

**0. Public Release Data Set Information**

This document details the Lab Protocol for NHANES 2001-2002 data.

A list of the released analytes follows:

<b>Lab</b>	<b>Analyte</b>	<b>SAS Label</b>	<b>Description</b>
I05_b	URXUGC	Gonorrhea, urine	Gonorrhea, urine

## 1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Gonorrhea is one of the most commonly reported sexually transmitted diseases in the United States with nearly 700,000 reported cases per year. *Neisseria gonorrhoeae* is the etiologic agent of gonorrhea. In men, gonorrhea infection usually results in acute anterior urethritis accompanied by a purulent exudate. In women, the infection is most often found in the cervix, but the vagina and uterus also may be infected. Frequently the infection is asymptomatic, especially in women. Without treatment, local complication of gonococcal infection can occur including pelvic inflammatory disease (PID) or acute salpingitis for women and epididymitis for men.

The LCx *N. gonorrhoeae* Assay (Abbott Laboratories, Abbott Park, IL) uses the nucleic acid amplification method LCR to detect the presence of *N. gonorrhoeae* DNA directly in clinical specimens. The four oligonucleotide probes in the LCx assay recognize and hybridize to a specific target sequence within the Opa gene of *N. gonorrhoeae* DNA. The oligonucleotides are designed to be complementary to the target sequence so that in the presence of target, the probes will bind adjacent to one another. They can then be enzymatically joined to form the amplification product which subsequently serves as an additional target sequence during further rounds of amplification. The product of the LCR reaction is detected on the Abbott LCx Analyzer.

## 2. SAFETY PRECAUTIONS

Consider all clinical specimens for analysis potentially positive for infectious agents. Observe universal precautions; wear protective gloves, eye wear, and lab coat during all steps of this method because of both infectious hazards. Place all plastic and glassware that come into contact with any clinical specimen in a plastic autoclave bag for disposal.

Some components of this product contain sodium azide as a preservative. The LCx Inactivation Diluent contains hydrogen peroxide. Material safety data sheets (MSDS) for sodium azide and hydrogen peroxide are available through the testing laboratory.

## 3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

- a. Each shipment of specimens received from the NHANES 2001-2002 mobile unit contains a corresponding transmittal sheet and an ANSI data file (XXXXXXX.TXT) is emailed as an attachment. The data file, containing the specimen ID, collection date, and type of sample (i.e. urine) is checked against the information on the transmittal sheet and specimen label prior to the assay.
- b. After the data is calculated and the final values are approved by the reviewing supervisor for release, all results are entered onto the NHANES ANSI data file by using Excel.
- c. After the results are entered, back-up copies are made and stored in locked areas.
- d. The results are emailed, as an attachment, to NCHS.

## 4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

- a. Urine specimens must be first catch (defined as the first 15-20mL of the urine stream). Specimens that are moderately bloody (greater than 0.5% v/v) or grossly mucoid (greater than 10% w/v) are unacceptable since they may cause inhibition of the LCx *N. gonorrhoeae* Assay. LCx uses the same urine specimen to test for the presence of *C. trachomatis* DNA.
- b. Urine specimens may be collected by using regular urine collection vials. Urine is transferred to 4-mL polypropylene screw-top vials and frozen at  $\leq -20^{\circ}\text{C}$ . Each week, batches of frozen urine samples are placed in a styrofoam-insulated shipping container with dry ice and sent to the laboratory by an overnight courier.
- c. Urine specimens are stable up to 24 hours at 4-8 $^{\circ}\text{C}$ . For longer periods, store the urine at  $\leq -20^{\circ}\text{C}$  in plastic vials, as long as the vials are tightly sealed to prevent desiccation of the sample.
- d. The optimal amount of urine is 3.0 mL to 4.0 mL. Specimen volumes of less than 3.0 mL are unacceptable.
- e. Avoid repeated freeze-thaw cycles, which may compromise specimen integrity.
- f. Specimens should arrive frozen.
- g. Residual samples are frozen at  $\leq -20^{\circ}\text{C}$ .

## 5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

## 6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALBRATORS (STANDARDS), AND CONTROLS

### a. Instrumentation

- (1) Eppendorf Microcentrifuge, model 5415C (Brinkmann Instruments, Westbury, NY).
- (2) Abbott LCx Dry Bath, model 8B23 (Abbott Laboratories, Abbott Park, IL).
- (3) Gilson Pipetman, 20- to 200- $\mu$ L (Rainin Instrument Co, Woburn, MA).
- (4) Gilson Pipetman, 200- to 1000- $\mu$ L (Rainin Instrument Co, Woburn, MA).
- (5) Vortex Genie mixer (Scientific Industries, Inc., Bohemia, NY).
- (6) Abbott LCx Thermal Cycler, model 8B24 (Abbott Laboratories, Abbott Park, IL).
- (7) Abbott LCx Analyzer, model 9A40 (Abbott Laboratories, Abbott Park, IL).

### b. Other Materials

- (1) LCx Urine Specimen Preparation, catalog number 3B21-24, including LCx urine specimen microfuge tubes with cap locks and LCx urine specimen resuspension buffer (Abbott Laboratories, Abbott Park, IL).
- (2) LCx Gonorrhea Amplification kit, catalog number 9B11-91, including LCx Gonorrhea Amplification vials, LCx Gonorrhea Negative Control, Calibrator and Activation Reagent (Abbott Laboratories, Abbott Park, IL).
- (3) LCx Gonorrhea Detection Reagent Pack (Abbott Laboratories, Abbott Park, IL).
- (4) Specimen Tube Racks (Fisher Scientific Co, Fairlawn, NJ).
- (5) Pipette tips with aerosol barrier (USA Scientific, Ocala, FL).
- (6) LCx Inactivation Diluent, catalog number 7B15-04 (Abbott Laboratories, Abbott Park, IL).
- (7) LCx System Diluent, catalog number 7B14-04 (Abbott Laboratories, Abbott Park, IL).

### c. Reagent Preparation

All reagents come prepackaged and are ready to use.

### d. Standards Preparation

- (1) LCx Calibration Standard
  - i. Prepackaged and ready to use. Activated by the addition of 100 $\mu$ L of LCx Gonorrhea Activation Reagent. After addition, the contents of the bottle is recapped and vortexed for 2-5 seconds. The bottle of activated LCx calibrator is designed to be used up to 48 hours if stored at 2-8 $^{\circ}$ C.

### e. Preparation of Quality Control Materials

- (1) LCx Negative Control
  - i. Prepackaged and ready to use. Activated by the addition of 100 $\mu$ L of LCx Gonorrhea Activation Reagent. After addition, the contents of the bottle is recapped and vortexed for 2-5 seconds. The bottle of activated LCx negative control is designed to be used up to 48 hours if stored at 2-8 $^{\circ}$ C.
- (2) LCx Positive Control
  - i. *N. gonorrhoeae* culture on a chocolate agar plate.
  - ii. Dilute *N. gonorrhoeae* to 1x10<sup>7</sup> CFU/mL in trypticase soy broth containing 20% glycerol. To store stock, aliquot in 100 $\mu$ L to 500 $\mu$ L volumes and keep at -20 $^{\circ}$ C or below.
  - iii. Resuspend *N. gonorrhoeae* in LCx Urine Specimen Resuspension Buffer and serially dilute to 1x10<sup>3</sup> CFU/mL. Heat the diluted cell preparation (1.0mL in LCx Urine Specimen Microfuge Tubes) in the LCx Dry Bath at 97 $^{\circ}$ C ( $\pm$ 2 $^{\circ}$ C) for 15 minutes ( $\pm$  1 minute). Perform amplification and detection in parallel with unknown specimens.

## 7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

### a. Assay Validity

The LCx analyzer automatically assesses validity of the LCx Gonorrhea Negative Control and Calibrator assay results

before proceeding to analyze specimen assay results. The LCx first verifies that the assay results of the Negative Controls and Calibrator are within the specified ranges of the LCx *N. gonorrhoeae* Assay Parameters by comparing the assay results of the Negative Control and Calibrator to the values listed in the assay parameters. A run is valid when the individual and average results are within the values listed for CAL HIGH, CAL LOW, CAL AVE HIGH, CAL AVE LOW, NEG LOW, NEG HIGH, NEG AVE HIGH, NEG AVE LOW parameters in the LCx *N. gonorrhoeae* Assay Parameters. In the event of an invalid Negative Control or Calibrator assay result, the assay results printout will identify the out-of-range result, the signal to cutoff (S/CO) ratio of the specimens will not be calculated and a flag indicating an invalid result will occur in the note column of the printout next to the specimen assay results.

If an amplification vial opens during thermal cycling, the sample is invalid and should not be used.

## 8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

### a. Sample Preparation

- (1) Allow urine specimen to completely thaw if frozen. Mix urine in the urine vial by vortexing for 2-5 seconds. It is not necessary for all particulate material to be fully dissolved.
- (2) Using a pipettor with aerosol barrier tips, transfer 1mL of the mixed urine into the Urine Specimen Microfuge Tube from the Urine Specimen Preparation kit.
- (3) Centrifuge at  $\geq 9,000xg$  for 15 minutes ( $\pm 2$  minutes) in a microcentrifuge.
- (4) Using a fine-tipped, plastic pipette, gently aspirate the urine supernatant off. Be cautious not to contact or dislodge the pellet, which may be translucent. The time between centrifugation and removal of the supernatant must not exceed 15 minutes.
- (5) Using a pipettor with aerosol barrier tips, add 1.0mL of LCx Urine Specimen Resuspension Buffer. Close lid of microfuge tube and resuspend pellet by vortexing until the pellet is off the bottom of the tube.
- (6) Secure tube closure with a cap lock until it clicks into place.
- (7) Insert specimen tubes in wells of preheated dry bath and allow the heat block temperature to stabilize to 97°C.
- (8) After the temperature of the heat blocks is stabilized to 97°C, heat specimens for 15 minutes ( $\pm 5$  minutes). Remove cap lock and discard.
- (9) Pulse-centrifuge the processed urine specimen in a microcentrifuge for a minimum of 10-15 seconds.
- (10) Test the processed urine specimen immediately, or store for up to 60 days at 2-8°C or -20°C or below prior to testing. If the processed urine specimen is stored frozen, it must be completely thawed prior to addition of the LCx Gonorrhea Amplification Vial.
- (11) Before opening the LCx Gonorrhea Amplification Vials, verify by visual inspection that no liquid is in the cap of the vial. The amplification reagent level should measure approximately two-thirds of the conical part of the vial. If necessary, the vial may be pulse centrifuged in a microcentrifuge for 10-15 seconds.
- (12) Using a pipettor with aerosol barrier tips, add 100 $\mu$ L of each processed urine specimen to the appropriately labeled LCx Gonorrhea Amplification Vial and make sure that each vial is securely closed. Immediately place the vial in the LCx Thermal Cycler for amplification.

### b. LCx Amplification

- (1) Turn the LCx Thermal Cycler on for at least 15 minutes prior to use.
- (2) LCx thermal cycling conditions should be edited to the following amplification parameters described below:

Assay Step-Cycle File:

Segment 1	93°C for 1 second
Segment 2	59°C for 1 second
Segment 3	62°C for 1 minute 10 seconds
Cycle count	40 cycles

The amplification run time is approximately 2 hours.

- (3) Place the amplification vials into the thermal cycler, and initiate run. After completion of the thermal cycler run, amplification product may remain at 15-30°C for up to 72 hours prior to LCx detection.

### c. LCx Detection and Inactivation of Amplification Product

The following LCx *N. gonorrhoeae* Assay parameters have been factory set in the Assay Module and may not be edited.

Table 1  
LCx Analyzer Factory Set Parameters

Parameter	Setting	
03	SAMPLE REP	1
32	MAX NRMSE	0.500
33	MIN CORR	0.950
34	MAX INTRCPT	12000.0
45	CAL HIGH	2230.00
46	CAL LOW	370.00
47	CAL AVE HIGH	2000.00
48	CAL AVE LOW	600.00
54	NUM POS CNTL	0
55	NUM NEG CNTL	2
61	% CUT OFF	25.000
83	NUM CAL	2
85	NEG HIGH	145.00
86	NEG LOW	0.00
87	NEG AVE HIGH	100.00
88	NEH AVE LOW	0.00

- (1) Before running the LCx Analyzer, check to see that LCx Inactivation Diluent contains a minimum of 100mL and the LCx Systems Diluent contains a minimum of 250mL.
- (2) Remove the LCx Gonorrhea Amplification Vials from the LCx Thermal Cycler.
- (3) Place the LCx Reaction Cells into a MEIA Carousel; lock the carousel.
- (4) Pulse centrifuge the LCx Gonorrhea Amplification Vials in a microcentrifuge for 10-15 seconds before placing into the LCx Reaction Cells.
- (5) Place the amplification vials into the LCx reaction Cells in the following order: Negative Controls in positions 1 and 2, Calibrators in positions 3 and 4, and the positive control and specimens in the remaining positions.
- (6) Place the carousel into the LCx Analyzer.
- (7) Lock the Amplification Vial Retainer by turning the handle counterclockwise.
- (8) Remove the LCx Gonorrhea Detection Reagent Pack from 2-8°C storage, gently invert 5 times, and open the reagent pack bottles in the numeric order: 1, 2, 3, 4.
- (9) Look for any film that may have formed over the openings of the reagent bottles. If present, remove using a long, clean pipette tip or a wooden applicator stick for each bottle.
- (10) Place the LCx Gonorrhea Detection Reagent Pack into the LCx Analyzer.
- (11) Press RUN on the LCx Analyzer control panel. Final assay results will be printed in approximately 60 minutes.
- (12) Remove the assay printout results from the LCx Analyzer.
- (13) After completion of the detection procedure, remove the LCx Gonorrhea Detection Reagent Pack, and close the caps in the numeric order: 4, 3, 2, 1. Store the detection reagent pack at 2-8°C.
- (14) Unlock the Amplification Vial Retainer by turning the handle clockwise until it is no longer over the MEIA carousel.
- (15) Remove the MEIA Carousel, individually remove the LCx Reaction Cells, and dispose appropriately.
- (16) Review the assay results and record the patient results.

**d. Recording of Data**

(1) Quality Control Data

The positive control should give a positive assay value (S/CO ratio  $\geq 1.20$ ).

(2) Analytical Results

A positive specimen has a S/CO of greater than 1.20 while a negative specimen is less than 0.8. Values between 0.8 and 1.20 are considered equivocal and repeat testing is recommended.

Following a valid run, enter the analytical results from the LCx Analyzer printout on the ANSI data file provided by the NHANES 2001-2002 Survey.

**e. Calculations**

- (1) The LCx *N. gonorrhoeae* Assay uses MEIA detection on the LCx Analyzer to detect *N. gonorrhoeae* DNA. All calculations are performed automatically. The presence or absence of *N. gonorrhoeae* is determined by relating the LCx Assay results for the specimen to the Cutoff value. The Cutoff value is the mean rate for the LCx calibrator duplicates multiplied by 0.45.

**9. REPORTABLE RANGE OF RESULTS**

Reportable results are expressed as positive or negative for *N. gonorrhoeae*.

**10. QUALITY CONTROL (QC) PROCEDURES**

The method described in this protocol is FDA cleared for the detection *N. gonorrhoeae* and is performed according to manufacturer guidelines. The sensitivity and specificity for female urine has been reported as greater than 92.0% and 99.1% respectively. The sensitivity and specificity for male urine has been reported as greater than 85.7% and 96.6% respectively.

**11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA**

- a. New reagent will be prepared and tested with control urine.
- b. If the run is declared "invalid," the entire run is repeated. If the "out of control" condition still exists, a new kit will be used and tested with control specimens. Specimens for that analytical run will be reassayed after the system has been reverified to be "valid."

**12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS**

- a. Some clinical specimens may contain endogenous inhibitors that interfere with amplification thus resulting in a false negative. Exogenous substances such as spermicides and feminine powder sprays may also interfere with amplification.
- b. DNA contamination from the Calibrator or clinical specimens must be avoided by good laboratory practice.

**13. REFERENCE RANGES (NORMAL VALUES)**

Not applicable to this procedure.

**14. CRITICAL CALL RESULTS ("PANIC VALUES")**

Not applicable to this procedure.

**15. SPECIMEN STORAGE AND HANDLING DURING TESTING**

Keep the specimens at 20-25°C during preparation and testing. Otherwise, store the serum at  $\leq -20^{\circ}\text{C}$ .

**16. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS**

In case of system failure, another FDA cleared amplified technology will be used to test the specimens.

**17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)**

Not applicable for this procedure.

**18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING**

For the NHANES 2001-2002 study, residual urine samples are stored at  $\leq -20^{\circ}\text{C}$  for 10 years after analysis and then discarded.

**19. Summary Statistics and QC graphs**

Qualitative assays are qualitative assays with a positive, negative or borderline/indeterminate result. The absorbance or reactivity values of specimens are compared with a cutoff value that is a ratio of the negative control mean and the positive control mean. Since the controls are read as cutoff values, plots of these values are not generated for quality control purposes.