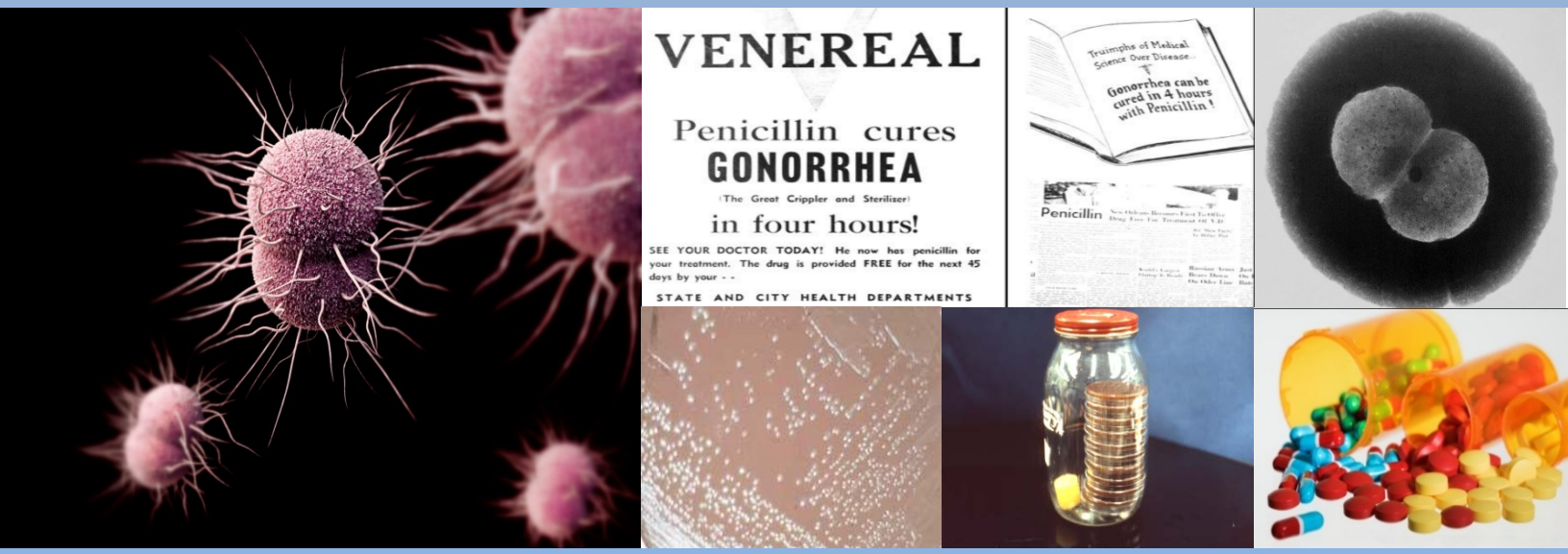
Gonococcal Isolate Surveillance Program (GISP)



Protocol

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1. Introduction

1.1. Background

The treatment and control of *Neisseria gonorrhoeae* infections have been complicated by the organism's ability to acquire antimicrobial resistance. The increasing prevalence of strains with plasmid-mediated resistance to penicillin (PPNG) prompted the abandonment of penicillin as single-dose therapy for gonorrhea in 1987. The development of plasmid-mediated resistance to tetracycline, i.e., TetM, caused the Centers for Disease Control and Prevention (CDC) to recommend, in 1985, that tetracycline not be used for the treatment of gonococcal infections. The prevalence of chromosomally-mediated resistance to fluoroquinolones reached levels in the United States at which fluoroquinolones were no longer recommended for gonorrhea treatment by 2007. Occasional chromosomal-mediated resistance to spectinomycin has also been reported. Minimum inhibitory concentrations (MICs) of oral cephalosporins, such as cefixime, have increased in many regions of the world, including the United States. Isolates with high azithromycin MICs have been detected sporadically.

The Gonococcal Isolate Surveillance Program (GISP) was established in 1986 to monitor antimicrobial susceptibility trends in *N. gonorrhoeae* strains in the United States and to establish a rational basis for the selection of gonococcal therapies. Data from this project have been reported and used to revise the CDC's STD treatment recommendations in 1989, 1993, 1998, 2002, 2006, 2010, and 2015. This protocol supersedes all previous protocols for the project.

1.2. Objectives

1. To monitor *N. gonorrhoeae* antimicrobial susceptibilities trends

2. To characterize male patients with urethral gonorrhea attending STD clinics, particularly those infected with *N. gonorrhoeae* that are not susceptible to recommended antimicrobials.

3. To phenotypically characterize isolates to describe the diversity of *N. gonorrhoeae* antimicrobial resistance.

2. Methods

The Gonococcal Isolate Surveillance Program is a collaboration between the CDC Division of STD Prevention (DSTDP) Surveillance & Data Management Branch (SDMB) and the Laboratory Reference & Research Branch (LRRB), with support from the Program Development and Quality Improvement Branch (PDQIB); regional laboratories; and selected public health STD programs and associated STD specialty care clinics in the United States. The responsibilities of each group of participants are detailed in this protocol.

GISP analyses are based on (1) demographic and clinical data from the first 25 male patients attending the sentinel clinics each month who have been identified to have a positive urethral culture for *N. gonorrhoeae*, and (2) antimicrobial susceptibility data from these urethral isolates.

3. Activities and Responsibilities

3.1. Sentinel Sites

3.1.1. Overview

A GISP sentinel site is responsible for the monthly collection and submission of (1) urethral gonococcal isolates from men to its assigned GISP regional laboratory, and (2) clinical/demographic data on GISP patients to CDC.

To participate in GISP, the sentinel STD specialty care clinics will routinely use gonococcal culture in lieu of or in addition to non-culture testing (such as nucleic acid amplification testing [NAAT]) on all or a subset of male patients with urethritis. Culture provides useful data on antimicrobial susceptibility that can benefit patients directly and is important for local disease control efforts.

At each sentinel site, an individual will be assigned to be responsible for data collection and appropriate gonococcal isolate collection, and to ensure that the isolates are sent to the appropriate regional laboratory and that the epidemiologic data are sent to CDC.

3.1.2. Sentinel Site Laboratory Collection, Handling, and Shipping of Isolates

Urethral *N. gonorrhoeae* isolates (based on a presumptive* or confirmed identification) will be collected from the first 25 men with urethral gonococcal infection (regardless of symptom status) each month. Usually, the isolates will be collected starting on the Monday of the first full week each month. Restricting sampling to symptomatic men with urethritis is acceptable.

Because there may be occasional month-to-month variability in the number of isolates submitted, a sentinel site may provide more than 25 isolates in any given month to make up for providing fewer than 25 isolates in other months; the overall goal is for each sentinel site to provide at least 300 isolates per year. Most isolates will be pre-treatment isolates; however, post-treatment isolates may be included in the sample.

> *A presumptive identification of *N. gonorrhoeae* will be based on the following criteria: (i) growth of typical appearing colonies on a selective medium such as Thayer-Martin at 35° C to 36.5° C in 5% CO_2, (ii) a positive oxidase test, and (iii) the observation of gram-negative, oxidase-positive diplococci in stained smears.

Gonococcal isolates will be subcultured from the selective primary medium to a non-inhibitory medium, e.g., chocolate agar with 1% IsoVitaleX to obtain a pure culture of the isolate. If the subcultured isolate is not pure, serial subcultures of individual colonies must be performed until a pure culture is obtained. After 18 to 20 hours of incubation, growth from the pure culture is suspended heavily in trypticase soy broth containing 20% (v/v) glycerol; duplicate frozen cultures of each isolate are prepared.

Isolates will be assigned sequential identifiers for each month. Each identifier will be composed of a three-letter designation for the sentinel site (see Appendix 1), followed by a six-digit number indicating the year and month of isolate collection (yyyymm), and a two digit number in the sequence from 01 through 25 or higher. For example, the 20th isolate selected in January 2014 in Atlanta will be given the number ATL-201401-20.

Each sentinel site laboratory should maintain a monthly log of GISP identification numbers and the corresponding patient name or identification number. This log is for local use only and is not to be shared with the regional laboratory or CDC. This information must be routinely shared with the sentinel site staff person who is responsible for abstracting demographic and clinical data on GISP patients. So that data can be properly merged at CDC, the GISP identification number of an individual isolate in the antimicrobial susceptibility data must match the GISP identification number of the isolate in the demographic and clinical data.

Isolates will be frozen to -70° C if possible. If a -70° C freezer is not available, isolates may be frozen to -20° C (freezer/dry ice chest) until shipped to the regional laboratory; isolates to be shipped must be placed in the coldest sections of the -20° C freezer (not in the door or at the front of a shelf) and should be stored in containers separate from any other frozen gonococcal cultures (including separate from duplicate frozen specimens). GISP isolates should not be subjected to changes in temperature, which may result in loss of viability during storage. A frost-free freezer should not be used.

Isolates should be shipped each month to the regional laboratory on Monday of the week immediately following completion of isolate collection, but no later than the first Monday of the month following the month of isolate

collection. Duplicate isolates must be kept until the regional laboratory confirms that viable isolates have been received.

If NAAT for gonorrhea is performed for a specific specimen and is found to be negative, the isolate should not be shipped to the regional laboratory as part of core GISP activities. If such non-gonococcal isolates are shipped, the sentinel site should notify the regional laboratory.

*Please e-mail or telephone the regional laboratory **prior** to shipping isolates to confirm when the isolates will be shipped and to ensure that someone in the regional laboratory will be available to receive the isolates. Ideally, isolates should be shipped no later than Wednesday in any week in which they are shipped to ensure that they are received at the regional laboratory before close of business on Friday.

Isolates must be packed in two leak-proof containers, one inside the other. The package containing the isolates should be packed in insulated styrofoam containers (to be provided by CDC) with dry ice (at least 10 lb.); dry ice should be packed on each side of the package of isolates. The containers should be shipped by overnight express (Priority Overnight), and the shipping costs charged to the FedEx account number provided by CDC. The container will be returned to the sentinel site by FedEx Express Saver 3-day delivery for future shipments. Sites should ship GISP isolates ***each month***; isolates should not accumulate for several months and then be shipped together because this prevents the regional laboratories from completing the susceptibility testing on schedule.

3.1.3. Sentinel Site Clinic or Program Activities

3.1.3.1. Reporting Demographic and Clinical Data

Demographic and clinical data should be submitted for each patient from whom a GISP isolate is submitted. A unique GISP number will be assigned to each isolate (see Section 3.1.2, item 3 above). Data may be obtained through review of medical records by clinic staff. Data will be submitted electronically using Form 1 (CDC 73.60 A) of the secure GISP web-based application and maintained locally in the clinic files (see Appendix 1 for description of requested data elements and the GISP Coding Guide). An example of Form 1 is available on the GISP website. Data may also be submitted electronically as comma-delimited (.csv) files. Demographic and clinical data reports should be received at CDC *no more than four weeks* after the end of the month in which the corresponding isolates were collected.

Appendix 1 provides detailed descriptions of the requested data elements. The GISP Coding Guide provides instructions on correct coding of responses. The following is a concise list of the requested demographic and clinical data elements:

- Sentinel site code
- Specimen collection (YYYY/MM)
- Sequential patient number (01–25 or higher)
- Clinic code (for those sentinel sites submitting GISP isolates from more than one clinic)
- Sex
- Ethnicity
- Race (census categories)
- Date of clinic visit
- Age
- Sex of sex partner
- Presence of symptoms
- Previous history of gonorrhea
- Number of previous confirmed episodes of gonorrhea in past year
- HIV status at time of clinic visit for gonorrhea (including results of HIV testing at the time of the clinic visit)
- Travel outside the United States during the previous 60 days
- History of giving or receiving drugs / money for sex in the previous 12 months

- Any antibiotic use during the previous 60 days
- History of injection drug use in the previous 12 months
- History of non-injection recreational drug use (excluding alcohol) in the previous 12 months
- Primary treatment for gonorrhea (such as ceftriaxone, if recommended dual therapy administered)
- Secondary treatment for gonorrhea (such as azithromycin 1 g, if recommended dual therapy administered; This variable was previously considered co-treatment for presumed chlamydia, if present)

3.1.3.2. Annual Process Measure Reporting

As described in the STD AAPPS Funding Opportunity Announcement (CDC-RFA-PS14-1402), sentinel sites are expected to monitor and report on process measures to document progress towards achieving GISP project outcomes. The data should be submitted to CDC as part of the Annual Progress Report (APR).

At a minimum, awardees are expected to monitor and report on the following measures:

• Number of cases of gonococcal urethritis diagnosed in men attending the STD clinic

• Number of isolates submitted to the GISP regional laboratory

• Percentage of submitted isolates that were found by the GISP regional laboratory to be non-viable or contaminated

• Percentage of monthly isolate batches shipped to the GISP regional laboratory within one week after the end of monthly collection

• Percentage of monthly data transmissions that were submitted to CDC within 4 weeks after the end of the month in which the corresponding isolates were collected

• Percentage of collected isolates for which the following data elements were reported: (a) age, (b) race/ethnicity, (c) gender of sex partner/sexual orientation, (d) HIV status, (e) antibiotic use, and (f) treatment

In addition, awardees should describe their plans to address challenges faced in enrollment, specimen quality and viability, timeliness of specimen or data transmission, and data completeness.

3.2. Regional Laboratories

The regional laboratories in Atlanta, Baltimore, Birmingham, Seattle, and Austin are responsible for determining ß-lactamase production and antimicrobial susceptibilities of GISP isolates received from the sentinel sites, and shipping selected isolates to CDC.

3.2.1. Receipt of Isolates

The isolates will be cataloged and frozen at -70º C until tested. Any problems with the isolates such as improper shipping, non-viability, or contamination should be reported as soon as possible to the sentinel site and to CDC. The insulated containers will be returned to the sentinel sites for future shipments by FedEx Express Saver charging the shipping costs to the FedEx account number provided by CDC.

3.2.2. Confirmatory Testing

The regional laboratories are not required to perform confirmatory tests on all isolates although it is recommended that confirmatory tests be performed on any isolate that exhibits atypical colonial morphologic characteristics or

aberrant susceptibility patterns. Because only urethral isolates are being tested, a significant problem with the inclusion of non-gonococcal isolates in the sample is unlikely. However, urethral *N. meningitidis* may occasionally be isolated from urethral specimens. Because gonococcal serologic reagents may cross-react with non-gonococcal *Neisseria* and related species, we recommend that strains be identified with tests that detect acid production from carbohydrates and/or enzyme substrate tests or with a probe culture confirmation test. Non-gonococcal isolates need not undergo susceptibility testing as part of core GISP activities and should not be included in GISP data (unless otherwise directed by the CDC GISP team)

3.2.3. ß-lactamase Tests

All isolates will be tested for ß-lactamase by the Nitrocefin test (Appendix 2).

3.2.4. Antimicrobial Susceptibility Testing

Antimicrobial susceptibilities to (reported as MICs of) penicillin G, tetracycline, gentamicin, cefixime, ceftriaxone, ciprofloxacin, and azithromycin will be determined by the agar-dilution procedure on Difco GC medium base (Becton Dickinson, Cockeysville, MD) inoculated with 10^4 colony forming units (CFU). Appendix 3 describes the current GISP testing ranges and details for preparation of antibiotic-containing media. Regional laboratories should include a set of three control strains (F-18 [ATCC 49226; quality control isolate mandated by the Clinical and Laboratory Standards Institute (CLSI)] and two blinded strains) with each run and should report control strain MIC data on Form 3 (CDC 73.60C) each month (see Appendix 4, CDC Reference Strains of *N. gonorrhoeae*). An example of Form 3 can be found on the GISP website. The results of Form 3 should be entered into the GISP web-based application and the receipt copy should be maintained in laboratory files (see Appendix 1 for description of Data Elements, Form 3 [CDC 73.60C]). It is expected that susceptibility testing will be completed within one month of receipt of isolates from a sentinel site, and reported to CDC on a monthly basis within one week of completion.

If isolates meeting *Alert* Value MIC criteria (as listed below) are identified, it is the responsibility of the regional laboratory to retest these isolates to confirm the MICs. Regional laboratory personnel may retest these isolates with the next batch of isolates, provided this is no longer than one month after the initial test.

3.2.4.1. Isolates with Alert Value MICs

Isolates with MICs defined as alerts require confirmation by retesting and prompt reporting.

Alert MIC Criteria

Ceftriaxone MIC ≥ 0.125 µg/ml*

Cefixime MIC ≥ 0.25 µg/ml*

Azithromycin MIC ≥ 2.0 µg/ml**

* For ceftriaxone and cefixime, isolates should be tested for growth on medium containing the antibiotics at concentrations ranging from two dilutions below the initial MIC to sufficient concentrations above the initial MIC to obtain an endpoint MIC.

** For azithromycin, isolates should be tested for growth on medium containing azithromycin at concentrations ranging from two dilutions below the initial MIC to sufficient concentrations above the initial MIC to obtain an endpoint MIC.

The regional laboratory should notify the GISP Project Officer at CDC, sentinel site, and the pertinent local and/or state STD program(s) **within one working day** by telephone or by e-mail of any isolate(s) identified to demonstrate an alert MIC. If the isolate(s) with an alert result is re-tested, the GISP Coordinator and Project Officer at CDC, sentinel site, and the pertinent local and/or state STD programs should be notified **within one working day** after the alert value(s) has been confirmed.

Alert isolates must be stored and shipped in ***triplicate*** to the CDC & ATSDR Specimen Packaging, Inventory and Repository (CASPIR) Facility on a quarterly basis (4 times per year). Two of the alert isolates will remain with CASPIR and one of the isolates will be sent to CDC Laboratory Reference and Research Laboratory (LRRB) for testing. By the end of 2017, isolates will be sent initially to the CDC STAT Laboratory and then sent to CASPIR and CDC LRRB. Alert isolates are to be shipped on dry ice by FedEx Priority Overnight delivery and shipping costs should be charged to the FedEx account number provided by CDC. A sheet marked "Alert Value Isolate Packing Form" that contains the original and the retest MIC values for each isolate should be included with the isolate shipment; a copy of this same sheet should **also** be sent separately to the GISP Project Coordinator.

The one alert isolate that is sent to LRRB will be accessioned by Pathogenesis and Antimicrobial Reference and Research Laboratory (PARRL) team. The isolate will be accessioned into an Enterprise Laboratory Information Management System (ELIMS) database. Although the local or state laboratories placed a label that contains two identifiers (GISP and CASPIR) on the isolate tube, the isolate will also receive a CSID number by the ELIMS database. Once accessioning and testing has been completed, the isolate is stored in the -70°C freezer for later use.

The GISP Project Officer may occasionally request that selected alert isolates (critical values) be sent rapidly to CDC/CASPIR for confirmatory testing or further evaluation/characterization by LRRB. These isolates may have susceptibility results that might warrant public health action (see below).

Ceftriaxone MIC ≥ 0.5 µg/ml

Cefixime MIC ≥ 1.0 µg/ml

Azithromycin MIC ≥ 8.0 µg/ml

3.2.4.2. Archive isolates

On an annual basis, CDC will request that regional laboratories ship additional GISP isolates (referred to as *archive* isolates) to CDC/CASPIR for long-term storage. These isolates may be of phenotypic interest or may be a random sample of isolates submitted to the regional laboratory. These isolates will contribute to the GISP isolate bank, which is one of the few remaining large collections of gonococcal cultures, and possibly the FDA-CDC Antimicrobial Resistance Isolate Bank.

Based on current archive isolate criteria, CDC will generate the list of requested isolates and send the list to each regional laboratory. This list will usually be generated in the autumn of the year following specimen collection: the list of 2014 archive isolates will be generated and sent to labs in late 2015.

Duplicate copies of each requested archive isolate should be shipped to CASPIR, CDC by priority overnight delivery, and the shipping costs changed to the FedEx account number provided by CDC. By the end of 2017, isolates will be sent initially to the CDC STAT Laboratory and then sent to CASPIR.

3.2.5. Isolate Preservation

All isolates should be suspended in trypticase soy broth containing 20% (v/v) glycerol and frozen at -70° C in

duplicate at the regional laboratories. When isolates are requested by CDC, **triplicate** (in the case of alert and critical isolates) or **duplicate** (archive isolates) copies of each requested isolate should be shipped to CASPIR, CDC. A copy of each shipped isolate must be maintained at the regional laboratory until notified by the GISP Coordinator or GISP Manager that the isolates have been received. This will generally require maintenance of isolates from the current year and the previous year.

3.2.6. Shipping isolates to CASPIR

To ship GISP specimens to CDC/CASPIR, specimens should be placed into **liquid nitrogen suitable cryotubes with screwcaps** (no snapcaps).

GISP isolates should be shipped to:

Robert J. Davidson
CDC CASPIR™ Facility
602 Webb Gin House Rd., Bldg. C
Lawrenceville, GA 30045
Office: 770-339-5950
Fax: 770-339-5943
E-mail: rum8@cdc.gov

GISP isolates should be shipped on Mondays or Tuesdays using FedEx. At the time that a GISP shipment is sent, Robert Davidson should be emailed with the date of shipment and the FedEx tracking number so that he can expect and monitor the shipment.

3.2.7. Data reporting

Upon completion of laboratory testing, the antimicrobial susceptibilities of isolates submitted from each sentinel site will be entered in the GISP web-based application. See Appendix 1 for data elements for Form 2, CDC 73.60 B. Instructions for coding are found in the GISP Coding Guide. The results of Form 2 should be entered into the GISP web-based application and the receipt copy of Form 2 should be maintained in the laboratory files. A copy of the results of Form 2 (both Excel file and pdf) should also be sent back to their respective sentinel sites.

3.2.8. External Quality Assessment (EQA)

Twice each year, a set of 15 coded cultures will be provided to the regional laboratories by CDC LRRB for antimicrobial susceptibility testing. These cultures will include strains selected to represent susceptible and resistant isolates of *N. gonorrhoeae* and may include more than one copy of some strains. With the isolate shipment, LRRB will include the date by which results are requested to be returned by the regional laboratories to LRRB. LRRB will report back to each regional laboratory within 30 days of receiving results from each site with a preliminary discussion of results to allow the regional laboratory to assess its intra-laboratory reproducibility. Some assessment of overall performance may be based on previously determined modal MICs for strains. Each preliminary report will indicate individual MICs that are ≥2 dilutions greater or less than the previously determined modal MIC for that strain and antimicrobial agent. If the EQA results suggest problems in MIC testing, the regional laboratory should identify and address these problems, and report to CDC LRRB on corrections made. This report should be made within 30 days of notification by CDC that corrective actions are necessary. Depending upon the extent of testing problems, a second set of EQA cultures may then be provided for testing, with another 60 days for completion. Customized testing procedures may be used to address specific testing difficulties. Finally, if proficiency problems cannot be solved, testing may have to be shifted to an alternate laboratory.

EQA Testing Range

Antimicrobial agents	Range (µg/ml)
penicillin	0.008 – 64.0
tetracycline	0.06 – 64.0
gentamicin	1.0 – 32.0
ceftriaxone	0.001 – 1.0
cefixime	0.002 – 1.0
ciprofloxacin	0.001 – 32.0
azithromycin	0.008 – 16.0

3.2.9. Training and Consultation for Sentinel Sites

The regional laboratories may need to perform training of sentinel site personnel or provide technical assistance consultation to sentinel sites to improve or optimize the quality of GISP isolates submitted. If a problem with non-viability or contamination is recognized by the regional laboratory, this should be brought to the attention of the sentinel site quickly, as these problems may indicate problems with sentinel site isolate collection, handling, storage, or shipping.

So that sentinel sites can monitor and report accurately on non-viability and contamination as part of their annual reporting process, regional laboratories are asked to develop systems to record and track non-viability and gross contamination of isolates and share these data with the sentinel sites.

3.2.10. Regional Laboratory Annual Process Measure Reporting for Annual Funding

As per the STD Laboratory-based Surveillance & Gonococcal Isolate Surveillance Project (FOA PS14-1401), regional laboratories must submit an Annual Progress Report (APR) annually. The APR functions as both a mechanism for reporting progress towards project objectives and for requesting continued funding for the following fiscal year.

For monitoring of progress towards project objectives, laboratories are asked to report the following items:

- Number of isolates received
- Number (and percentage) of isolates received from each sentinel site that were non-viable
- Number (and percentage) of received isolates from each sentinel site that were contaminated
- Number of isolates tested for antimicrobial susceptibility
- Percentage of monthly batches of isolates that were tested within 1 month of receipt of isolates
- Whether the laboratory achieved a passing grade of ≥80% agreement with the modal MIC ±1 dilution of each EQA assessment for which results are available

3.3. Centers for Disease Control and Prevention

The administrative duties and technical assistance responsibilities relating to GISP will be performed by DSTDP SDMB and LRRB, with support from PDQIB.

3.3.1. Description of SDMB and PDQIB activities

1. Perform site visits, as needed, to sentinel sites and regional laboratories.
2. Implement data collection protocols, including modification of data collection forms when necessary and complying with Office of Management and Budget requirements.
3. Perform data management.
4. Provide an annual report to each sentinel site describing the percentage of the sentinel site data that are incomplete or missing.
5. Review and analyze demographic, clinical, and antimicrobial susceptibility data; communicate important clinical findings to STD programs and others.
6. Provide regional and site-specific data in electronic format to sites participating in GISP on a per request basis.
7. Prepare and distribute an annual report summarizing project findings.
8. Request GISP isolates from regional laboratories for archival storage in CASPIR.
9. Evaluate Annual Progress Reports (APRs) and budget requests from sentinel sites and regional laboratories.
10. Recruit new sites as needed.
11. Address human subject research issues for the project.
12. Update the protocol, coding guide, data collection forms, and GISP website, as needed.

3.3.2. Description of LRRB Activities

1. Perform site visits to regional laboratories as needed.
2. Accession all isolates into the ELIMS database.
3. Train regional laboratory personnel when necessary.
4. Select, quality control, evaluate, and distribute to regional laboratories (1) Difco GC medium base for antimicrobial susceptibility testing, (2) antimicrobial powders that do not require Material Transfer Agreements (e.g., penicillin, etc.), and (3) control strains.
5. Confirm antimicrobial susceptibility results for alert isolates, and other isolates as needed, and provide MICs to GISP Coordinator within 4 weeks of receipt of isolates in LRRB.
6. Distribute External Quality Assessment (EQA) cultures twice annually; Prepare and distribute biennial EQA reports.
7. Perform molecular epidemiologic characterization of selected isolates (e.g. isolates with cefixime MICs $\geq$0.25 µg/ml, ceftriaxone MICs $\geq$0.125 µg/ml, or azithromycin MICs $\geq$2.0 µg/ml, and others as deemed appropriate). Molecular characterization of isolates collected under this protocol may include genome sequencing and other advanced molecular detection approaches.
8. Perform identification of novel antimicrobial susceptibility patterns among isolates that require further investigation.
9. Assist with analysis of antimicrobial susceptibility data.

10. Conduct Etest® (bioMérieux, Durham, NC) and agar dilution confirmatory testing for endpoints of isolates exhibiting azithromycin MICs ≥16 µg/ml by agar dilution and cefixime MICs of ≥0.5 µg/ml

4. General Project Issues

4.1. Quality Assurance

It is expected that sentinel sites, regional laboratories, and CDC will perform the tasks described in this protocol in a timely and efficient manner within the prescribed deadlines. A summary of the GISP timelines for project participants may be found in Appendix 5. Difficulties in adhering to the protocol with regards to isolate collection at the sentinel sites should be reported to the regional laboratories; difficulties in adhering to the protocol with regards to clinical/demographic data collection at the sentinel sites and difficulties in adhering to the protocol at the regional laboratories should be referred to the GISP Coordinator at CDC.

The duties listed in this protocol for the various GISP participants may overlap in many areas. Frequent communications among GISP participants are to be conducted to monitor the day-to-day activities of the project. On an annual basis, a meeting of the Principal Investigators will take place. Additional meetings are to be scheduled as required.

4.2. Human Subjects

The GISP protocol was reviewed by the Office of the Associate Director for Science (ADS), NCHHSTP, CDC (most recently in May 2014) and the CDC Institutional Review Board (IRB). GISP is considered a surveillance and disease control activity, and not human subjects research.

4.3. Office of Management & Budget

The GISP protocol has been reviewed and approved by the Office of Management and Budget (OMB expiration 8/31/2019).

4.4. Publication of GISP Data

In order to make GISP data widely available, CDC will publish GISP data in the annual STD Surveillance Report, annual GISP profiles, and other GISP reports and peer-reviewed manuscripts.

Reports of analyses of overall GISP susceptibility trends and prevalence of resistance will include CDC and one principal investigator from each regional GISP laboratory as collaborators and co-authors.

Papers describing analyses of data from an individual sentinel site or an outbreak investigation at a specific sentinel site should involve staff from the relevant sentinel site.

Local use of GISP data is encouraged. Sentinel sites can develop abstracts and manuscripts for peer-reviewed publication based on local GISP data. In such cases, sentinel sites should acknowledge GISP as the source of data in the Methods Section, and if appropriate, sentinel sites are encouraged to collaborate with the regional laboratory that conducted the susceptibility data. Sentinel sites are asked to provide the GISP Project Officer at CDC with courtesy copies of accepted abstracts and manuscripts.

For papers that combine GISP data with other data sources or for which the described analyses expand substantially beyond GISP susceptibility data, GISP and the regional laboratories should at least be acknowledged. Authorship will be decided on a case-by-case basis.

4.5. Use of GISP Isolates and GISP Data

GISP isolates are collected primarily for surveillance of *N. gonorrhoeae* susceptibility, but some uses of GISP isolates and GISP data not described in this protocol may be desirable and may enhance the public health usefulness of this project.

To ensure adequate communication and address any human subjects issues which may arise with the use of isolates or data collected for public health surveillance, proposals by external parties for use of GISP isolates or GISP data not described in this protocol should be initiated through the following process: 1) a brief (i.e., 1–2 page) written proposal should be provided to the GISP Project Officer for CDC review, 2) if appropriate, consent and/or collaboration of the relevant sentinel site state or local STD programs that provided the isolates should be sought (and appropriateness can be determined by the CDC GISP team based on the nature of the project), and 3) Institutional Review Board (IRB) review should be sought as appropriate. Submission of the proposal to the GISP Project Officer at CDC is requested as a first step to ensure that projects do not overlap with work already in progress and to allow an assessment of whether the proposed project fits within the non-human subject research determination at CDC or requires IRB review.

An exception to this process is when isolates are already collected dually under GISP and another ongoing protocol. In that case, appropriate consents and/or collaborations of the persons collecting and processing the isolates should already have been obtained. Local IRB review should be sought as appropriate.

Sentinel sites and regional laboratories are asked to notify the CDC GISP Project Officer of proposed local uses of isolates collected through GISP.

4.6. Selection Criteria for Project Expansion

Sentinel sites and regional laboratories were chosen through a competitive application and objective review panel process (Funding Opportunity Announcements [FOAs] 14-1402 for the sentinel sites and 14-1401 for the laboratories). Potential sentinel sites that are not part of GISP will be able to apply for participation in the next funding cycle (that will begin in 2019).

Appendix 1

GISP Data Elements

Demographic/Clinical Data (Form1 or CDC 73.60A)

Variable Name	Type/Length	Description	Values
CLINIC	[Char, 3]	Sentinel site code	ALB=Albuquerque, ATL=Atlanta, BOS=Boston, BUF=Buffalo, BHM=Birmingham, CHI=Chicago, COL=Columbus, CLE=Cleveland, DAL=Dallas, GRB=Greensboro, HON=Honolulu, IND=Indianapolis, KCY=Kansas City; LAX=Los Angeles, LVG=Las Vegas, MIN=Minneapolis, NOR=New Orleans, NYC=New York City, ORA=Orange County, PHI=Philadelphia, PHX=Phoenix, PON=Pontiac, POR=Portland, SDG=San Diego, SEA=Seattle, SFO=San Francisco, TRP=Tripler Army Medical Center
YRMO	[Char, 6]	Year/Month of patient's visit	YYYYMM
ID	[Char, 2]	Patient of isolate number	01, 02, 03, 04…50
CLINID	[Char, 1]	Clinic identifier number	1, 2, 3…9
SEX	[Char, 1]	Sex	1=male, 2=female, 9=unknown
ETHNIC	[Char, 1]	Hispanic	1=Hispanic or Latino, 2=not Hispanic or Latino, 9=unknown
AMIND	[Char, 1]	American Indian/Alaskan Native	1=yes, 2=no, 9=unknown
ASIAN	[Char, 1]	Asian	1=yes, 2=no, 9=unknown
BLACK	[Char, 1]	Black	1=yes, 2=no, 9=unknown
NAHAW	[Char, 1]	Native Hawaiian/Pacific Islander	1=yes, 2=no, 9=unknown
WHITE	[Char, 1]	White	1=yes, 2=no, 9=unknown
ORACE	[Char, 1]	Other race	1=yes, 2=no, 9=unknown
DATEVIS	[Char, 10]	Date of clinic visit	MM/DD/YYYY
AGE	[Num, 2]	Age in years	99= unknown
SEXOR	[Char, 1]	Sex of sex partner	1=women only 2=men only 3=women and men 9=unknown
SYMP	[Char, 1]	Symptoms of gonorrhea	1=discharge and/or pain, 2=no discharge and no pain, 9=unknown
HISTORY	[Char, 1]	Previous history of gonorrhea (ever)	1=yes, 2=no, 9=unknown
EPSDS	[Num, 2]	Number of previous episodes within the past 12 months	0=no documented episodes 99=unknown
HIVSTAT	[Char, 1]	HIV status at time of clinic visit for gonorrhea	1=positive, 2=negative, 3=indeterminate, 9=unknown
TRAVEL	[Char, 1]	Travel outside of US in past 60 days	1=yes, 2=no, 9=unknown

Variable Name	Type/Length	Description	Values
SEXWK	[Char, 1]	History of giving or receiving drugs/money in the past 12 months	1=yes, 2=no, 9=unknown
ANTIBIOT	[Char, 1]	Antibiotic use in the past 60 days	1=yes, 2=no, 9=unknown
IDU	[Char, 1]	History of injection drug use in the past 12 months	1=yes, 2=no, 9=unknown
NONIDU	[Char, 1]	History of non-injection drug use in the past 12 months	1=yes, 2=no, 9=unknown
TRMT1	[Char, 2]	Primary treatment for gonorrhea	00=none 03=spectinomycin (Trobicin) 2 gm 04=ceftriaxone (Rocephin) 250 mg 05=ceftriaxone (Rocephin) 125 mg 06=ciprofloxacin (Cipro) 500 mg 07=cefoxitin (Mefoxin) 2 gm 12=cefixime (Suprax) 400 mg 14=cefpodoxime proxetil (Vantin) 200 mg 15=ofloxacin (Floxin) 400 mg 17=ceftizoxime (Cefizox) 500 mg 18=cefotaxime (Claforan) 500 mg 21=azithromycin (Zithromax) 2 gm 22=levofloxacin (Levaquin) 250 mg 23=cefpodoxime proxetil (Vantin) 400 mg 24=ceftibuten (Cedax) 400 mg 25=cefdinir (Omnicef) 300 mg 26=cefdinir (Omnicef) 600 mg 27= gemifloxacin 320 mg 28= gentamicin 240 mg (or weight-based dosage) 88=other (please indicate in Other Treatment 1) 99=unknown
OTHTRMT1	[Char, 15]	Other treatment not listed as code for TRMT1	If code “88” was entered for Treatment 1, please type in the name and dosage of the drug used for primary treatment of gonorrhea.
TRMT2	[Char, 2]	Second antibiotic used as part of dual therapy for gonorrhea (and treatment of chlamydia)	00=none 01=ampicillin/amoxicillin 09=doxycycline (Vibramycin)/tetracycline 10=erythromycin 11=azithromycin (Zithromax) 1 gm 15=ofloxacin 21=azithromycin (Zithromax) 2 gm 22=levofloxacin 88=other 99=unknown

Antimicrobial Susceptibility Testing (Form 2 or CDC 73.60B)

Variable Name	Type/Length	Description	Values
CLINIC	[Char, 3]	Sentinel site code	ALB=Albuquerque, ATL=Atlanta, BOS=Boston, BUF=Buffalo, BHM=Birmingham, CHI=Chicago, COL=Columbus, CLE=Cleveland, DAL=Dallas, GRB=Greensboro, HON=Honolulu, IND=Indianapolis, KCY=Kansas City; LAX=Los Angeles, LVG=Las Vegas, MIN=Minneapolis, NOR=New Orleans, NYC=New York City, ORA=Orange County, PHI=Philadelphia, PHX=Phoenix, PON=Pontiac, POR=Portland, SDG=San Diego, SEA=Seattle, SFO=San Francisco, TRP=Tripler Army Medical Center
YRMO	[Char, 6]	Year/Month of patient's visit	YYYYMM
ID	[Char, 2]	Patient of isolate number	01, 02, 03, 04…50
B_LAC	[Char, 1]	Beta-lactamase test	1=positive, 2=negative
PEN	[Num, 6]	Penicillin MIC	0.008, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0
TETRACY	[Num, 6]	Tetracycline MIC	0.06, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0
GEN	[Num, 6]	Gentamicin MIC	1.0, 2.0, 4.0, 8.0, 16.0, 32.0
CFX	[Num, 6]	Cefixime MIC	0.002, 0.004, 0.008, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1.0, 2.0
CRO	[Num, 6]	Ceftriaxone MIC	0.001, 0.002, 0.004, 0.008, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1.0, 2.0
CIPRO	[Num, 6]	Ciprofloxacin MIC	0.001, 0.002, 0.004, 0.008, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0
AZI	[Num, 6]	Azithromycin MIC	0.008, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0
DATETEST	[Date, 10]	Date isolate tested	MM/DD/YYYY
CONTROL	[Char, 1]	Control ID	A, B, C, D

NOTE: MIC values listed as possible responses may include values that are no longer included in the current recommended testing ranges.

Control Strain Susceptibility Testing (Form 3 or CDC 73.60C)

Variable Name	Type/Length	Description	Values
LAB	[Char, 3]	Regional laboratory	AUS=Texas Dept. of State Health Services, Austin, TX BID=Beth Israel Deaconess Medical Center, Boston, MA EMO=Emory University, Atlanta, GA UAB=Univ. of Alabama at Birmingham UWA=Univ. of Washington, Seattle, WA
CONTROL	[Char, 1]	Control ID	A, B, C, D
STRAIN	[Char, 9]	Strain number	F-18 1 (blinded) 2 (blinded)
B_LAC	[Char, 1]	Beta-lactamase test	1=positive, 2=negative
PEN	[Num, 6]	Penicillin MIC	0.008, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0
TETRACY	[Num, 6]	Tetracycline MIC	0.06, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0
GEN	[Num, 6]	Gentamicin MIC	1.0, 2.0, 4.0, 8.0, 16.0, 32.0
CFX	[Num, 6]	Cefixime MIC	0.002, 0.004, 0.008, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1.0, 2.0
CRO	[Num, 6]	Ceftriaxone MIC	0.001, 0.002, 0.004, 0.008, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1.0, 2.0
CIPRO	[Num, 6]	Ciprofloxacin MIC	0.001, 0.002, 0.004, 0.008, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0
AZI	[Num, 6]	Azithromycin MIC	0.008, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0, 64.0
DATETEST	[Date, 10]	Date isolate tested	MM/DD/YYYY

NOTE: MIC values listed as possible responses may include values that are no longer included in the current recommended testing ranges.

Appendix 2

ß-lactamase Testing

Purpose

ß-Lactamases are enzymes produced by bacterial species including *N. gonorrhoeae* that inactivate penicillin. Strains of *N. gonorrhoeae* that produce ß-lactamase are termed penicillinase-producing *Neisseria gonorrhoeae* (PPNG). Six different ß-lactamase plasmids in *N. gonorrhoeae* confer high-level resistance to penicillins, but not to cephalosporins. Patients infected with PPNG strains are expected to fail therapy with penicillins such as ampicillin or amoxicillin at a high rate. The level of plasmid-mediated penicillin resistance is usually much higher than that conferred by chromosomal resistance genes; the latter confer resistance (or moderate resistance) to penicillin and decreased susceptibility to cephalosporins. ß-Lactamases hydrolyze the amide bond in the β-lactam ring of penicillins including chromogenic cephalosporins such as Nitrocefin. A ß-lactamase test is performed by mixing a few colonies of a strain with a few drops of Nitrocefin solution in a tube, or by dropping Nitrocefin solution onto isolated colonies on a culture plate. A ß-lactamase-positive strain hydrolyzes the substrate and produces a color change from yellow/colorless (negative) to red (positive) after incubation at room temperature for between 15 seconds and no longer than 1 minute. The speed of the ß-lactamase reaction may be affected by the plasmid contained in the strain; PPNG strains possessing a 4.4-MDa plasmid may produce positive reactions much faster than strains possessing a 3.2-MDa plasmid. If no reaction occurs (no color change), the strain is ß-lactamase-negative.

Reagents and Media

- Nitrocefin powder
- Dimethyl Sulfoxide [DMSO; $(CH_3)SO_4$] *Hazard rating: NFPA 325M* (Dimethyl Sulfoxide. (DMSO; methyl sulfoxide).). Liquid. ACS Reagent Grade)
- Sodium Dihydrogen Phosphate (NaH_2PO_4), Reagent Grade
- Sodium Dihydrogen Phosphate (NaH_2PO_4), Reagent Grade (Sodium Phosphate, Dibasic†, Anhydrous, Guaranteed Reagent, 99% min. by acidimetry)
- Sodium dihydrogen phosphate
- Disodium hydrogen phosphate
- 24-h. pure culture, on nonselective medium, of strains to be tested

Supplies, Other Materials

- Beaker (50ml) or weigh paper or weigh boat
- Graduated cylinder (50ml)
- Screw-cap / snap-capped tubes (5 ml capacity)
- Pasteur pipettes (sterile)
- DMSO
- Beaker (50ml) or weigh paper or weight boat
- Sterile 100 ml. or 500 ml. bottles (storage of phosphate buffer)
- Tube/microtiter plate (nonsterile OK)
- Sterile inoculating loop
- Pasteur pipettes (sterile)

Safety Precautions

- Always wear the appropriate PPE (lab coats, safety glasses or goggles, and gloves) when working in the laboratory.
- Disposable gloves are <u>one-time</u> use items and should not be re-used. Inspect gloves for defects before donning. Gloves should be checked for proper fit and should not be too large or too small.
- Work surfaces should be routinely disinfected after completion of work and after any spill or splash.

10% bleach must be used as a disinfectant and 70% ethanol must be used only for cleaning purposes.

	WARNING	
	DIMETHYL SULFOXIDE (DMSO) MAY IRRITATE THE NOSE AND THROAT AND IS ABSORBED RAPIDLY THROUGH THE SKIN OR MUCOUS MEMBRANES. IT MAY CAUSE HEADACHES, GARLIC-LIKE BREATH, NAUSEA, VOMITING, DIARRHEA, AND COMA. CHRONIC EXPOSURE MAY RESULT IN ALLERGIC SENSITIZATION AND SCALING DERMATITIS. DMSO MAY ALSO ACCELERATE THE SKIN ABSORPTION OF OTHER MATERIALS INCLUDING TOXIC SUBSTANCES.	
	INGESTED	DO NOT INDUCE VOMITING. CALL A PHYSICIAN.
	INHALED	REMOVE TO FRESH AIR. IF NOT BREATHING, GIVE ARTIFICIAL RESPIRATION. CALL A PHYSICIAN.
	SKIN CONTACT	IMMEDIATELY WASH SKIN WITH LOTS OF SOAP AND WATER. REMOVE CONTAMINATED CLOTHING AND SHOES; WASH BEFORE REUSE. GET MEDICAL ATTENTION IF IRRITATION PERSISTS AFTER WASHING
	EYE CONTACT	FLUSH AFFECTED AREA IMMEDIATELY WITH LOTS OF RUNNING WATER (15 MIN), LIFTING THE UPPER AND LOWER LIDS OCCASIONALLY. GET IMMEDIATE MEDICAL ATTENTION.

Sample Information / Processing

Optimum specimen:

- A pure culture of the organism grown on nonselective (chocolate or equivalent) medium at 37ºC ±1ºC for 18-24 h in a 5% ±1% CO_2 incubator.

Substitute specimen:

- Four or five isolated colonies of similar morphology from a culture grown on a nonselective (chocolate or equivalent) medium or a selective (Thayer Martin or equivalent) at 37ºC ±1ºC for 18-24 h in a 5% ±1% CO_2 incubator.

Unacceptable specimen:

- Growth from cultures grown on a nonselective (chocolate or equivalent) medium or a selective (Thayer Martin or equivalent) at 37ºC ±1ºC for >24 h in a 5% ±1% CO_2 incubator.

Criteria for declaration of "Unacceptable" specimen:

- Culture held at room temperature longer than 1 h. after removal from incubator.
- Culture is older than 24 h.

Compromising factors affecting test results:

- Growth used to inoculate test must be pure. Mixture of strains that may include a ß-lactamase-positive contaminating strain with a ß-lactamase-negative gonococcal isolate may give a false-positive result.
- ß-Lactamase is an enzyme which will become inactive under adverse conditions
- Enzyme may be inactive in older culture of gonococci which has lysed.
- Enzyme activity may be reduced if culture has been held at room temperature for longer than 1 h.

Stability of specimen:

- Test should be performed within 1 h. of removal of culture from incubator. Enzyme activity will diminish with time after incubation for 24 h. or upon removal of culture from incubator to room

temperature.

Quality Control

Control strains:

- ß-Lactamase positive: *Neisseria gonorrhoeae* strain P681-E:
- ß-Lactamase negative: *Neisseria gonorrhoeae* ATCC 49226 (F-18)
- QC strains are stored at -70ºC in a solution of trypic soy broth containing 20% glycerol.
- Confirm ß-lactamase reaction of control strains before prepared frozen specimens. Specimens may be stored at -70ºC for 2 years.

Quality Control Procedure:

- QC strains are tested in the same manner as test strains. QC strains should be subcultured at least once after the initial culture from the frozen specimen before the test is performed.
- Streak onto plates of chocolate agar or supplemented GC agar for isolation. Incubate at 37ºC ±1ºC in a 5% ±1% CO_2 incubator for 18 - 24 h.
- Perform ß-lac by either plate or tube test.
- Record results on the reagent quality control antibiotic susceptibility test control sheet.

Quality Control Schedule:

- Test QC strains each day the test is performed and with each new lot of reagent.

Tolerance limits & Corrective Action:

- *Problem*: The Nitrocefin reagent should be colorless/yellow before inoculation with bacterial growth. If the reagent is pink, the substrate has been hydrolyzed during storage.
- *Corrective action*: The reagent must be discarded. A new tube should be used.
  The ß-lactamase-positive control strain will turn the reagent pink within 1 minute after growth is added. The color of the reagent to which growth from the ß-lactamase-negative control strain is added will remain unchanged.

- *Problem*: If the ß-lactamase-positive control strain gives a negative reaction and the ß-lactamase-negative control strain gives a positive reaction, the frozen specimen tubes or the culture plates may have been mislabeled.
- *Corrective Action*: Open another frozen specimen; retest to determine if the tubes may have been misnumbered. *Alternatively*, prepare new frozen specimens from known ß-lactamase-positive and negative cultures.

- *Problem*: If both the ß-lactamase-positive and -negative control strains give a negative reaction in the test, it is possible that the 9-lactamase-positive strain has spontaneously lost its plasmid. (This is a rare event.) Alternatively, a ß-lactamase-negative strain may have been inadvertently frozen and labeled as a ß-lactamase-positive strain.
- *Corrective Action*: Prepare new frozen QC specimens from a known ß-lactamase-positive strain. Discard previously prepared frozen specimens.

Preparation of Stock Nitrocefin Solution

1. **Prepare 10x Stock Solution**
   - Dissolve 0.5g Nitrocefin with a few drops of DMSO
   - Add 100ml of 0.1M phosphate buffer (pH 7.2)
   - Aliquot 2ml per tube, label with date prepared and store frozen at -20ºC
   - The stock solution can be stored for up to 1 year

2. **Prepare Working Solution**
   - Add 18.0ml of 0.1M phosphate buffer (pH 7.2) to 2ml of the 10x Nitrocefin stock solution
   - Label with date prepared and store remaining working solution at -4ºC for up to 2 months

Examination (Test) Procedure

1. **Plate Test**
   - Remove Nitrocefin solution (500 µg/ml) from the freezer /refrigerator and warm to room temperature.
   - Using a Pasteur pipette, drop one drop of the nitrocefin solution onto well-isolated colonies.
   - Observe the colonies for a color change to pink. This change should occur within 15 sec.
   - Read/record results.
     - Positive = yellow/colorless to red
     - Negative = no color change

2. **Tube/well Test**
   - Remove working Nitrocefin solution (25 µg/ml) from the freezer/ refrigerator and warm to room temperature.
   - Using a Pasteur pipette, dispense 2-3 drops of Nitrocefin reagent into tube/well.
   - Using a sterile inoculating loop, mix a loopful of growth with the reagent in the tube/well
   - Observe the suspension for a color change to pink. This change should occur within 15s

3. **Read/record results.**
   - Positive = yellow/ colorless to red
   - Negative = no color change

4. **Procedure Notes**
   - Mixed cultures of ß-lactamase-positive and -negative isolates are the major source of error in a test that has been accurately quality controlled. For this reason the test should be performed only on pure cultures of an isolate.
   - In an instance where a rapid result is required, a PRESUMPTIVE test may be performed on colonies on selective medium or on a mixed culture on a nonselective medium. Such a test must be clearly indicated as a PRESUMPTIVE ß-LACTAMASE POSITIVE and should be followed by a CONFIRMED test performed on a pure culture.
   - It is important that sufficient organisms be tested in the tube/well test. Insufficient organisms may result in either a weak, inconclusive result or a delayed reaction that may not be read until after the time allowed for a properly performed test.

5. **Interpretation of Results**
   - Negative reaction: ß-Lactamase-negative
   - Positive reaction: ß-Lactamase-positive

Sample Retention and Storage
- Nitrocefin solution is stored in a -20º C freezer.

Appendix 3

Methods for Antimicrobial Susceptibility Testing

Purpose / Principle

The treatment and control of *Neisseria gonorrhoeae* infections have been complicated by the organism's ability to acquire antimicrobial resistance. The development of plasmid-mediated resistance to tetracycline (TetM), i.e., in 1985 the detection of this plasmid caused CDC to recommend that tetracycline not be used for the treatment of uncomplicated gonococcal infections. The increasing prevalence of strains with plasmid-mediated resistance to penicillin (PPNG) prompted the abandonment of penicillins as single-dose therapies for gonorrhea in 1987. The prevalence of chromosomally-mediated resistance to fluoroquinolones in *N. gonorrhoeae* in Hawaii and California reached levels at which fluoroquinolones were no longer recommended for gonorrhea treatment in Hawaii in 2000 and in California in 2002. Occasional chromosomal-mediated resistance to spectinomycin has also been reported. Sporadic isolates with high minimal inhibitory concentrations (MICs) to azithromycin and cefixime have emerged more recently.

Agar-dilution susceptibility testing is the reference method for determining antimicrobial susceptibilities of gonococcal isolates. This method measures the ability of isolates to grow on agar medium containing an antimicrobial agent. Because isolates of *N. gonorrhoeae* are fastidious in their growth requirements and sensitive to toxic agar components, antimicrobial susceptibilities of gonococci are determined on GC base medium supplemented with 1% IsoVitaleX (or equivalent supplement) inoculated with 10^4 colony forming units/ml. The susceptibility of an isolate is defined by the MIC; i.e., the minimum concentration (µg/ml) of an antimicrobial agent that is required to inhibit its growth on the medium.

Antimicrobial Agents and Range of dilutions (µg/ml) (last revised February 2015)

Standard panel:
Penicillin G: 1.0 to 4.0
Tetracycline: 0.25 to 16.0
Gentamicin: 1.0 to 32.0
Cefixime: 0.015 to 0.5
Ceftriaxone: 0.008 to 0.5
Ciprofloxacin: 0.015 to 16.0
Azithromycin: 0.03 to 16.0
β-lactamase: positive or negative

Retest criteria for isolates with alert value MICs:

If the MICs of strains are not determined at the highest concentration of agent tested, the MIC should be retested for susceptibility to a higher range of two-fold dilutions. An endpoint should be determined. For specific repeat testing criteria, see page 10.

Equipment

- Incubator 37°C ±1°C, 5% ±1% CO_2
- Scale
- Steers replicator, base and inoculator (sterilized)

Reagents and Media

- GC II base medium plus IsoVitaleX
- Mueller-Hinton broth
- Antibiotic powders
    - Penicillin G; Sigma-Aldrich, Inc. Catalog# P7794-100MU

    - Tetracycline; Sigma-Aldrich, Inc. Catalog# T7660-5G
    - Ceftriaxone, Sigma-Aldrich, Inc. Catalog# C-5793
    - Cefixime; Sigma-Aldrich, Inc. Catalog# 18588-100MG-F
    - Ciprofloxacin; Sigma-Aldrich, Inc. Catalog# 17850-50-F
    - Azithromycin; Sigma-Aldrich, Inc. Catalog# 75199-25MG-F
    - Gentamicin; Sigma-Aldrich Inc. Catalog# G1264-1G
- McFarland Standard, 0.5 on the scale

Supplies, Other Materials

- Square 100mm x 10mm Petri plates
- Cotton tipped swabs
- result sheets; 1 per test
- Marking pen
- Sterile loops

Safety Precautions

- All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure. Refer to "CLSI® M29- *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline* - Current revision". For additional handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories - CDC/NIH - Latest edition", or to the regulations currently in use in each country.
- Follow procedures for infectious or potentially infectious products for the disposal of all used materials.

Sample Information / Processing

- Use a pure culture of a gram-negative, oxidase-positive diplococci grown on a nonselective medium (chocolate or supplemented GC base medium) and incubated for 16–18 hours at 37ºC ±1ºC in a 5% ±1% CO_2-enriched atmosphere.

Preparation of Antibiotic Containing Media

GC II agar base medium supplemented with 1% IsoVitaleX

- Prepare the required volume of GC base medium in single strength according to the manufacturer's directions. This may be done by weighing the correct amount of agar base into each bottle or by preparing the medium in bulk and dispensing it in the required volumes.
- Autoclave the medium at 121ºC for 15 min. Cool to 50ºC in a waterbath.
- Reconstitute the dehydrated IsoVitaleX with the provided diluent according to the manufacturer's directions.
- Add 10 ml of supplement per liter of base medium i.e., 1% (v/v); mix thoroughly. This medium is referred to as ‘Supplemented GC II’ medium.
- Dispense the required volume of Supplemented GC II medium into individual containers for the addition of antimicrobial solutions. Maintain at 50ºC in a waterbath.
- Prepare the working solutions and dilutions of antimicrobial agents from the stock solutions or standard powder (see Calculations). Working solutions are 10X concentrations and are prepared by serially diluting in sterile waterbath.
    - Note: It is important that no longer than 1 hour elapse between the time that the stock solution is thawed, the dilutions are prepared and added to the base medium and the plates are poured.

Agent	Dilution Range (µg/ml)	Concentration of Stock Solutions (µg/ml)
Penicillin	0.004 –4.0	400.0
Tetracycline	0.03 – 16.0	1600.0
Ceftriaxone	0.001 – 0.5	50.0
Cefixime	0.001 – 0.5	50.0
Ciprofloxacin	0.002 – 16.0	1600.0
Azithromycin	0.03 – 16.0	1600.0
Gentamicin	1.0 – 32.0	3200.0

- Add the required volumes of the prepared working solutions and dilutions of the antimicrobial agents to the respective bottles of GC medium, mix thoroughly and dispense into clearly labeled plates (see Calculations). Thorough mixing of the antibiotics in the medium can be accomplished by swirling the contents 3 times in a clockwise and counterclockwise motion followed by inverting the bottle 3 times, minimizing bubble formation.
- Pre-label plates (bottom of plate) with antibiotic and dilution. Record the Lot # of GC II agar base and IsoVitaleX supplement on the result worksheet for each run.
- Pour the number of plates corresponding to the volume required for each plate.
- Allow the plates to cool. Invert the plates and store them in sealed plastic bags at 4°C to 8°C for no longer than 8 weeks.

Calculations

Preparation of Stock Solutions of Antimicrobial Agents

- Stock solutions of antibiotics are prepared 100-fold more concentrated than the highest concentrations to be incorporated into the medium.
- Working antibiotic stock solutions are labeled with the date prepared and stored at -70°C for up to 6 months.
- The standard antibiotic powders may not be 100% active. The activity of the agent is provided with the standard powder. It is necessary to calculate the amount of powder to be weighed to prepare the standard stock solutions. This is done using the following formula:

$$\text{Volume (ml)} = \frac{\text{Weight (mg) x Activity (µg/mg)}}{\text{Stock Solution Concentration (µg/ml)}}$$

- Example:
  For Cefixime, the highest concentration of antimicrobial agent added to the medium is 4 µg/ml. Thus, the concentration of stock solution prepared will contain 400 µg/ml. If the activity of the cefixime powder is stated to be 750 µg/ml, the calculation would be used for 5ml of stock solution:

$$5 \text{ ml} = \frac{x \text{ mg x } 750\mu\text{g/mg}}{400\mu\text{g/ml}}$$

$$x \text{ mg} = \frac{5 \text{ ml x } 400\mu\text{g/ml}}{750\mu\text{g/mg}}$$

x mg = 2.7mg

Quality Control

Quality Control Strains:

- *Neisseria gonorrhoeae*, strain ATCC 49226 (F-18) (Susceptible)

GC strains are stored at -70°C in a solution of trypic soy broth containing 20% glycerol.
Reactions of QC strains should be confirmed at the time the frozen stocks are prepared. QC strains may be stored at -70°C for 2 years.

Procedure: Quality Control:

- Thaw vials of QC strains stored at -70°C.
- Streak inoculate strains onto plates of chocolate agar or supplemented GC agar medium for isolation. Incubate plates at 37°C ±1°C in a 5% ±1% CO_2 incubator for 18-24 h.
- Perform tests as described in Procedure section.
- Read and record results. Compare the QC results with expected MICs to assess the accuracy of QC testing.
  Note: The MIC of an organism as determined by agar dilution testing may vary ±1 dilution from the modal MIC. For highly reproducible testing, the range of MIC values for an individual isolate should not span more than 3 dilutions.

Quality Control Schedule:

- A QC test is performed each time clinical isolates are tested.
- QC results are recorded on the run sheet.

Tolerance Limits & Corrective Action:

- Problem: None of the reference strains give the expected MIC values. If the values are consistently "off" by the same number of tubes, this suggests that the concentrations are incorrect; this most probably occurred during preparation of the plates rather than through mislabeling of plates. If only one set of values is incorrect, it is possible that the reference strain is misnumbered or contaminated.
- Corrective action: If all values are "off" but the concentrations of the media can be deduced from the values obtained, the plates may be relabeled. If the value for only one strain is "off," the plates may be used. However, the offending reference strain should be rechecked or replaced for future testing.

- Problem: The MIC values obtained for the reference strains are "off," but there is no pattern to the results.
- Corrective action: Discard plates and prepare a new batch of medium.

Examination (Test) Procedure

- Use a cotton-tipped applicator to suspend isolated colonies (or cells from less dense areas of growth on the plate) in 5 ml of Mueller-Hinton broth.
- Adjust the density of the suspension to contain approximately 108 colony forming units (CFU)/ml by comparison with a 0.5 McFarland BaSO4 turbidity standard.

- Dilute this suspension 1:10 in MH broth to give 10^7 CFU/ml.
- Dispense an equal volume of each suspension into wells of a replicating device, e.g., Steer's replicator. These replicating devices deliver 0.001 - 0.005 ml of the bacterial suspension to the surface of the medium i.e., 10^4 CFU.
- Inoculate each plate of the set of antibiotic containing media plus a plate of GC II base medium containing 1% IsoVitaleX (as a control to determine that all isolates grew).
    - Type of Plate: Antimicrobial susceptibility testing is performed using square 100 mm x 10 mm Petri dishes. A total of 36 isolates can be tested per plate when inoculated with a Steer's replicator. Seven reference strains with known susceptibilities are included with each run. Therefore a total of 29 specimens can be tested with each run.
    - Note: Optionally, a control plate may be inoculated between each set of antibiotic-containing medium to ensure against carry-over of antimicrobial agents from one medium to another. However, there is no evidence to suggest that antibiotics are carried from one plate to the next. These plates also allow for monitoring for contamination of the inocula during the inoculation process.
    - Note. The time elapsing between the preparation of the strain suspensions and inoculation of the plates should not exceed 1 h.
- Dry the inoculated plates at room temperature for approximately 15 min. Invert the plates incubate at 37°C ±1°C in a 5% ±1% CO_2 incubator for 24 h.
- Examine the plates for growth. Use a separate sheet to record the results for each antibiotic tested and record the growth for each isolate on each dilution tested. Record the growth as good (+), poor (±), or no growth (-). By using this scheme, the results can be reviewed at a later date for transcription errors and, when isolates grow on the highest concentration tested, the degree of growth will indicate whether the isolate is growing well or not.
- Record the results for the QC strains and clinical strains on the run sheet

Method Performance Specifications

- The MIC of an organism as determined by agar dilution testing may vary ±1 dilution from the modal MIC. For highly reproducible testing, the range of MIC values for an individual isolate should not span more than 3 dilutions.

Appendix 4

Reference Values

Strain	ß	Pen	Tet	Cro	Cfx	Cip	Azn	Gen
ATCC 49226	NEG	0.25–1.0	0.25–1.0	0.004–0.015	0.004–0.03	0.001–0.008	0.25–1.0	N/A

Appendix 5. Summary of GISP Timelines for Project Participants

Sentinel Sites

1. **Demographic and clinical data** (Form 1) - Due monthly to CDC, no more than 4 weeks after the end of the month in which the corresponding isolates were collected.
2. **Isolates -** Due monthly to assigned regional laboratory, shipping out on Monday or Tuesday of the week immediately following completion of isolate collection but no later than the first Monday of the following month.
3. **AAPPS (14-1402) grant application reporting** - Due annually, when AAPPS (14-1402) grant applications are due in late summer or early fall.

Regional Laboratory

1. **Testing of isolates** - Should be completed within one month of receipt of isolates.
2. **Susceptibility test data** (Form 2) - Due monthly to CDC and to sentinel sites, within one week of completion of testing.
3. **Control strain susceptibility test data** (Form 3) - Due monthly to CDC, within one week of completion of testing (together with the sentinel site Form 2 susceptibility data obtained from the same run).
4. **Notification of Alert Value Isolates -** Reporting of the identification of alert Value Isolates to CDC should take place within one working day of when the test results are read. Confirmatory testing should be done within a month of first test.
5. **Shipping of batched alert value isolates** – quarterly
6. **Shipping of selected critical alert value isolates to CDC** – ad hoc
7. **Annual Progress Reports and funding applications reporting** - Due annually, usually due in late summer or early fall.
8. **Shipping of archive isolates -** yearly

CDC

1. **Final date for receiving corrections or updates to previous year's GISP data from sentinel sites and regional laboratories** - Mid-March
2. **Electronic GISP data files for sentinel sites -** Available by August upon request to GISP Data Manager.
3. **Publish annual GISP Report** - By Fall/Winter of the year following the year of isolate collection.
4. **Notification of Alert Value Isolates** - Will ensure that appropriate sentinel site(s) and the local and/or state STD Program(s) are notified

Appendix 6. Project Personnel

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Appendix 7

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Participating Jurisdictions and Sentinel Sites

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Arizona (Maricopa County/Phoenix)

California (Orange County and County of San Diego)

Chicago, Illinois

Georgia (Fulton County/Atlanta)

Hawaii (Honolulu)

Indiana (Indianapolis)

Los Angeles County, California

Louisiana (New Orleans)

Massachusetts (Boston)

Michigan (Oakland County/Pontiac)

Minnesota (Hennepin County/Minneapolis)

Missouri (Kansas City)

Nevada (Southern Nevada Health District/Las Vegas)

New Mexico (Albuquerque)

New York (Erie County/Buffalo)

New York City, New York

North Carolina (Guilford County/Greensboro)

Ohio (Cleveland and Columbus)

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