2; p.4 paragraphs 1&5; p.5 paragraph 1; p.7 paragraphs 3&4]	F
7.	As soon as I am discharged from the hospital research unit, I can resume sexual activity without risk to my partner(s). [p.3 paragraph 2; p.4 paragraphs 1&5; p.5 paragraph 1; p.7 paragraphs 3&4]	F
8.	The gonorrhea bacteria used to infect me are killed by antibiotics, and all subjects previously infected in this research have been cured. [p.6 paragraph 4]	T
9.	Although the bacterial inoculum has been extensively tested for purity, it is still possible that it may contain other infectious agents that could infect me. [p.6 paragraph 5]	T
10.	Taking some other medications during the course of this study may influence the safety of the experiment. [p.3 paragraph 1]	T
11.	While admitted to the hospital research unit, I will be allowed to leave during the day, but must return to give specimens and stay overnight on the ward. [p.4 paragraphs 1&5]	T
12.	The morning urine specimens collected for the study may be from the second or third urination of the day. [p.4 paragraph 5; p.5 paragraph 2]	F
13.	Blood and urine specimens that I provide will be stored indefinitely for future research use, unless I indicate otherwise. [p.5 paragraph 5; p.9 paragraph 3]	T
14.	If I have any concerns about my participation in this study, I can call the UNC Institutional Review Board at 919-966-3113. [p.11 paragraph 1]	T

18.3 Appendix C: Toxicity Table

Eligibility criteria are based on Grade 1 values in **bold** below.

Clinical Adverse Events						
Blood, Serum, or Plasma Chemistries	Reference Range¹	Eligibility Range²	L O/ HI /N³	Mild (Grade 1)⁴	Moderate (Grade 2)	Severe (Grade 3)
Creatinine (mg/dL)	0.7-1.3	0.7-1.7	HI	>ULN- 1.7	1.8-2.0	>2.0
ALT (U/L)	18 yr 19+ yr	10-40 10-105	HI	44-105	106-175	>175
		19-72 19-105				
Hematology						
Hemoglobin (Males) (g/dL)	13.5-17.5	12.0-17.5	LO	12.0-12.5	10.0-11.9	<10.0
White Blood Cell Count (WBC) (x10 ⁹ /L)	4.5 – 11.0	2.5-15.0	HI	11.0-15.0	15.1-20.0	>20.0
			LO	2.50-3.50	1.50-2.49	<1.50
Neutrophils (ANC) (x10 ⁹ /L) ⁵	2.0-7.5	1.5-7.5	LO	1.50-2.00	1.00-1.49	<1.00
African Americans	1.3-7.5	1.3-7.5				
Urine						
Protein (dipstick)	Negative	< 2+	HI	1+	2+	>2+
Blood (microscopic) – red blood cells per high power field (rbc/hpf)	0-3	0-10	HI	5-10	11-50	>50 and/or gross blood

¹Reference range from UNC Hospitals' McLendon Clinical Laboratories or reference (25) for ANC for African Americans

²Laboratory values acceptable for eligibility for enrollment

³Low, High, Not Graded

⁴If initial bound of Grade 1 has gap from reference range or eligibility range, calculations based on NEJM reference ranges

-
- Urinary frequency
 - Testicular pain
 - Feverish
 - Chills
 - Malaise
 - Diarrhea
 - Rash

Objective signs:

- Urethral discharge

A first-void urine specimen will be collected. All subjects will receive antibiotic treatment before discharge from the inpatient portion of the study. Subjects are treated if (1) he requests treatment regardless of signs, symptoms or positive cultures (2) purulent urethral discharge is observed by the examining clinician or reported by the subject or (3) 5 days after inoculation, regardless of infection status. On the last day of the inpatient portion of the trial, or the day on which a subject receives treatment for gonococcal infection, a urethral swab will be obtained. Subjects will be observed for approximately 20 minutes following administration of study antibiotics.

Subjects return to the CTRC within one week for re-examination by a physician or physician's assistant licensed to treat gonococcal infections and their complications, and a urine CT/NG NAAT test of cure. The test of cure is a nucleic acid amplification test. In the unlikely event of a positive test of cure, we will obtain a urethral specimen for culture to enable antibiotic susceptibility testing.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

- Serum CH50 test for complement deficiency, creatinine, ALT and HIV, syphilis, HBV and HCV serologies and CBC at screening
- Urine-based nucleic acid amplification testing for CT/NG and TV and urinalysis at screening; urine CT/NG and TV tests 2-4 days prior to enrollment; urine CT/NG test and urinalysis at 1 week follow-up (Day 5-13).

8.2.2 Special Assays or Procedures

- Daily urine sediment culture for *N. gonorrhoeae* to monitor infection (Study Days 2-6). Bacteria isolated from urine cultures will be enumerated, and individual colonies will be stored for research testing to compare numbers and the genotypes and phenotypes of output compared to input organisms.

-
- Daily urine dipstick analysis for hematuria (Study Days 2-6).
 - Daily microscopic examination of urine sediment and quantitation of WBCs to monitor inflammation (Study Days 2-6).
 - Stored urine (collected 2-4 days prior to enrollment, on Study Days 2-6, and at 1 week follow-up [Study Days 5-13]) and blood (collected 2-4 days prior to enrollment, on day of antibiotic treatment, and at 1 week follow-up [Study Days 5-13]) from subjects who provide permission for stored specimens will be used to measure cytokines, chemokines and other molecules that may mediate host immune responses to gonococcal infection. Levels of individual cytokines will be assessed using commercially available ELISAs. Multiplexed cytokine analysis will be measured using slide-based anticytokine antibody arrays or microparticle based arrays. Activation of inflammatory cell death programs will be assessed by staining with a fluorescent stain excluded by viable cells followed by quantitation of staining by flow cytometry. Assays of immunological parameters are purely for pursuit of research objectives, and results will not be used for clinical purposes.

8.2.3 Specimen Preparation, Handling, and Shipping

- Approximately 30 mL of blood will be obtained at screening. Screening labs will be sent to the NC Hospitals McLendon Clinical Laboratories for CH50, CBC, ALT, creatinine and HIV, syphilis, HBV and HCV serology tests. Blood and urine obtained at the eligibility testing visit and at followup will be sent to Dr. Hobbs' Laboratory where specimens will be processed and stored at $\leq -70^{\circ}\text{C}$ according to laboratory SOPs.
- Urine obtained at screening, before enrollment and at followup will be sent to the NC Hospitals McLendon Clinical or UNC CFAR VIM Core Laboratories for nucleic acid amplification testing for *C. trachomatis*, *N. gonorrhoeae* and *T. vaginalis*. Excess urine will be transferred to Dr. Hobbs' Lab, aliquoted and stored frozen at $\leq -70^{\circ}\text{C}$.
- Daily urine specimens will be processed by Dr. Hobbs or other study personnel in the investigators' laboratory at the CTRC. Urine dipstick analysis will be performed on whole urine (8-10 mL). This volume will then be centrifuged in the investigators' lab at the CTRC, and sediment will be examined microscopically to enumerate WBCs. The remaining urine from the original 50-mL specimen will be centrifuged separately. The supernatant from the spun urine will be aliquoted and stored frozen at $\leq -70^{\circ}\text{C}$ in Dr. Hobbs' Lab. The urine sediment will be inoculated onto GCB agar plates for *N. gonorrhoeae* culture; inoculated plates will be loaded into candle jars for transport from the CTRC to Dr. Hobbs' Lab. Individual *N. gonorrhoeae* colonies isolated from urine cultures (up to 100 per subject per day) will be stored frozen at $\leq -70^{\circ}\text{C}$ in Dr. Hobbs' Lab.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

All AEs that occur from inoculation through the 2 week final follow-up phone call (Study Days 12-23) will be reported. At each scheduled visit, AEs will be assessed and any AEs identified during this period will be reported.

The Investigator is responsible for reporting all AEs that are observed or reported during the study, regardless of their relationship to study product.

Adverse Event: ICH E6 Good Clinical Practice Guidelines defines an Adverse Event (AE) as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an adverse event may come to the attention of study personnel during study visits and interviews or by a study recipient presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for “serious adverse events” should be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician’s assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include MD, PA, Nurse Practitioner, DO, or DDS), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the patient is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

All adverse events will be graded for severity and relationship to study product.

Severity of Event: All adverse events will be assessed by the investigator according to the DMID Toxicity Table (May 2015) as follows:

- Mild: events do not interfere with the patient’s daily activities.
- Moderate: events may cause some interference with activity not requiring medical intervention.
- Severe: events prevent daily activity and require medical intervention.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Relationship to study products: The investigator's assessment of an AE's relationship to study procedure or drug is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All adverse events must have their relationship to study product assessed using the following terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- **Related** – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- **Not Related** – There is not a reasonable possibility that the administration of the study product caused the event.

Relatedness to study product must specify whether to *N. gonorrhoeae* inoculum, cefixime, ceftriaxone or ciprofloxacin.

Expected AEs are those that occur during or following the study procedures (i.e. urethral inoculation, discomfort from collecting specimens, bruising from blood draw) or study medication administration which are outlined in the potential risks of the study medication package inserts and have no other definitely related explanation. Unexpected AEs are those events that occur and are not listed in the package insert for the study medication or events that are not the usual side effects associated with the study procedures.

9.2.2 Reactogenicity (for Vaccine Studies and Some Therapeutic Trials)

Not applicable

9.2.3 Serious Adverse Events

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse event or unexpected suspected adverse reaction. An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator

9.2.4 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

Abnormal urine dipstick values will be reported to the study physician who will follow through with appropriate clinical testing, care and treatment.

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

Adverse Events

Adverse Events including local and systemic reactions not meeting the criteria for “serious adverse events” will be captured in the investigator’s records for the subject. Information to be collected includes event description, time of onset, investigator assessment of severity, relationship to study product, time of resolution of the event, seriousness, and outcome. All adverse events occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the patient is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study it should be recorded as an AE.

Serious Adverse Events

All serious adverse events will be:

- recorded on the appropriate serious adverse event case report form
- followed through resolution by a study physician
- reviewed by a study physician

9.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

All SAEs will be submitted within 24 hours of site awareness on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20814, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The DMID medical monitor and clinical protocol manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID medical monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the DMID Pharmacovigilance Group.

9.3.3 Regulatory Reporting for Studies Not Conducted Under DMID-Sponsored IND

Not applicable

9.3.4 Other Adverse Events (if applicable)

The occurrence of transmission of *N. gonorrhoeae* to a non-study subject will be reported. This has not occurred in prior studies. If an enrolled subject were to report having had sex during the timeframe relevant to study activities, the partner(s) would be referred to the UNC infectious diseases clinic, evaluated and given empiric treatment. Specimens would be obtained from the partner(s) to determine whether the infecting organism matched the inoculated subject's infection. The partner would be asked to provide informed consent to undergo non-standard of care procedures to evaluate the infection.

The occurrence of *N. gonorrhoeae* inoculum contamination and, if applicable, clinical events resulting from contamination, microbiological failure of cefixime or ceftriaxone treatment post infection, and response to ciprofloxacin in treatment failures will be reported to DMID Pharmacovigilance to be forwarded to DMID and the SMC and to the on-site ISM within 2 weeks of occurrence. If these events meet criteria for serious adverse event, they will be reported on an SAE form according to SAE reporting procedures.

- Site SAEs will be reported to DMID Pharmacovigilance according to the protocol and, separately, to the on-site ISM. DMID Pharmacovigilance will forward SAEs to DMID and, separately, to the DMID SMC Coordination staff for forwarding to the SMC.
- Any serious and unexpected (not listed in the package insert) adverse events considered to be related to licensed products that are administered in the course of the study (cefixime, ceftriaxone, ciprofloxacin, lidocaine, epinephrine) will be reported via DMID Pharmacovigilance to the FDA and separately to the on-site ISM. DMID Pharmacovigilance will forward SAEs to DMID and, separately, to the DMID SMC Coordination staff for forwarding to the SMC.

9.3.5 Reporting of Pregnancy

Not applicable

9.4 Type and Duration of Follow-up of Subjects after Adverse Events

Subjects who experience an adverse event will be followed until symptoms resolve or stabilize. Follow-up will continue through the duration of scheduled study visits. If a subject has completed the required study visits and symptoms persist, the subject will be monitored by telephone or clinic visit until the adverse event resolves or stabilizes.

9.5 Halting Rules

The risk of major complications to subjects in the experimental setting is remote based on the natural history of gonorrhea and previous human experimentation. However, the study will be monitored by a Safety Monitoring Committee (SMC) to ensure safety and proper performance of the study. If concerns affecting subject safety or data integrity are identified by the SMC, no new subjects will be enrolled, but protocol procedures will continue, to ensure the safety of the subjects who have been inoculated. The trial will be halted (at least temporarily) if at any time during the study:

1. One SAE related to study procedure or study product(s)
2. Three or more severe AEs of the same type (described by the same MedDRA SOC and Preferred Term) related to study procedure or study product(s)
3. One antibiotic treatment failure (so that susceptibility of the isolate may be evaluated)
4. Documented contamination of inoculum
5. A late, or systemic complication of gonococcal infection including any of the following is reported:
 - a. recovery of the infecting *N. gonorrhoeae* strain(s) from a normally sterile extragenital site
 - b. petechial or pustular acral skin lesions
 - c. asymmetric polyarthralgia
 - d. tenosynovitis
 - e. oligoarticular septic arthritis

For competitive infections with mixed inocula, sample sizes are determined by the expected standard deviation of the mean proportion of recovered WT gonococci. Because calculations are based on the numbers of bacteria, constituting larger sample sizes than infectious dose calculations based on the number of infected subjects, mixed infections can provide a more accurate indication of attenuation using fewer subjects than traditional comparisons of infectivity using separate, inocula with individual strains (24). Note that competitive infections will not necessarily be conducted for isogenic mutants that demonstrate statistically significant differences in the primary and secondary outcomes specified for noncompetitive infections. The total number of subjects specified in the current protocol ($n = 32$) refers to the maximum number of participants to be prospectively enrolled in noncompetitive and competitive studies for the MtrD mutant. The total of 32 prospectively enrolled subjects for the combined MtrD mutant studies provides sufficient sample sizes for both noncompetitive and competitive analyses as described.

11.3 Planned Interim Analyses (if applicable)

11.3.1 Infectivity Outcomes Review

Interim analyses for noncompetitive infection studies will include results from up to 8 evaluable subjects inoculated with the WT *N. gonorrhoeae* strain and at least 4 evaluable subjects inoculated with the isogenic mutant (See Table 2 above). For interim analyses of noncompetitive infections, data for WT may include results from the current working bank, regardless of the proximity in time of the specific isogenic mutant group. See Section 5.3.4 for handling of withdrawals.

At the interim analysis, the following decision rule for the noncompetitive analysis will be used:

- If the p-value from Fisher's Exact Test is less than 0.025, we will consider stopping the mutant group.
- If the p-value from Fisher's Exact Test is greater than 0.025 and the p-value will still be greater than 0.025 regardless of the results from additional subjects in the mutant group, we may consider closing enrollment for the noncompetitive infection study early for futility and proceeding with the competitive infection study.
- If the p-value from Fisher's Exact Test is greater than or equal to 0.025, we will continue to enroll until evaluable data are obtained from at least 7 subjects in the mutant group.

If additional subjects are enrolled in the mutant group, a second interim analysis will include results from all evaluable subjects in the mutant and WT groups. At the second interim analysis, the following decision rule for the noncompetitive analysis will be used:

- If the p-value from Fisher's Exact Test is less than 0.025, we will consider stopping studies for the isogenic mutant.

-
- If the p-value from Fisher's Exact Test is greater than or equal to 0.025, we will conclude that a difference in infectivity cannot be determined in noncompetitive experimental human infections.

If the infectivity of the two strains is not significantly different at that point, we will proceed with competitive infections to determine the relative fitness of the isogenic mutant. Interim analyses for competitive infections will be conducted after at least 5 subjects have been inoculated with a specific mixture of WT and mutant. The following decision rule will be used for the interim analysis:

- If the p-value from the sign test of the competitive index is less than 0.025, we will consider stopping the competitive infection group
- If the p-value from the sign test of the competitive index is greater than or equal to 0.025, we will continue to enroll up to 16 subjects.

11.3.2 Safety Review

The SMC will convene to review subject safety as described in section 9.6. Safety outcome measures include adverse events, protocol deviations and any unexpected events that occurred since the previous review. Primary and secondary study outcomes for subject groups enrolled to test an individual mutant and statistical rules for halting noncompetitive infections will be reviewed to determine whether or not to proceed to competitive infections.

11.3.3 Immunogenicity or Efficacy Review

Not applicable

11.4 Final Analysis Plan

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.

Baseline and demographic characteristics will be summarized. All subjects inoculated with a dose of *N. gonorrhoeae* within 1 log₁₀ of the intended dose and with necessary data elements available will be included in the analyses. Data from subjects who are enrolled in the trial but who receive an unintended inoculum dose or who withdraw from the study before objective study endpoints are reached will not be included in final analyses. For noncompetitive experimental infections with individual WT or isogenic mutant strains of *N. gonorrhoeae*, the primary outcome is the proportion of subjects that become infected after inoculation with each

the trial. 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.

14.3.1 Informed Consent/Assent Process (in Case of a Minor)

Not applicable; individuals below the age of 18 will be excluded from the study.

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

Females will not be included in the study because of the risk of complications from ascendant gonococcal infection. Male subjects of any race will be included. Children under 18 years of age are deemed inappropriate for inclusion in a study of a sexually transmitted infection.

14.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. Results of study research may be published, but subject identities will not be revealed. After written informed consent is provided, consecutive study code numbers will be assigned to the subjects to ensure confidentiality. This study code number will be used for laboratory transport and processing, result reporting, and data recording. Additionally, the principal investigator will keep records locked in cabinets. Data entered into computerized files will be stored only in a password protected file on a computer in the PI's office, which is accessible only by authorized personnel directly involved with the study. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

This research is covered by a Certificate of Confidentiality from NIH. The researchers with this Certificate may not disclose or use information, documents, or biospecimens that may identify subjects in any federal, state, or local civil, criminal, administrative, legislative, or other action, suit, or proceeding, or be used as evidence, for example, if there is a court subpoena, unless subjects have consented for this use. Information, documents, or biospecimens protected by this Certificate cannot be disclosed to anyone else who is not connected with the research except, if there is a federal, state, or local law that requires disclosure (such as to report child abuse or communicable diseases but not for federal, state, or local civil, criminal, administrative, legislative, or other proceedings, see below); if subjects have consented to the disclosure, including for medical treatment; or if it is used for other scientific research, as allowed by federal regulations protecting research subjects.

The Certificate cannot be used to refuse a request for information from personnel of the US federal or state government agency sponsoring the project that is needed for auditing or program evaluation by NIAID, which is funding this project, or for information that must be disclosed in order to meet the requirements of the US FDA. The study monitor or other authorized representatives of the sponsor such as the investigator and her research associates, the UNC-CH