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POLICY AND PROCEDURE	
SUBJECT/TITLE:	Clinical Laboratory Testing Procedures
APPLICABILITY:	All clinical testing performed by laboratory staff
CONTACT PERSON & DIVISION:	Christina R Henning, Lab
ORIGINAL DATE ADOPTED:	08/18/2006
LATEST EFFECTIVE DATE:	08/01/2023
REVIEW FREQUENCY:	Every 5 years
BOARD APPROVAL DATE:	N/A
REFERENCE NUMBER:	400-001-P

A. PURPOSE

“There are very few activities in life which are free from some form of constraint or limitation. Laboratory practice is no exception to this.” (Jim Ames, Bacteriologist) This procedure manual has been developed to provide guidance for the Canton City Public Health laboratory as it fulfills its’ responsibilities to the people of the city of Canton. It is not intended to substitute for proper laboratory practice or good judgment but rather help direct the analyses that take place in this facility.

B. POLICY

Clinical testing performed at and/or by Canton City Public Health Laboratory (CCPHL) Staff will be done in compliance with Centers for Medicare & Medicaid Services (CMS) 42 Code of Federal Regulations Part 493 Clinical Laboratory Improvement Amendments of 1988 (CLIA) regulations, brochures, and interpretive guidelines.

C. BACKGROUND

CCPHL is licensed under the authority of the Ohio Department of Health (ODH) as a laboratory meeting both federal CLIA regulations and state requirements to perform clinical laboratory testing: CLIA ID 36D0672229.

D. GLOSSARY OF TERMS

CCPHL – Canton City Public Health Laboratory

CLIA - Clinical Laboratory Improvement Amendments of 1988.

EMR - Electronic Medical Record System

ODH – Ohio Department of Health



E. PROCEDURES & STANDARD OPERATING GUIDELINES

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The CCPHL tests clinical specimens following varying procedures. Specific testing procedures are referenced in individual Appendices with general information provided in the following sections. The procedures for each test conducted by CCPHL staff are outlined in accordance with CLIA regulations as stated below.

§493.1251 Standard: Procedure manual.

1. *A written procedure manual for all tests, assays, and examinations performed by the laboratory must be available to, and followed by, laboratory personnel. Textbooks may supplement but not replace the laboratory's written procedures for testing or examining specimens.*
2. *The procedure manual must include the following when applicable to the test procedure:*
 1. *Requirements for patient preparation; specimen collection, labeling, storage, preservation, transportation, processing, and referral; and criteria for specimen acceptability and rejection as described in §493.1242.*
 2. *Microscopic examination, including the detection of inadequately prepared slides.*
 3. *Step-by-step performance of the procedure, including test calculations and interpretation of results.*
 4. *Preparation of slides, solutions, calibrators, controls, reagents, stains, and other materials used in testing.*
 5. *Calibration and calibration verification procedures.*
 6. *The reportable range for test results for the test system as established or verified in §493.1253.*
 7. *Control procedures.*
 8. *Corrective action to take when calibration or control results fail to meet the laboratory's criteria for acceptability.*
 9. *Limitations in the test methodology, including interfering substances.*
 10. *Reference intervals (normal values).*
 11. *Imminently life-threatening test results or panic or alert values.*
 12. *Pertinent literature references.*
 13. *The laboratory's system for entering results in the patient record and reporting patient results including, when appropriate, the protocol for reporting imminently life-threatening results, or panic, or alert values.*
 14. *Description of the course of action to take if a test system becomes inoperable.*
3. *Manufacturer's test system instructions or operator manuals may be used, when applicable, to meet the requirements of paragraphs (b)(1) through (b)(12) of this section. Any of the items under paragraphs (b)(1) through (b)(12) of this section not provided by the manufacturer must be provided by the laboratory.*

4. *Procedures and changes in procedures must be approved, signed, and dated by the current laboratory director before use.*
5. *The laboratory must maintain a copy of each procedure with the dates of initial use and discontinuance as described in §493.1105(a) (2).*

Prior to the addition of an unmodified nonwaived, FDA-cleared or approved test system the laboratory will meet the requirements of §493.1253. The laboratory is responsible for verifying the performance specifications of the test system that it introduces, prior to reporting patient test results. The verification of method performance should provide evidence that the accuracy, precision, and reportable range of the procedure are adequate to meet the clients' needs, as determined by the laboratory director and clinical consultant. A plan will be developed by the clinical consultant and laboratory director to meet the requirements of §493.1253 based on the test system being verified.

1. INSTRUCTIONS FOR SPECIMEN COLLECTION

Instructions for specimen collection are provided in the individual lab procedure.

2. LABELING SPECIMENS

- The primary specimen container/tube must be labeled with a minimum of the patient Unique ID code AND patient number, and if collected outside of the normal clinic hours the date and time of collection. The code is made up of the patient's initials from their first and last name and the two-digit day month and year of birth: FL010292. If the patient's name is hyphenated, use only the first initial of the first part of the last name: FL010292. If the patient's last name has two parts without a hyphen, use both initials from the last name: FLL010292. Note that use of a patient name alone on the specimen is inadequate to uniquely identify the specimen, adding a date of birth or another identifier is required. Note that microscope slides submitted for identification purposes are considered primary specimen containers and must be labeled appropriately. Exceptions may apply, but at a minimum all samples must have a minimum of two unique forms of identification.

3. TEST REQUESTS

All patient specimens submitted to the CCPhL for testing must be accompanied by an appropriately completed test requisition. Tests requisitions will be submitted in writing or electronically using the Canton City Electronic Medical Record System (EMR) unless the system is not in use during specimen collection.



LABORATORY REQUISITION FORM

This form is used for submission of patient specimens/isolates when the EMR system is not available. Test requisition forms for patient testing must be completed in their entirety and contain the following information (CLIA Regulation 42 CFR 493.1241) prior to submission:

1. Patient's name or unique patient unique ID,
2. Patient's sex,
3. Patient's Date of Birth (DOB) or age; if unique ID is not used,
4. Test(s) to be performed,
5. Source of the specimen, when appropriate,
6. Date and, if appropriate, time of specimen collection,
7. Submitting individuals name or approved initials, and
8. Any additional information relevant and necessary for a specific test to ensure accurate and timely testing and reporting of results, including interpretation, if applicable.

Note: Information provided on the test requisition form will be cross-referenced with information appearing on the label on the specimen container/tube/slide.

All patient-specific identifiers (i.e., patient name, date of birth, patient number, or other unique number or code) provided on the test requisition form must match those provided on the specimen container/tube/slide; any discordance will result in the specimen being held prior to testing to verify information with submitting authority. This could potentially lead to the specimen being deemed unsatisfactory for testing.

Also, if other information (e.g., sex, Date of Collection (DOC), time of collection) is discordant between the specimen container/tube and test requisition form that potentially affects the acceptability of the specimen, the submitter will be contacted for clarification.

Information provided on the test requisition may be changed following confirmation from the submitting authority. A corrected requisition form or other suitable documentation must be provided by the submitter before the specimen can be accepted for testing.

VERBAL REQUESTS

The laboratory will accept oral requests for laboratory tests and will solicit a written or electronic authorization immediately. A written or electronic authorization must be received within 30 days of the oral request. Verbal requests for add-on testing to previously submitted specimens are accepted, as appropriate (see Add-on Test Requests section below).

ADD-ON TEST REQUESTS

Additional testing may be added after submission of an original test request, if volume of the original submitted specimen is adequate. Requests for add-on testing may be received verbally but must be followed with a written or electronic request within 30 days of the verbal request. All verbal requests



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for add-on testing require test order 'read-back' to ensure accuracy. Additional testing may be delayed in the absence of a written or electronic request. A test report will not be issued for any additional requested testing in the absence of a written or electronic request.

STAT REQUESTS

STAT testing must be specified in the original test requisition or verbally with subsequent electronic or written request as indicated in the Verbal Requests section above.

TEST CANCELLATIONS

Testing can only be cancelled by the original submitting authority of the specimen. This can be done verbally or in writing.

REFERRAL TESTING

When indicated, the CCPHL will refer specimens to specific reference laboratories for additional testing. Test results from reference laboratories will be reported directly to the submitter.

Specimens referred for testing to a reference laboratory should be submitted using the reference laboratory's test requisition form, and collected, labeled, stored, and shipped as instructed by the reference laboratory. A Specimen Referral Log will be used to track dates and time as applicable of collection and shipping. Prior to submitting specimens to a reference laboratory, the referring laboratory will have on-hand a copy of the CLIA certificate of the reference laboratory to verify that the reference laboratory is certified for testing in the applicable specialty/subspecialty. Laboratory certificates are maintained as appendix 400-001-09-05-A_CLIA Reference Laboratory Certificates (note this is available only upon request).

4. SPECIMEN STORAGE AND SHIPMENT

Following collection, specimens must be appropriately pre-processed and stored (as necessary) and transported to ensure that they arrive at the CCPHL in a satisfactory state for testing. For detailed information on appropriate pre-processing, storage, packaging, and shipping of samples for submission to the CCPHL, refer to the individual test descriptions. Some general guidance on these topics is provided below.

1. Specimens must be packaged in a securely sealed, water-tight, primary container appropriate for the specimen being collected (e.g., blood tube, screw-capped plastic tube, etc.). This primary container must be appropriately labeled.
2. The primary container must be placed in a secondary container (clear rectangular plastic boxes, coolers or Ziploc bags are commonly used) that is capable of being closed to form a water-tight seal. The secondary container should be labeled with a biohazard warning sign.
3. Multiple samples may be placed in a single secondary container.



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COURIER SERVICE

The CCPHL utilizes department staff as well as Aultman's courier to transport specimens to CCPHL, local reference laboratories and the Ohio Department of Health's courier for specimens sent to the Ohio Department of Health Laboratory. To schedule a pickup for the Aultman courier call 330-438-6327 and place the locked box with the sample on one of the back doors. For samples being sent to the Ohio Department of Health, refer to ODHL for courier information and handling instructions.

Shipping Regulations

For shipping specimens, specimens should be packaged and labeled in compliance with applicable state, and federal, and international regulations covering the transport of clinical specimens and etiologic agents/infectious substances. Specific rules and regulations set forth by the U. S. Department of Transportation (Code of Federal Regulations 49 (CFR 49) part 173.196, Category A infectious substances and part 173.199, Category B infectious substances) should be followed to ensure safe transport of potentially infectious substances. Staff preparing specimens for shipment must have a minimum of training in Packaging and Shipping Division 6.2 Materials. (400-009-06-P)

5. SPECIMEN RECEIPT

SPECIMEN REJECTION

Specimens may be rejected for the following reasons:

- Inappropriate specimen (e.g., type; patient age; patient gender),
- Inappropriate specimen container or collection device/media,
- Insufficient volume for analysis (i.e., Quantity Not Sufficient (QNS)),
- No or illegible patient name or another unique identifier on specimen container,
- No or illegible patient name or other unique identifier on requisition form,
- Inability to match at least one unique identifier between the test requisition form and the specimen container due to absence or illegibility of others,
- No test requisition,
- Specimen received outside of timeframe appropriate for testing,
- Specimen handled improperly after collection (e.g., improper temperature during specimen shipment; specimen container leaked, broke, or otherwise compromised during transport),
- Laboratory accident (e.g., spilled sample during accessioning),
- Other reasons as outlined in the individual test descriptions of this Test Directory.

All specimens deemed unsatisfactory must have documentation stating the reason on the Lab Order and Results Log (400-004-03-F). Do not discard the specimen prior to consulting with the clinician for the sample status. This permits the clinician to clarify any potential concerns as well as make possible exceptions which must be clearly documented.



Missing Information

When any of the following information is missing from the test requisition form or specimen container, or is otherwise illegible or unclear (e.g., orders are non-specific or non-standard), the submitter will be contacted by the CCPHL, as appropriate:

- Date of Birth (DOB) or age, if appropriate,
- Date of Collection (DOC),
- Sex,
- Address of submitter,
- Test requested,
- Source of specimen, if appropriate.

6. TEST REPORTS

REPORT RESULTS

Results are reported in accordance with the parameters found in 400-001-09-02-A_Reference Range and Results Interpretation.

ISSUE OF TEST REPORTS

Reports are issued via the EMR. If the EMR is not available results will be provided on the original test requisition form.

CHANGES TO INFORMATION ON TEST REPORTS

Corrections to test reports, after original issue of test results to the submitter, may be made by the CCPHL, as appropriate. Changes will be documented in the Lab Orders and Results Log in the Quality Assessment and Assurance Policy binder. Once a result has been entered into the EMR and cleared for patient review, the original result cannot be changed. If a change is needed, utilize the comments field to note the needed change and that the current result is invalid and enter a new result with the correct information.

PATIENT ACCESS TO LABORATORY TEST RESULTS

On February 6th, 2014, the Centers for Medicare & Medicaid Services (CMS) published a final rule that amended both the Clinical Laboratory Improvement Amendments (CLIA) and the Health Insurance Portability and Accountability Act (HIPAA) to provide patients with direct access to laboratory test results. Under the final rule, laboratories that operate as covered entities under HIPAA are required to provide individual patients, or their representatives, with laboratory test results for those tests performed by the laboratory upon the patient's request.

The CCPHL is unable to provide laboratory test results directly to individuals presenting at this location. Patients, or their legal representatives, may obtain copies of their laboratory test reports for testing



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performed at the CCPHL by presenting at the Nursing Division of the Canton City Health Department or other health care facility where medical care was provided. The patient, or their legal representative, will be asked to complete an appropriate form, provide a photo ID and/or authorization code prior to release of laboratory test results.

Additionally, patients are provided access to their electronic medical record (EMR) which contains all patient encounter information, including their laboratory testing results.

Alternatively, patients, or their legal representatives, can contact the Nursing Division of the Canton City Health Department by phone to obtain further information.

4. GENERAL DOCUMENTS APPENDICIES & ATTACHMENTS

- 400-001-09-01-P_Electronic Laboratory Reporting Procedure
- 400-001-09-02-A_Reference Range and Results Interpretation
- 400-001-09-03-A_Lab Slip Legend
- 400-001-09-04-F_Control and reagent log.doc
- 400-001-09-05-A_Reference Lab CLIA Licenses
- 400-001-09-06-P_Shipping Policy

GRAM STAIN

1. MATERIALS REQUIRED

Glass microscope slides (one end frosted)
Gram stains: Crystal Violet, Gram Iodine, Safranin
Staining tray or rack
Decolorizer (95% Alcohol)
Heat source for slide fixation: burner, alcohol lamp, candle
Bibulous paper
Water (for rinsing)
Microscope with 10X and 100X oil immersion lens
Immersion oil
Commercially Prepared Control Slide
Biohazard waste container
Sharps container
Appropriate PPE

2. PATIENT PREPARATION

The patient will have been taken into a CCPH STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CCPH STI Clinic protocol. Urethral exudate will be collected on a glass microscope slide and brought to the STI Clinic STAT lab. Exudate may be expressed from the urethral meatus onto the slide; alternatively, a urethral swab may be used to collect the specimen. The slide must be labeled with the patient's unique ID and clinic number. It may be exposed to air and ambient temperatures until transport; no preservative is required. It is placed into a disposable plastic cup which is placed into a covered plastic transport box clearly marked with a biohazard label and taken to the STAT lab. If testing is to be delayed the slide may be stored in a covered container until it can be stained.

Once the slide is in the STAT lab all pertinent information is entered in the STAT lab logbook utilizing the STI Clinic Stat Lab Log Sheet (400-001-01-02-F), and the slide itself is examined for suitability for Acceptance: properly labeled: accompanied by a lab slip or electronic order: not cracked, damaged, or broken; evidence that a specimen is actually present. There is no referral for Gram Stains so it must meet criteria for Acceptance or Rejection. Grounds for Rejection are: No specimen on the slide; slide not fully and properly labeled; no accompanying lab slip or electronic order; slide is damaged, contaminated with a foreign object, or otherwise not intact. Rejected samples will be entered in the specimen log along with the reason for rejection. Note: The laboratory may accept oral requests for laboratory tests if it solicits a written or electronic authorization. Do not discard the specimen prior to consulting with the clinician for the sample

status. This permits the clinician to clarify any potential concerns as well as make possible exceptions which must be clearly documented.

Prepared slides can be stored indefinitely in slide boxes at ambient temperatures once they have been prepared (6 months minimum is desirable). Slides which present difficulties for the analyst can be preserved in this way until reviewed by another analyst or the Laboratory Director.

3. MICROSCOPIC EXAMINATION

A properly collected, prepared, labeled, and identified specimen, properly examined by the laboratory staff, will assist the clinical staff in determining what treatment should be provided to the patient. This is accomplished by:

Staining

1. Heat-fix the glass slide containing the specimen. This prevents the material on the slide from rinsing off during the staining process. The slide is passed through the flame of an alcohol burner (specimen side up away from flame); it should feel warm but not hot to the back of the hand. Too hot can distort cellular morphology. It is then allowed to cool.
2. Immerse the slide in the Crystal Violet in the Coplin jar; alternatively, you can flood the fixed slide with crystal violet. Allow 1 minute for staining.
3. Gently rinse the slide under running tap or Deionized water.
4. Immerse the slide in the Gram's Iodine Solution in the Coplin jar; alternatively, flood slide with the iodine solution. Allow 1 minute for the stain to be fixed.
5. Decolorize the slide by gently rinsing it with 95% alcohol, letting it flow over the stained material while the slide is held at an angle. Stop applying the decolorizer when the rinse just runs clear.
6. Gently rinse the slide under running tap or Deionized water
7. Immerse the slide in the Safranin in the Coplin jar; alternatively flood the slide with Safranin. Allow 1 minute for staining.
8. Gently rinse the slide under running tap or Deionized water
9. Drain the slide and blot it dry with bibulous paper.

Reading and Interpretation

10. Scan the slide with the microscope set on low (10X) power, looking for areas where White Blood Cells (WBCs), Epithelial cells (Epi's), and/or exudates are present.
11. Once suitable areas are located switch to the oil immersion (100X) lens and examine at least 10 representative fields. Note the average apparent number of WBCs per observed field; if Gram Negative Diplococci are present; and whether they are Intracellular (inside WBCs), Extracellular (outside WBCs), or both.

12. Note these observations in the STAT Lab log and on the patient's EMR or Lab slip.

5. MATERIAL PREPARATION

The microscope slides should be glass, one end frosted, 3 x 1" or (25 x 75mm) precleaned by the manufacturer. Our preferred decolorizer is 95% Denatured Ethanol; student, reagent or USP grade will suffice. Gram stains are prepared with reagent grade stains and chemicals according to published formulae (see appendix 400-001-01-03-P). Gram Reaction control cards are commercially obtained and utilized per manufacturer's directions.

6. CALIBRATION PROCEDURES

There are no calibrations performed for this procedure.
Follow routine operational instructions for the use of a microscope.

7. REPORTABLE RANGE

Refer to the most up-to-date version of the Reference Range Results and Interpretation for allowable reporting parameters. In general, observations can be reported for:

- a) WBCs per Oil Immersion Field (OIF), ranging from 0 to >50.
- b) Gram Stain Reaction, ranging from Gram Negative Diplococci Not Found to Gram Negative Diplococci (Intracellular or Extracellular) Found.

8. CONTROL PROCEDURES

In order to:

- (1) Detect immediate errors that occur due to test system failure, adverse environmental conditions, and operator performance, and
- (2) Monitor over time the accuracy and precision of test performance that may be influenced by changes in test system performance and environmental conditions, and variance in operator performance, the following control procedure will be used:

A commercially prepared control slide containing a minimum of two control materials, a gram-positive control and a gram-negative control will be processed according to manufacturer's instructions (400-001-01-04-A) each week of testing. Observed reactions will be noted in the QC Log, 400-001-01-01-F. The Daily QC Log will be reviewed quarterly utilizing the Quarterly QA Checklist to monitor overtime trends or concerns in the test system performance.

9. CORRECTIVE ACTION

If the Gram Stain control slide fails to give the expected results it should be repeated to determine if there was a mistake in performing the procedure. Examine the control slide to ensure that test material is present. If the second stain fails, the working stains in the jars should be replaced with

fresh and a new control run. If there is another failure, a slide of laboratory stock cultures should be prepared and run to determine if the fault is with the stain system or the control slides. A failure at this point requires that all prepared stains be discarded and replaced with new.

10. LIMITATIONS OF THE PROCEDURE

Because gram-negative diplococci have a distinct appearance they are not as easily confused as other organisms. But the gram stain technique is not as sensitive as others because of the masking effects of the mucus, WBCs, and other artifacts commonly found in the specimens. Analyst experience and technique have a major effect as improper attention to times, over- or under decolorization, and improper microscope technique can affect observations and interpretation. Additionally, the technique does not differentiate between viable and non-viable cells. See references for more information.

11. EXPECTED RESULTS AND NORMAL VALUES

Gram positive organisms will appear blue to violet in color; gram negative organisms will be red. Gram negative diplococci (two red, spherical organisms lying closely opposed, resembling 'two red kidney beans side by side') are presumptive of *Neisseria gonorrhea*. They may be either intracellular (inside white blood cells) or extracellular (outside of white blood cells) and noted as such. WBCs are much larger than diplococci, and often contain multiple nuclei. Epithelial cells are larger yet, Gram Negative, and usually have a visible nucleus. Normal Values are 0–4 WBC/OIF and Gram-Negative Diplococci Not Found. Report of anything else is clinically significant.

12. PANIC OR ALERT VALUES

There is no Panic or Alert Value.

13. DATA ENTRY AND REPORTING

The results of Control Slide runs are entered into the Daily QC Log, as well as other pertinent information such as date of working stain changes. Patient specimen observations are entered into the STAT Lab logbook as the analyst performs the examination, and initialed by the analyst upon completion. Results are provided to the Nursing Division by transcribing the results from the log into the EMR. To enter a result into the EMR, the scheduler page on the left toolbar is selected. The date of the clinic is then selected from the calendar. The patients' names for the current clinic are displayed and the correct patient's name can be selected by clicking on the name and scrolling down to the "Facesheet" option. Once the "Facesheet" page opens, the current laboratory tests can be found in the "Pending Services" section. To result a test, the "Pending Services" section can be expanded by clicking on the "Expand to Detail View" icon or the test can be result directly from the "Pending Service" section by clicking on the flask icon which is the "Add Result" button. In the expanded view, the "Add Result" option must be selected for the desired test. The first field on

the resulting page is the “Observation Identifier” and the patient’s unique ID is entered into this field. The next field to be entered is the “Observation Date.” The “Observation Date” field will automatically populate with the current date and will only need to be modified if the date of testing is different from the current date. The following field is the “Reference Range” and Negative will be selected for Gram Stain tests. The “Result Value” field will need to be filled in with the WBC range and whether or not gram-negative diplococci were observed. The WBC count will be 0-4, 5-50, or >50. If Intracellular diplococci or extracellular diplococci are observed, then this will be notated as “I GND” and “EGND” respectively. If no diplococci are observed, then “No GND” will be typed into this field. The “Result Flag” field will be entered with either Negative, Positive, or Abnormal. If no intracellular gram-negative diplococci were present and the WBC was 0-4, then Negative can be entered. If intracellular gram-negative diplococci were found, regardless of how many WBC, the result will be entered as Positive. If either no intracellular diplococci were found or only extracellular diplococci were found and the WBC was 5 or greater, then Abnormal can be selected for the “Result Flag” field. The “Acknowledge Result” will always have “yes” selected and the “Units” field can be left blank. The last field is the “Comments” section, and it will usually remain empty unless a specific comment for the sample is necessary. Once the fields are filled in, the “Add” button can be selected. A “To Do” is sent to the Lab Results group when the final test of the clinic session is completed so that the clinician, using the EMR may initiate appropriate treatment. Alternately if the EMR is not available, transcribe the results onto the Lab Requisition Form and hand carry the results to the appropriate nursing staff.

Proficiency Testing specimens are recorded in the patient Log as if they were an actual patient. Results are then transcribed into the Proficiency Testing Providers Electronic Reporting System at the earliest convenience and prior to the testing close date.

14. SYSTEM FAILURE OR INOPERABILITY

If Gram stains cannot be performed the Medical Director and Director of Nursing must be immediately informed so that STI Clinic practice can be modified to accommodate this situation.

15. GRAM STAIN APPENDICIES & ATTACHMENTS

- 400-001-01-01-F_Gram Stain Quality Control Log
- 400-001-01-02-F_Stat Lab Log Sheet
- 400-001-01-03-P_Gram Stain Reagent Preparation
- 400-001-01-04-A_Product Insert Fisher Gram-Check control slides



WET PREP

1. MATERIALS

- Glass microscope slides (1 end frosted)
- Cover slips (optional)
- Glass screw-top test tubes
- Disposable cotton swabs, sterile
- 0.9% Saline solution
- 10% Potassium Hydroxide solution (KOH)
- Transfer pipette
- Microscope with 10X and 40X
- Test Tube Rack
- Biohazard waste container
- Appropriate PPE

2. PATIENT PREPARATION

The patient will be taken into a CCPH STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CCPH STI Clinic protocol. The clinician will have collected the specimen (endocervical discharge) on a cotton swab, placed it in a screw-top test tube containing normal saline, labeled it with the patient's Unique ID and clinic number, and brought it to the STI Clinic STAT lab in a covered plastic transport container marked with a Biohazard label. Maintaining the specimen at room temperature and prompt transportation will help maintain trichomonad motility.

Once the specimen is in the STAT lab all pertinent information is entered into the STAT lab logbook. It is examined for suitability for Acceptance, as there is no referral for Vaginal Wet Preparations- recollection is the only recourse for unacceptable specimens. A swab or swabs must be present in the tube, and there must be sufficient saline to cover the cotton. Some blood is permissible but, if too much is present that the saline is opaque, the sample should be discarded. The Specimen should be accompanied with a lab slip or electronic order. Lack of any of these is a basis for sample Rejection, which will be noted in the logbook along with an explanation as to why. Do not discard the specimen prior to consulting with the clinician for the sample status. This permits the clinician to clarify any potential concerns as well as make possible exceptions which must be clearly documented.

There is no practical way to store Vaginal Wet Preparation specimens, but if care is taken to keep them at room temperatures and to keep the screw-top tube capped to prevent desiccation, motility and morphology of clinically significant features may be prolonged for many hours.



3. MICROSCOPIC EXAMINATION

A properly collected, prepared, labeled, and identified specimen, properly examined by the laboratory staff, will assist the clinical staff in determining what treatment should be provided to the patient. It should provide information on the presence or absence of *Trichomonas vaginalis*, yeast or fungal elements, abnormal bacteria, epithelial cells, and white blood cells. This is accomplished by:

1. The tube is swirled to thoroughly mixing the contents with the swab. They are then removed and rolled on a microscope slide (which may have been labeled with the patient number) expressing any fluid and material onto the slide. The use of a cover slip is optional; it prevents the objective lens from contacting the specimen and may make visualization of thick specimens easier but requires the use of another slide to perform the KOH and Whiff tests and may retard trichomonad motility.
2. Place the prepared slide on the microscope, select low (10x) power, focus, and scan the slide, looking particularly for motile trichomonads and fungal pseudohyphae.
3. Change the magnification to high (40X) power. Examine approximately 10 fields, noting presence or absence of trichomonads, clue cells, fungal pseudohyphae or budding yeast and the average number of white blood cells per field. Note these values in the Stat Lab Logbook.
4. KOH Addition to the Vaginal Wet Preparation. Remove the slide from the microscope stage and add a drop of 10% Potassium Hydroxide (KOH) solution. Immediately bring it near the nose and sniff; a “fishy”, amine odor is a positive Whiff test. Note such in the Logbook.
5. Replace the slide on the microscope stage. Examine at low (10X) and high (40X) powers. Bacteria, trichomonads, WBCs and RBCs will have been dissolved, epithelial cells will be distended and cleared, and pseudohyphae and budding yeast cells will be very evident. Enter positive findings in the logbook.

4. MATERIAL PREPARATION

The microscope slides should be glass, one end frosted, 3 x 1” or 25 x 75mm, precleaned by the manufacturer. Coverslips (optional) may be 22 x 22mm and of #1 (approx. 0.1 mm) thickness. Screw-top tubes of 20 x 150mm will accommodate swabs, which should be sterile, and cotton tipped. Approximately 2.0 to 3.0 ml. of sterile saline should be present in the tube before collection. The transfer pipette used for KOH addition should be of 5 – 10 ml. capacity and may be disposable plastic. 10% KOH and saline are prepared with reagent grade materials according to published formula (400-001-02-01-P).

5. CALIBRATION

There are no calibration materials or calibrations performed on the Vaginal Wet Preparation.

Follow routine operational instructions for the use of a microscope.

6. REPORTABLE RANGE

Refer to the most up-to-date version of the Reference Range Results and Interpretation for allowable reporting parameters. In general, observations can be reported for:

KOH: Negative (Not observed) or Positive (pseudohyphae/budding yeast seen)

Trich: Negative (Not observed) or Positive (motile *T. vaginalis* seen)

Whiff: Negative (Not observed) or Positive (fishy/amine odor noted w/KOH addition)

Other: Negative (Not observed) or significant feature per CCPH clinical staff observation

WBCs: the average number of White Blood Cells observed in 10 High Power Fields; 0-10 of >10.

7. CONTROL PROCEDURES

In order to:

- (1) Detect immediate errors that occur due to test system failure, adverse environmental conditions, and operator performance, and
- (2) Monitor over time the accuracy and precision of test performance that may be influenced by changes in test system performance and environmental conditions, and variance in operator performance, the following control procedure will be used:

1. When the first Vaginal Wet Preparation of a clinic arrives in the laboratory it will be prepared and read by the analyst on duty per this protocol and observations entered into the Vaginal Wet Preparation Quality Control log (400-01-02-02-F).
2. A second analyst will read the same specimen and also enter observations into the Vaginal Wet Preparation Quality Control log. If a second analyst is not available, the first analyst will repeat the first findings as if they were the second analyst.
3. The analysts will then perform a KOH addition and log those results.
4. If the analyst observations agree clinical analysis will proceed. If they disagree:

The analysts will repeat the procedure, comparing the same microscopic field.

Agreement allows clinical analysis to proceed; disagreement results in:

Both analysts consult with the Technical Supervisor or 3rd analyst and review training materials.

In addition to this, the materials and challenges provided as part of Vaginal Wet Preparation Proficiency Testing events may be retained and reviewed as supplementary Quality Control resources.

8. CORRECTIVE ACTION

If the control procedure outlined above does not provide the expected agreement:

1. Vaginal Wet Preparation analysis must be suspended at once,

2. The Clinician in charge must be informed at once so that STI clinic operations may be adjusted to allow for the suspension of testing, and
 3. The Clinical Director must be immediately contacted so that guidance and direction in remediation may be provided,
 4. An investigation as to causes, documented on appropriate forms, must be initiated. All materials and reagents must be checked for suitability, and the microscope for function.
- All findings and discoveries, along with all remedial actions, will be documented and reviewed with the laboratory staff to help preclude future occurrences.

9. LIMITATIONS OF THE PROCEDURE

This procedure is very dependent upon the adequacy of the specimen. Too many red blood cells (specimen collected during menses) will interfere with visualizing clue cells, trichomonads, or white blood cells. Opaque, bloody specimens should be avoided. Living trichomonads exhibit a characteristic motility (this is a diagnostic criteria); specimens need to be maintained at room temperature and tested as soon as feasible. Yeasts can be confused with air bubbles or occasionally with cotton strands from swabs. Residues from vaginal lubricants, douches, or Over The Counter (OTC) medications can interfere with interpretation of field of views. The analyst needs to be very familiar with yeast morphology. The characteristic amine odor of a Gardnerella infection may be weak enough to be masked by the smell of latex gloves, and it dissipates rapidly after KOH addition. Clue cells may be absent or hard to spot due to cellular debris. According to our proficiency test provider, at least 75% of the cell margin must be covered by bacteria to be considered a clue cell. Analyst experience plays a major role in the successful use of this test. See references for more information.

10. EXPECTED RESULTS AND NORMAL VALUES

Trichomonas vaginalis exhibits a distinctive morphology and motility. Both fungal pseudohyphae and budding yeasts also possess a distinct morphology and are unaffected by the addition of KOH. Clue cells also exhibit a distinctive presentation, as do White Blood Cells. Normal values are:

- Trichomonas vaginalis (Trich): Negative
- Yeast (KOH): Negative
- Vaginosis (Whiff): Negative
- Clue cells (Other): Negative
- White Blood Cells (WBCs): ≤ 30 /HPF (High Power Field)

11. PANIC OR ALERT VALUES

There are no Panic or Alert values for the Vaginal Wet Preparation or KOH Addition.

12. DATA ENTRY AND REPORTING

The results of Vaginal Wet Preparations and KOH daily quality control are entered into the Daily Wet Prep QC Log. Patient specimen observations are entered into the STAT Lab logbook as the analyst performs the examination, and initialed by the analyst upon completion.

Results are provided to the Nursing Division by transcribing the results from the log into the EMR. To enter a result into the EMR, the scheduler page on the left toolbar is selected. The date of the clinic is then selected from the calendar. The patients' names for the current clinic are displayed and the correct patient's name can be selected by clicking on the name and scrolling down to the "Facesheet" option. Once the "Facesheet" page opens, the current laboratory tests can be found in the "Pending Services" section. To result a test, the "Pending Services" section can be expanded by clicking on the "Expand to Detail View" icon or the test can be resultated directly from the "Pending Service" section by clicking on the flask icon which is the "Add Result" button. In the expanded view, the "Add Result" option must be selected for the desired test. The first field on the resulting page is the "Observation Identifier" and the patient's unique ID is entered into this field. The next field to be entered is the "Observation Date." The "Observation Date" field will automatically populate with the current date and will only need to be modified if the date of testing is different from the current date. The following field is the "Reference Range" and Negative will be selected for the Trichomonas, BV (Whiff), and KOH (yeast) tests. The "Reference Range" field should remain empty for the WBC count. The "Result Value" field requires data entry and a dash (-) is sufficient for the Trichomonas, BV (Whiff), and KOH (yeast) tests. For the WBC count, the range of ≤ 30 or > 30 will need to be entered for the "Result Value" field. The "Result Flag" field will be entered with the result of the test, as either Negative or Positive, or will remain empty for the WBC count. The "Acknowledge Result" will always have "yes" selected and the "Units" field can be left blank. The last field is the "Comments" section, and it will usually remain empty unless a specific comment for the sample is necessary. Once the fields are filled in, the "Add" button can be selected. A "To Do" is sent to the Lab Results group when the final test of the clinic session is completed so that the clinician, using the EMR may initiate appropriate treatment. Alternately if the EMR is not available, transcribe the results onto the Lab Requisition Form and hand carry the results to the appropriate nursing staff.

Proficiency Testing specimens are recorded in the patient Stat Lab Log as if they were an actual patient. Results are then transcribed into the Proficiency Testing Providers Electronic Reporting System at the earliest convenience and prior to the testing close date.

13. SYSTEM FAILURE OR INOPERABILITY

If Vaginal Wet Preparations and KOH additions cannot be performed the Clinician in charge must be informed so that STI Clinic practice can be modified to accommodate this situation. An



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investigation into the cause of failure or inoperability must be initiated at once with the aim of restoring operability as soon as possible.

14. WET PREP APPENDIX

- 400-001-02-01-P_Wet Prep Reagent Preparation
- 400-001-02-02-F_Wet Prep QC Log

PREGNANCY PROCEDURE (HUMAN CHORIONIC GONADOTROPIN-HCG) (WAIVED)

1. MATERIALS REQUIRED

hCG test kit, which includes:

- Test strips
- Package insert

Timer (at minimum capable of a 3-minute interval)

Urine collection containers – screw-top

hCG positive and negative controls

Appropriate PPE

2. PATIENT PREPARATION, SAMPLE COLLECTION, AND RELATED REQUIREMENTS

The patient will be taken into a CCHP STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CCHP STI Clinic protocol. The clinician will have asked the patient to self-collect the specimen (urine) in a provided sample container, labeled it with the patient's Unique ID and clinic number, and transported it to the STAT lab. The specimen should be maintained at room temperature unless testing is to be delayed, in which case specimens may be held up to 48 hours at 2-8° C.

Once the specimen is in the STAT lab all pertinent information is entered into the hCG patient logbook. It is examined for suitability for Acceptance, as there is no referral for hCG (Pregnancy) tests-recollection is the only recourse for unacceptable specimens. Specimens must be properly and legibly labeled, provided in a timely fashion and Specimens exhibiting visible precipitates should be allowed to settle, or be centrifuged to provide a clear specimen. Lack of any of these is a basis for sample Rejection, which will be noted in the logbook along with an explanation as to why. Do not discard the specimen prior to consulting with the clinician for the sample status. This permits the clinician to clarify any potential concerns as well as make possible exceptions which must be clearly documented.

3. MICROSCOPIC EXAMINATION AND PROCEDURE

There is no microscopic examination for the hCG (Pregnancy) test procedure; it is a macroscopic technique.

Follow the Package Insert for complete directions for kit use.

4. MATERIAL PREPARATION

Follow Package Insert for material preparation (400-001-03-01-A Urine hCG strip pkg insert).

5. CALIBRATION

The timer used for the test should be verified annually and meet manufacturer's specifications.

6. REPORTABLE RANGE

This is a qualitative test; there are three (3) possible results: Positive, Negative, or Invalid. See Package Insert for additional information.

7. CONTROL PROCEDURES

In order to:

- (1) Detect immediate errors that occur due to test system failure, adverse environmental conditions, and operator performance, and
- (2) Monitor over time the accuracy and precision of test performance that may be influenced by changes in test system performance and environmental conditions, and variance in operator performance, the following control procedure will be used:

Utilize a positive hCG control and a negative hCG control to verify proper test performance with each new lot, each new shipment, monthly as a check on storage, each new untrained operator and as otherwise required by your lab internal quality system procedures. It is acceptable to utilize confirmed positive/negative controls, patients, or proficiency testing materials for quality control testing. Record all quality results in the hCG QC log (400-001-03-04-F).

8. CORRECTIVE ACTION

If a test fails to meet Control Criteria for acceptability (a red line in the control region of the device viewing window with a clear background – white to light pink) the test procedure should be reviewed and the test re-run with a new device. If the problem persists discontinue testing, notify the Medical Director and/or the Nursing Director (or appropriate clinical personnel), and contact the manufacturer's Technical Support (1-877-441-7440) for further directions.

9. LIMITATIONS OF PROCEDURE

Follow Package Insert.

10. EXPECTED RESULTS AND NORMAL VALUES

Follow Package Insert.

11. PANIC OR ALERT VALUES

There are no Panic or Alert Values.

12. DATA ENTRY AND REPORTING

The results of hCG Control runs are entered into the hCG QC Log (400-001-03-04-F), as well as other pertinent information such as date of run, lot numbers and expirations of test kits and controls, and



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analyst performing the run. Patient specimen observations are entered into the hCG logbook (400-001-03-03-F) as the analyst performs the examination and initialed by the analyst upon completion. Results are provided to the Nursing Division by transcribing the results from the log into the EMR. To enter a result into the EMR, the scheduler page on the left toolbar is selected. The date of the clinic is then selected from the calendar. The patients' names for the current clinic are displayed and the correct patient's name can be selected by clicking on the name and scrolling down to the "Facesheet" option. Once the "Facesheet" page opens, the current laboratory tests can be found in the "Pending Services" section. To result a test, the "Pending Services" section can be expanded by clicking on the "Expand to Detail View" icon or the test can be result directly from the "Pending Service" section by clicking on the flask icon which is the "Add Result" button. In the expanded view, the "Add Result" option must be selected for the desired test. The first field on the resulting page is the "Observation Identifier" and the patient's unique ID is entered into this field. The next field to be entered is the "Observation Date." The "Observation Date" field will automatically populate with the current date and will only need to be modified if the date of testing is different from the current date. The following field is the "Reference Range" and Negative will be selected for the Pregnancy test. The "Result Value" field requires data entry and a dash (-) is sufficient. The "Result Flag" field will be entered with the result of the test, as either Negative or Positive or Invalid. The "Acknowledge Result" will always have "yes" selected and the "Units" field can be left blank. The last field is the "Comments" section, and it will usually remain empty unless a specific comment for the sample is necessary. Once the fields are filled in, the "Add" button can be selected. A "To Do" is sent to the Lab Results group when the final test of the clinic session is completed so that the clinician, using the EMR may initiate appropriate treatment. Alternately if the EMR is not available, transcribe the results onto the Lab Requisition Form and hand carry the results to the appropriate nursing staff. New lots of test kits or controls are entered into the Reagent Log.

13. SYSTEM FAILURE OR INOPERABILITY

If hCG Pregnancy tests cannot be performed the Clinician in charge must be informed so that STI Clinic practice can be modified to accommodate this situation. Patients suspected of being pregnant may need to be referred to other providers for appropriate treatment.

14. HCG APPENDIX

- 400-001-03-01-A Urine hCG strip pkg insert
- 400-001-03-03-F_hCG Patient Log
- 400-001-03-04-F_hCG QC Log

HIV-1/2 ANTIBODY PROCEDURE (WAIVED)

1. MATERIALS REQUIRED:

HIV test kit (OraQuick *Advance* Rapid HIV-1/2), which includes:

- HIV test device
- Developer Solution
- Package Insert

HIV test stand (reusable)

HIV specimen collection loop

Controls for HIV-1, HIV-2, and HIV-Negative

Package Insert for Controls

Timer capable of measuring 20 to 40 minutes

Biohazard waste container

Appropriate PPE

2. PATIENT PREPARATION, SAMPLE COLLECTION, AND RELATED REQUIREMENTS

The patient will be taken into a CCPH STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CCPH STI Clinic protocol. The clinician will collect a venous blood sample in a blood sample tube containing either EDTA (purple top) or sodium heparin (green top), labeled it with the patient's unique ID and clinic number, and transported it to the STAT lab in a sealable container with a biohazard label. The specimen should be maintained at room temperature (15-30° C) unless testing is to be delayed, in which case whole blood specimens may be held up to 5 days at 2-30° C. It must be mixed by gently inverting several times prior to testing to ensure a homogeneous mixture.

Once the specimen is in the STAT lab all pertinent information is entered into the STAT lab logbook. It is examined for suitability for Acceptance, as there is no referral for HIV Antibody tests- recollection is the only recourse for unacceptable specimens. There must be sufficient sample to allow complete submersion of the loop on the end of the specimen collection device. Clotted specimens will be rejected, and a new sample will need to be collected. Any rejected sample will be noted in the logbook along with an explanation as to why. Do not discard the specimen prior to consulting with the clinician for the sample status. This permits the clinician to clarify any potential concerns as well as make possible exceptions which must be clearly documented. Confirmatory testing is performed using the INSTI HIV-1/HIV-2 Antibody Test Kit.

3. MICROSCOPIC EXAMINATION AND PROCEDURE

There is no microscopic examination for the HIV Antibody test procedure; it is a macroscopic technique.

Note: This procedure utilizes potentially infectious patient specimens. Gloves, eye protection, and protective clothing are required.

Follow the Package Insert (400-001-04-05-A) for complete directions utilizing a venipuncture whole blood sample for kit use.

4. MATERIAL PREPARATION

All necessary materials should be assembled prior to testing. The test kits and testing related materials and equipment should all be at operating temperature (15-37° C) prior to performing the test. The test kits themselves should be stored at room temperature. The HIV controls should be stored at 2-8° C. Ensure that all testing materials and controls are within their expiration dates. Check the timer for a readable display (adequate battery). If an HIV test kit is opened and not needed, it is allowed to be used for testing during the next clinic. In this case, the test kit would need to remain in the package with the desiccant pouch present to keep any moisture out.

5. CALIBRATION

The timer used for the test should be verified annually and meet manufacturer's specifications. There is no calibration required for the test device (Waived test).

6. REPORTABLE RANGE

This is a qualitative test; there are three (3) possible results: Non-Reactive, Reactive, or Invalid. Refer to the Package Insert for additional reporting information.

7. CONTROL PROCEDURES

In order to:

- (1) Detect immediate errors that occur due to test system failure, adverse environmental conditions, and operator performance, and
- (2) Monitor over time the accuracy and precision of test performance that may be influenced by changes in test system performance and environmental conditions, and variance in operator performance, the following control procedure will be used:

Follow Control Package Insert (400-001-04-06-A) and record all quality results in the HIV QC log (400-001-04-04-F).

External Quality Control:

- OraQuick ADVANCE[®] Rapid HIV-1/2 Antibody Test Kit Controls are available separately for use only with the OraQuick ADVANCE[®]. This material is ordered and provided through the HIV grant.
- Rapid HIV-1/2 Antibody Test. The Kit Controls are specifically formulated and manufactured to ensure performance of the Test, and are used to verify analysts ability to properly perform the test and interpret the results. The HIV-1 and HIV-2 Positive Controls

will produce a reactive test result and have been manufactured to produce a very faint Test (“T”) line. The Negative Control will produce a nonreactive test result. Use of kit control reagents manufactured by any other source may not produce the required results, and therefore, will not meet the requirements for an adequate quality assurance program for the OraQuick ADVANCE® Rapid HIV-1/2 Antibody Test.

Run the Kit Controls under the following circumstances:

- Each new operator prior to performing testing on patient specimens,
- When opening a new test kit lot,
- Whenever a new shipment of test kits is received,
- If the temperature of the test kit storage area falls outside of 2°- 27°C (36°- 80°F),
- If the temperature of the testing area falls outside of 15°- 37°C (59°- 99°F), and
- At intervals as requested by the HIV Coordinator.

Room Temperature Control-verify room temperature, at least daily, and enter on 400-001-04-01-F_HIV Room Temp Log.

8. CORRECTIVE ACTION

If a test fails to meet Control Criteria for acceptability (a red line in the control region of the device viewing window with a clear background – white to light pink) the test procedure should be reviewed and the test re-run with a new device. If the problem persists discontinue testing, notify the Medical Director and/or the Nursing Director (or appropriate clinical personnel), and contact the manufacturer’s Customer Service (1-800-672-7873) for further direction.

9. LIMITATIONS OF PROCEDURE

Follow Package Insert.

10. EXPECTED RESULTS AND NORMAL VALUES

Follow Package Insert.

11. PANIC OR ALERT VALUES

There are no Panic or Alert Values, however reactive results must immediately be brought to the attention of the clinician.

12. DATA ENTRY AND REPORTING

The results of HIV-1/2 Control runs are entered into the HIV QC Log, as well as other pertinent information such as date of run, lot numbers and expirations of test kits and controls, and analyst performing the run. Patient specimen observations and OPSCAN number are entered into the HIV Patient Log (400-001-07-03-F) as the analyst performs the examination and initialed by the analyst upon completion. To enter a result into the EMR, the scheduler page on the left toolbar is selected.

The date of the clinic is then selected from the calendar. The patients' names for the current clinic are displayed and the correct patient's name can be selected by clicking on the name and scrolling down to the "Facesheet" option. Once the "Facesheet" page opens, the current laboratory tests can be found in the "Pending Services" section. To result a test, the "Pending Services" section can be expanded by clicking on the "Expand to Detail View" icon or the test can be result directly from the "Pending Service" section by clicking on the flask icon which is the "Add Result" button. In the expanded view, the "Add Result" option must be selected for the desired test. The first field on the resulting page is the "Observation Identifier" and the patient's unique ID is entered into this field. The next field to be entered is the "Observation Date." The "Observation Date" field will automatically populate with the current date and will only need to be modified if the date of testing is different from the current date. The following field is the "Reference Range" and Non-Reactive will be selected for the HIV test. The OPSCAN number (no letters) is entered in the "Result Value" field. The "Result Flag" field will be entered with the result of the test, as either Non-Reactive or Reactive. The "Acknowledge Result" will always have "yes" selected and the "Units" field can be left blank. The last field is the "Comments" section, and it will usually remain empty unless a specific comment for the sample is necessary. Once the required fields are filled in, the "Add" button can be selected. The HIV Patient Log form includes an OPSCAN line which necessitates first confirming that the OPSCAN number in the EMR by the nursing staff matches the OPSCAN number recorded on the patient test result within the EMR and the OPSCAN preprinted label all match, prior to placing a checkmark in the column.

Send a "To Do" to the Lab Results group when the final test of the clinic day for each patient is completed so that clinician or Medical Director may initiate appropriate treatment. Alternatively, if the EMR is not available, transcribe the results onto the Lab Requisition Form and hand carry the results to the appropriate nursing staff.

13. SYSTEM FAILURE OR INOPERABILITY

If HIV-1/2 Antibody tests cannot be performed the Medical Director and Director of Nursing must be informed so that STI Clinic practice can be modified to accommodate this situation. Patients suspected of being exposed to or infected with the HIV-1/2 virus may need to be referred to other providers for appropriate testing and treatment.

14. HIV APPENDIX

- 400-001-04-01-F_HIV Room Temp Log
- 400-001-04-03-F_HIV Patient Log
- 400-001-04-04-F_OraQuick QC Log
- 400-001-04-05-A_OraQuick Advance Rapid HIV ½ Version (3001-1215 rev 04/20)
- 400-001-04-06-A_OraQuick Controls product insert (3001-1202 rev 03/16)



INSTI HIV-1/HIV-2 ANTIBODY TEST KIT (WAIVED)

1. MATERIALS REQUIRED:

- INSTI test kit
- Controls for HIV-1, HIV-2, and HIV-Negative
- Package insert for controls
- Biohazard waste container
- Appropriate PPE

2. PATIENT PREPARATION, SAMPLE COLLECTION, AND RELATED REQUIREMENTS

The patient will be taken into a CPH STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CPH STI Clinic protocol. The clinician will collect a fingerstick whole blood sample in a capillary pipette and perform the test procedure in the exam room following the INSTI package insert instructions. The clinician must examine the sample for suitability for Acceptance, as there is no referral for HIV Antibody tests-recollection is the only recourse for unacceptable specimens. The specimen should be maintained at room temperature (15-30° C) and there must be sufficient sample to fill the capillary pipette. Lack of any of these is a basis for sample Rejection, which will be noted in the logbook along with an explanation as to why. The clinician will fill out a lab slip with the patient's results and bring it to the STAT lab. Once the lab slip is in the STAT lab all pertinent information is entered into the STAT lab logbook.

3. MICROSCOPIC EXAMINATION AND PROCEDURE

There is no microscopic examination for the HIV Antibody test procedure; it is a macroscopic technique.

Note: This procedure utilizes potentially infectious patient specimens. Gloves, eye protection, and protective clothing are required.

Follow the Package Insert, 400-001-04-07-A_INSTI HIV-1/HIV-2 Antibody Test Product insert, for complete directions for kit use.

4. MATERIAL PREPARATION

All necessary materials should be assembled prior to testing. The test kits and testing related materials and equipment should all be at operating temperature (15-30° C) prior to performing the test. The test kits themselves should be stored at room temperature. Ensure that all testing materials are within their expiration dates.



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5. CALIBRATION

There is no calibration required for the test device (Waived test).

6. REPORTABLE RANGE

This is a qualitative test; there are three (3) possible results: Non-Reactive, Reactive, or Invalid. Refer to the Package Insert for additional reporting information.

7. CONTROL PROCEDURES

In order to:

- (1) Detect immediate errors that occur due to test system failure, adverse environmental conditions, and operator performance, and
- (2) Monitor over time the accuracy and precision of test performance that may be influenced by changes in test system performance and environmental conditions, and variance in operator performance, the following control procedure will be used:

Follow Control Package Insert (400-001-04-08-A) and record all quality results in the HIV QC log (400-001-04-04-F).

External Quality Control

INSTI HIV-1/HIV-2 Antibody Test Kit Controls are available separately for use only with the INSTI HIV-1/HIV-2 Antibody Test Kit. This material is ordered and provided through the HIV grant.

The Kit Controls are specifically formulated and manufactured to ensure performance of the Test and are used to verify your ability to properly perform the test and interpret the results. The HIV-1 and HIV-2 Positive Controls will produce a reactive test result and have been manufactured to produce a faint blue color in the test spot. The Negative Control will produce a blue color in the control spot, but no color in the test spot, for a non-reactive test result. Use of kit control reagents manufactured by any other source may not produce the required results, and therefore, will not meet the requirements for an adequate quality assurance program for the INSTI HIV-1/HIV-2 Antibody Test.

Run the Kit Controls under the following circumstances:

- Each new operator prior to performing testing on patient specimens,
- When opening a new test kit lot,
- Whenever a new shipment of test kits is received,
- If the temperature of the test kit storage area falls outside of 2°- 30°C (35.6°- 86°F),
- If the temperature of the testing area falls outside of 15°- 30°C (59°- 86°F), and
- At intervals as requested by the HIV Coordinator.



8. CORRECTIVE ACTION

If a test fails to meet Control Criteria for acceptability (a blue color in the control spot) the test procedure should be reviewed and the test re-run with a new device. If the problem persists discontinue testing, notify the Medical Director and/or the Nursing Director (or appropriate clinical personnel), and contact the manufacturer's Customer Service (1-866-674-6784) for further direction.

9. LIMITATIONS OF PROCEDURE

Follow Package Insert.

10. EXPECTED RESULTS AND NORMAL VALUES

Follow Package Insert.

11. PANIC OR ALERT VALUES

There are no Panic or Alert Values, however reactive results must immediately be brought to the attention of the clinician.

12. DATA ENTRY AND REPORTING

The results of HIV-1/HIV-2 Control runs are entered into the HIV QC Log, as well as other pertinent information such as date of run, lot numbers and expirations of test kits and controls, and analyst performing the run. Patient specimen observations and OPSCAN number are entered into the HIV Patient Log (400-001-07-03-F).

13. SYSTEM FAILURE OR INOPERABILITY

If HIV-1/HIV-2 Antibody tests cannot be performed the Medical Director and Director of Nursing must be informed so that STI Clinic practice can be modified to accommodate this situation. Patients suspected of being exposed to or infected with the HIV-1/HIV-2 virus may need to be referred to other providers for appropriate testing and treatment.

14. INSTI APPENDIX

- 400-001-04-01-F_HIV Room Temp Log
- 400-001-04-03-F_HIV Patient Log
- 400-001-04-04-F_OraQuick QC Log
- 400-001-04-07-A_INSTI HIV-1/HIV-2 Antibody Test Kit Product insert Version (51-1080J rev 11/10/2020)
- 400-001-04-08-A_INSTI Controls product insert (51-1183F (30 Apr-2021))
- 400-001-04-009-A_INSTI Quick Reference Guide



SYPHILIS

SYPHILIS-HEALTH CHECK

1. MATERIALS:

Each kit contains items needed to perform 20 tests.

- | | |
|--|------|
| - SYPHILIS HEALTH CHECK Test devices : | 20 |
| - Disposable plastic pipettes : | 20 |
| - Diluent in a dropper bottle containing saline buffer, detergent and sodium azide (NaN ₃ , 0.1%) : | 5 mL |
| - Package insert | 1 |

Additional items needed include:

- Timer – 10-15 min.
- External Positive and Negative Controls (see Control Procedures for details)

2. PATIENT PREPARATION, SPECIMEN COLLECTION, ACCEPTANCE AND REJECTION

For Fingerstick Whole Blood Collection:

1. Rub the chosen finger towards the tip and wipe the end of the finger with an alcohol wipe and a sterile pad.
2. Alcohol will affect the test. Let dry thoroughly.
3. Two drops of whole blood (50 µL) is required to perform the test.
4. Stick fingertip with a lancet.
5. The first drop of blood should be wiped clean with a sterile pad. NOTE: It is important that the first drop should NOT be used to avoid any potential interference from the alcohol.
6. Rub the finger towards the tip for two more drops of blood.
7. Using the fixed volume pipette provided in the kit, touch the end of the pipette to the drop of blood.
8. Holding the pipette horizontally, allow the blood to flow into the pipette on its own, making sure that there are no air bubbles or empty spaces or gaps in the specimen. If air bubbles or empty spaces or gaps are present, collect another sample.
9. It may be necessary to rub the finger for an additional drop of blood to get two drops.
10. Whole Blood samples collected by finger-stick should be used on the Syphilis Health Check test devices immediately after collection.

3. MICROSCOPIC EXAMINATION AND PROCEDURE

There is no microscopic examination for the RPR test procedure; it is a macroscopic technique.

Note: This procedure utilizes potentially infectious patient specimens. Gloves, eye protection, and protective clothing are required.

WARNINGS AND PRECAUTIONS

1. Do not use test cassettes if foil pouches are opened or defective.
2. Make sure the materials in the kit are at room temperature before use.
3. Always wear gloves before performing Syphilis Health Check.
4. Place the device on clean flat surface facing up.
5. Use the pipette included in the kit only.
6. This test is designed for "*in vitro diagnostic*" use
7. Read instructions carefully before using this test
8. A positive test must be followed by or reflexed to a laboratory non-treponemal syphilis assay with titer information.
9. Clinical judgment is necessary for interpreting the test results
10. A positive result may not be useful for establishing a diagnosis of syphilis infection. In some situations, such a result may reflect a prior treated infection; a negative result can exclude a diagnosis of syphilis except for cases of incubating or early primary disease where syphilis antibodies are not yet detectable.
11. Blood specimens may be potentially infectious. Avoid contact with skin by wearing gloves and proper laboratory attire. Properly handle and discard all used test devices in an approved biohazard container.
12. Avoid any contact between hands and eyes or nose during specimen collection and testing
13. Do not use the buffer or cassette after the expiration date printed on the outside of each foil pouch.
14. Test cassettes are single use only.
15. Adding sample and buffer in the wrong order will result in an incorrect result.
16. Test buffer and Controls contains sodium azide as preservative that is a poison and may be harmful if swallowed. Seek medical help if buffer is swallowed.
17. Persons performing the test must be tested for colorblindness before performing the test.

PROCEDURE

1. Allow the Syphilis Health Check test device to come to room temperature prior to testing. Finger-stick whole blood samples should be used immediately after collection.
2. Remove the reaction device from its protective wrapper by tearing along the notch.
3. Label device with the patient's name or control number.
4. Fill the pipette with specimen (whole blood (50 µL), serum or plasma (25 µL)).
5. Hold the pipette vertically, dispense one drop (25 µl) of serum or plasma into the sample well (small circle). If whole blood is used, dispense two drops (50 µl) into the sample well.
6. Allow sample to be absorbed into the pad.
7. Hold the diluent dropper bottle vertically and slowly add 4 full drops (200 µL) into the sample well (small circle) at an interval of 2-3 seconds between each drop. One more drop can be added, if the sample does not flow down the membrane. **DO NOT USE WATER OR OTHER LIQUIDS.**
8. Set the cassette on a flat surface and incubate at room temperature (15 - 30°C) for 10 minutes.
9. Read the results after 10 minutes. The result can be read up to 15 minutes.

PLEASE NOTE: Do not read after 15 minutes.

4. MATERIAL PREPARATION

All SYPHILIS HEALTH CHECK kit components should be stored at (4° - 30°C).

Test cassettes should be stored in their sealed pouch.

Note: a single bottle of diluent is found within each kit. The diluent is interchangeable with any other box of kits as long as they have not expired.

Do not freeze the test kit.

5. CALIBRATION

The timer and centrifuge used for the test should be calibrated annually and meet manufacturer's specifications. There is no calibration required for the test device.

6. REPORTABLE RANGE

The reportable range of the Syphilis Health Check procedure is Nonreactive for a Negative test result and Reactive for a positive result. In addition, an invalid result is reported as invalid by selecting "No range applies" in the electronic entry system and describing the reason for the lack of a nonreactive or reactive result. This could include, control line failure, specimen rejected due to hemolysis, etc.

7. CONTROL PROCEDURES

Built –in Controls:

Syphilis Health Check contains built-in quality control features. A pink line in the Control Zone should always be seen and shows: 1) that enough volume is added and that 2) that proper flow is obtained. If this line is missing, the test was not run correctly or failed to function correctly. The test is invalid and the test should be repeated using a new cassette.

External Controls:

The Positive and Negative Controls, which are provided separately from the manufacturer, should be run according to laboratory requirements. These controls should be run like an unknown patient specimen, and as required by your laboratory's standard QC procedures or Medical / Lab Director selected from any of the following examples:

- Each new lot

- Each new shipment (even if from the same lot previously received)

- Each new operator (an individual who has not run the tests for at least two weeks)

- Monthly, as a continued check on storage conditions

- Whenever problems (storage, operator, or other) are identified

- If the controls do not give expected results (Positive or Negative), patient results must not be reported, and the test should be re-run.

If the test does not show any Control or Test line in the window or a smudged or partial line, the test cassette should be discarded. Do not report the results. Run the test again with a new cassette and follow the procedure exactly. If the second test does not show lines, please contact Technical Services at 866-358-9282. For any other concerns regarding Syphilis Health Check please call 866-358-9282 8am -6pm EST. Problems may also be reported using the MedWatch reporting system at <http://www.fda.gov/Safety/MedWatch/HowToReport/> or calling 1-800-FDA-1088 (1-800-332-1088).

Refrigerator temperatures must be checked and recorded daily.

Ambient (room) temperatures must be checked and recorded daily.

8. CORRECTIVE ACTION

If a test fails to meet Control Criteria for acceptability (a red line in the control region of the device viewing window with a clear background – white to light pink) the test procedure should be reviewed and the test re-run with a new device. If the problem persists discontinue testing

immediately, notify the Medical Director and/or the Nursing Director (or appropriate clinical personnel), and contact the manufacturer's Customer Service (866-358-9282) for further direction.

9. LIMITATIONS OF PROCEDURE

- 1- The results obtained from this assay are intended to aid in diagnosis only. As with all serological treponemal tests for syphilis, interpretation of results obtained with the Syphilis Health Check Treponemal Antibody test must be used in conjunction with a nontreponemal syphilis serologic test with titer, the patient's clinical symptoms, medical history and other clinical and/or laboratory findings to produce an diagnosis of syphilis by stage.
- 2- A positive treponemal test requires a reflexive second test with a nontreponemal assay with titer, such as RPR, along with a clinical evaluation, for diagnosis of syphilis.
- 3- Very early stage of infection could lead to false negative results, due to the low concentration of anti-Treponema pallidum antibodies in the serum, plasma or whole blood samples.
- 4- A positive result does not exclude the presence of other pathogens. A positive result can also be obtained in cases of other treponemal diseases such as yaws, pinta and bejel.
- 5- The Syphilis Health Check test is specific for detecting Treponema pallidum antibodies (IgG and IgM) in serum, plasma or whole blood samples. It does not detect T. pallidum directly.
- 6- All treponemal tests tend to remain reactive following treatment and cannot be quantified; therefore, they should not be used to evaluate responses to therapy. Because of the persistence of reactivity, probably for the life of the patient, the treponemal tests are of no value to the clinician in determining relapse or re-infection in a patient who has had a treated infection.
- 7- Treponemal antibodies after treatment are not indicative of immunity to future syphilis infections.
- 8- Performance characteristics of this device have not been established for matrices other than whole blood, serum or plasma.
- 9- Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients, cord blood, neonatal specimens, or infants.
- 10- Performance characteristics of this device have not been established with specimens containing heterophile antibodies which are known to cause false positive results in various immunoassays.
- 11- Treponemal tests are not recommended in neonates to diagnose congenital syphilis as passive transfer of maternal antibodies can cause false positive results. The results obtained from this assay are intended to aid in diagnosis only. As with all serological treponemal tests for syphilis, interpretation of results obtained with the Syphilis Health Check Treponemal Antibody test must be used in conjunction with a nontreponemal syphilis serologic test with titer, the patient's clinical symptoms, medical history and other clinical and/or laboratory findings to produce an diagnosis of syphilis by stage.

10. EXPECTED RESULTS AND NORMAL VALUES

INTERPRETATION OF RESULTS

The assay is calibrated against commercially available serum "standardized" against the WHO Reference Material and the cut-off confirmed with results obtained with uninfected patient samples and borderline treponemal positive samples diluted to assess the imprecision around the cut-off of the assay.

A. Negative

One pink/red line of any intensity appears in the "C" control area. This indicates a Non-Reactive result that is interpreted as Negative for Syphilis antibodies. No visible pink / red line in the test area is considered a negative result.

B. Positive

A pink/red line of any intensity appears in the device window adjacent to "T" Test and a second line of any intensity appears adjacent to "C" Control. This indicates a Reactive result that is interpreted as Presumptive Positive for Syphilis antibodies. Any visible pink/red only line is considered positive. 3 4

C. Invalid

If there is no color line visible in the "C" control area, whether or not there is a line in the "T" test area, the test is invalid and cannot be interpreted. In this case, repeat the test with a fresh specimen using a fresh device. Contact Diagnostic Direct Technical Services at 866-358-9282 if you are unable to produce a valid result upon repeat testing. The assay is calibrated against commercially available serum "standardized" against the WHO Reference Material and the cut-off confirmed with results obtained with uninfected patient samples and borderline treponemal positive samples diluted to assess the imprecision around the cut-off of the assay.

Contact Diagnostic Direct Technical Services at 866-358-9282 if you are unable to produce a valid result upon repeat testing.

A positive SHC result is not diagnostic of syphilis without additional nontreponemal serologic testing and a full clinical evaluation.
A new venous whole blood specimen must be obtained for further testing.

IMPORTANT: In addition to the pink line by the Control, ANY line that is seen near the Test mark of the cassette at the 10-minute time is considered a positive result. The intensity of the line does not matter.

The following Table provides an algorithm to aid in interpreting and reporting syphilis serology results for diagnosis of *T. Pallidum* infection status, using both a treponemal test and a non-treponemal test .

Non-Treponemal Result (NT)	Treponemal Result	Report/interpretation for all except neonates or infants*
Nonreactive	Negative (Nonreactive)	No serologic evidence of infection with <i>T. pallidum</i> (incubating or early primary syphilis cannot be excluded).
Reactive	Negative (Nonreactive)	Current infection unlikely; probability of Biological False Positive (BFP) secondary to other medical conditions (febrile diseases, immunizations, intravenous drug use, autoimmune diseases, etc.). Recommend repeat testing (nontreponemal, and treponemal by other test method.)
Nonreactive	Positive (Reactive)	Probably past treated infection or untreated latent infection (e.g., if no history of previous treatment); rarely due to potential cross-reactivity with other spirochetes/related antigens. Recommend to treat (if



		untreated latent infection) or additional testing consistent with clinical findings/history.** Possibility of false negative NT due to incubating syphilis or prozone in secondary syphilis, late latent syphilis, or late neurosyphilis.
Reactive	Positive (Reactive)	Presumptive evidence of current infection or inadequately treated infection, persistent infection, reinfection, or serofast if prior history of treated syphilis). Recommend additional testing consistent with clinical assessment.**
Nonreactive	Not done	Current infection unlikely; effectively treated infection if previously treated; cannot exclude incubating or early primary syphilis; cannot exclude latent or neurosyphilis. Treponemal testing advised if clinical suspicion is present of latent or neurosyphilis. Treponemal testing advised if clinical suspicion is present of latent or neurosyphilis. Recommend repeat testing if risks are present.
Not done	Negative (Nonreactive)	Current or past treated infection unlikely unless treated early in the incubating or early primary syphilis stage; cannot exclude incubating or early primary syphilis. Recommend repeat testing if risks are present.

*HIV-infected individuals may have delayed sero-reactivity or negative serology albeit very rarely.

**Nontreponemal testing with titer; clinical history; repeated (sequential) serological testing for changes in titer

11. PANIC OR ALERT VALUES

There are no Alert or Panic Values for the Syphilis procedure, however reactive results must immediately be brought to the attention of the clinician so that a subsequent sample for confirmatory testing can be obtained.

12. DATA ENTRY AND REPORTING

- The results of Syphilis Health Check Control runs are entered into the Syphilis Health Check Control Log (400-001-05-08-A).
- Daily temperatures do not have a separate log for the Health Check system, this information will be obtained from the HIV room temperature log or the Syphilis RPR QC log.
- Patient specimen observations are entered into the Syphilis log book as the analyst performs the examination and initialed by the analyst upon completion. Enter HC into the STAT column of the log in order to clarify that this is a Health Check test being completed. If the patient is positive and an RPR is run, enter the patient name on a second line in the patient log-the patient will have two separate entries in the patient log.

Results are provided to the Nursing Division by transcribing the results from the log into the EMR. See attachments 400-001-09-01-P_Electronic Laboratory Reporting Procedure, 400-001-09-02-

F_Reference Range and Results Interpretation, and 400-001-09-03-F_Lab Slip Legend additional details.

13. SYSTEM FAILURE OR INOPERABILITY

If Syphilis Health Check tests cannot be performed the Medical Director and Director of Nursing must be immediately informed so that STI Clinic practice can be modified to accommodate this situation. Patients suspected of being exposed to or infected with Syphilis may need to be referred to other providers for appropriate testing and treatment.

14. SYPHILIS HEALTH CHECK APPENDIX

- 400-001-05-03-F_RPR Patient Log
- 400-001-05-08-A_Syphilis Health Check Control Log
- 400-001-05-09-A_Syphilis Health Check Package Insert
- 400-001-05-10-A_HealthCheckQuickReference
- 400-001-05-11-A_HealthCheckControl Package Insert

SYPHILIS-RAPID PLASMA REAGIN (RPR)

1. MATERIALS:

RPR antigen suspension, in dispensing bottle w/ 20ga. unbeveled siliconized needle
RPR Dispensstirs dispensing devices
RPR 18mm circle test cards
Centrifuge
Deionized Water (DI Water)
Rotator (capable of 100±2 RPM and circumscribing a .75-inch circle)
Pipettors capable of 20, 50 and 1000ul
Pipette tips
0.9% Saline solution
RPR Control Card
Incandescent light source
Biohazard Waste Container
Appropriate PPE

2. PATIENT PREPARATION, SPECIMEN COLLECTION, ACCEPTANCE AND REJECTION

The patient will be taken into a CPH STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CPH STI Clinic protocol. The clinician will collect the specimen (whole venous blood) in either a) a plain ("red top") blood collection tube, or b) a tube containing EDTA anticoagulant ("Purple top") or c) serum separator tube (SST "gold top"). The tube will be labeled with the patient's unique ID and clinic number. It will be taken to the STI Clinic STAT lab in a covered plastic transport container marked with a Biohazard label. The specimen will be centrifuged to produce serum or plasma for testing, refer to 400-001-05-13-P_Procedure for Spinning Blood Samples for further information.

Once the specimen has been received into the STAT lab all pertinent information is entered into the RPR Patient Log (400-001-05-03-F). The properly labeled specimen is examined for suitability for Acceptance: a serum volume of at least 0.5 to 1.0 ml. is desirable. Reject samples with volumes of less than 0.4 ml, excessively hemolyzed, contaminated, or lipemic. A specimen is too hemolyzed for testing when printed material cannot be read through it. If testing is not being performed within 4 hours of receipt, follow steps 1 and 2 in the procedure to prepare the specimen for refrigerated storage. If nursing staff deliver a sample after laboratory staff have left for the day, refer to policy 200-012-P Centrifuging Blood Samples After Hours for storage procedure. If a delay of more than 48 hours is anticipated before testing, remove serum and store at refrigerator temperature (2-8°C). Ensure that the specimen is at 23-29°C before testing. The lack of any of these criteria is a basis for sample Rejection and the sample submitter will be notified as soon as possible. Note reason for sample rejection in the logbook. Do not discard the specimen prior to consulting

with the clinician for the sample status. This permits the clinician to clarify any potential concerns as well as make possible exceptions which must be clearly documented.

A specimen which exhibits any result other than “non-reactive” may be referred for further testing to an outside laboratory (e.g. Aultman Lab Services) at the direction of the Medical Director. If the original specimen was plasma, it must be recollected in a plain or serum separator tube so that serum may be obtained for testing by an outside laboratory.

3. MICROSCOPIC EXAMINATION AND PROCEDURE

There is no microscopic examination for the RPR test procedure; it is a macroscopic technique.

Procedure

Note: This procedure utilizes potentially infectious patient specimens. Gloves, eye protection, and protective clothing are required.

1. Bring all reagents and serum samples to room temperature (23-29°C) before beginning test. For Red top tubes allow time for a clot to form, for purple top tubes no processing delay is needed.
2. Centrifuge the specimens for 5 minutes at 3200 rpms to separate the serum or plasma from the cells. The ZiplQ centrifuge has two preset timers: P1 is set for 5 minutes and P2 is set for 1 minute. The back-up centrifuge will need to have the speed setting at an 8 to provide 3,200 to 4,500 rpms.
3. The RPR control card, with Reactive, Minimally Reactive and Non-Reactive controls must be run every day that samples are tested.

All samples are initially tested undiluted (Qualitative), unless otherwise specified by the Medical Director or clinic staff or patients' prior history.

Qualitative Procedure

4. Place 50 µL of serum or plasma onto an 18-mm circle of the RPR test card, using a disposable Dispensitir held vertically.
5. Using the inverted Dispensitir, spread the serum or plasma with the closed end to fill the entire circle. Do not spread the specimen beyond the confines of the circle.
6. Gently agitate the antigen dispensing bottle to re-suspend the particles.
7. Holding the dispensing bottle and needle in a vertical position, dispense several drops of antigen to clear the needle of air. (Redraw drops back into the bottle prior to returning bottle to storage) Then add exactly 1 free-falling drop (17 µL) of antigen suspension to each circle containing serum or plasma. Do not mix. The RPR Antigen Package Insert (400-001-05-05-A) is available for further information.
8. Place the card on the mechanical rotator under a humidifying cover. Rotate the card for 8 minutes at 100 ± 2 rpm.

9. Immediately remove the card from the rotator and briefly rotate and tilt the card by hand (three or four to-and-fro motions) under an incandescent light to aid in differentiating nonreactive from minimally reactive results.
10. Record the results of testing in the Stat Lab RPR Logbook as "Reactive" or "Nonreactive" and on the patient Laboratory Slip or into the EMR.

Specimens showing any degree of reactivity (clumping or "roughness") must be tested by the Quantitative procedure.

Quantitative Test:

This can be done in multiple quantitation formats, 1:1 through 1:16, 1:32 through 1:1024, in an order or combination that is relevant to the sample being tested. For example, if this is a prior positive patient with a titer of 1:256, testing from 1:32 through 1:1024 may be appropriate. At the discretion of the Medical Director additional dilutions may be added.

1:1 through 1:16

1. Using a pipettor place 50 μ L of 0.9% saline in circles numbered 2 through 5. Do not spread the saline.
2. Again, using the pipettor place 50 μ L of serum in circle 1 and 50 μ L of serum into the saline in circle 2.
3. Mix the saline and the serum in circle 2 by drawing the mixture up and down in the pipettor eight times. Avoid forming bubbles. This produces a 1:2 dilution.
4. Transfer 50 μ L from circle 2 (1:2) to circle 3, and mix as above.
5. Transfer 50 μ L from circle 3 (1:4) to circle 4, and mix.
6. Transfer 50 μ L from circle 4 (1:8) to circle 5 (1:16), mix; discard the last 50 μ L.
7. Using the flat end of a clean Dispensstir spread the diluted serum to fill the entire surface of the circle, starting with the highest dilution (circle 5, 1:16). Using the same Dispensstir, repeat for circle 4(1:8), 3(1:4), 2(1:2), and 1 (undiluted) in that order.
8. Gently agitate the dispensing bottle to re-suspend the antigen particles.
9. Holding the antigen dispensing bottle in a vertical position, dispense 1 or 2 drops of antigen to clear the needle of air. Then add exactly 1 free-falling drop (17 μ L) of antigen suspension in each circle. DO NOT MIX.
10. Place the card on the rotator under the humidifying cover and rotate the card for 8 minutes at 100 ± 2 rpm.
11. Immediately remove the card from the rotator; briefly rotate and tilt the card by hand (three or four to-and-fro motions) under an incandescent light to aid in differentiating nonreactive from minimally reactive results.

12. Record the results of testing in the Stat Lab RPR Logbook and on the patient Laboratory Slip or EMR.

If the highest dilution tested (1:16) is reactive, continue as follows:

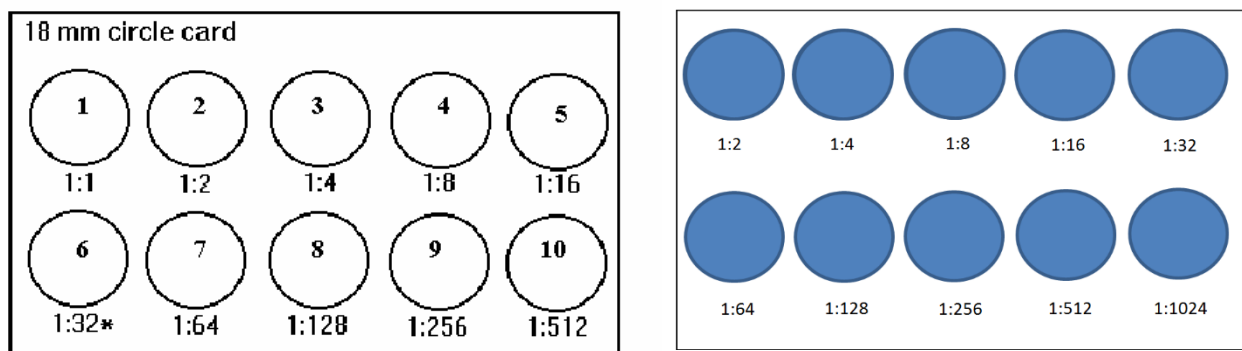
1:32 through 1:1024

- Prepare a 1:50 dilution of nonreactive serum in 0.9% saline to be used for making 1:32 and higher dilutions of the specimen to be tested. This is made by mixing 20µL of nonreactive patient serum with 1000µL (1.00 ml) of sterile 0.9% saline. See 400-001-05-06-A_Syphilis Dilution Sheet for more detailed information.
13. Prepare a 1:16 dilution of the test specimen by adding 0.1ml of serum to 1.5ml of 0.9% saline. Mix thoroughly.
 14. Place 50 µL of the 1:50 nonreactive serum diluent in labeled circles on the RPR card with the pipettor.
 15. Using the pipettor place 50 µL of the 1:16 test specimen dilution into circle 6.
 16. Mix the test specimen dilution and the serum in circle 6 by drawing the mixture up and down in the pipettor eight times. Avoid forming bubbles. This produces a 1:32 dilution.
 17. Using the same pipettor and tip, make serial twofold dilutions. Complete test as described in steps 4 through 12 (see “Quantitative Test”) for the next 5 circles. Dilutions higher than 1:1024 are not usually performed and are reported as >1:1024.

1:1 through 1:1024

18. Prepare a 1:50 dilution of nonreactive serum in 0.9% saline to be used for making 1:32 and higher dilutions of the specimen to be tested. This is made by mixing 20µL of nonreactive patient serum with 1000µL (1.00 ml) of sterile 0.9% saline. See Appendix A for more detailed information.
19. Using a pipettor place 50 µL of 0.9% saline in circles numbered 2 through 5. Do not spread the saline.
20. Place 50 µL of the 1:50 nonreactive serum diluent in circles 6 through 10 of the RPR card with the pipettor.
21. Again, using the pipettor place 50 µL of serum in circle 1 and 50 µL of serum into the saline in circle 2.
22. Mix the saline and the serum in circle 2 by drawing the mixture up and down in the pipettor eight times. Avoid forming bubbles. This produces a 1:2 dilution.
23. Transfer 50 µL from circle 2 (1:2) to circle 3, and mix as above.
24. Transfer 50 µL from circle 3 (1:4) to circle 4, and mix.
25. Transfer 50 µL from circle 4 (1:8) to circle 5 (1:16), and mix.
26. Transfer 50 µL from circle 5 (1:16) to circle 6 (1:32), and mix

27. Complete test as described in steps 24 through 27 for the next 5 circles, producing dilutions of 1:64, 1:128, 1:256, 1:512 and 1:1024. Dilutions higher than 1:1024 are not usually performed and are reported as >1:1024. Discard the last 50 µL.
28. Using the flat end of a clean Dispensstir, starting with the highest dilution spread the diluted serum to fill the entire surface of the circle, (circle 10, 1:512). Using the same Dispensstir, repeat for circle 9(1:256), 8 (1:128), 7 (1:64), 6 (1:32), 5 (1:16), 4(1:8), 3(1:4), 2(1:2), and 1 (undiluted) in that order.
29. Gently agitate the dispensing bottle to re-suspend the antigen particles.
30. Holding the antigen dispensing bottle in a vertical position, dispense 1 or 2 drops of antigen to clear the needle of air. Then add exactly 1 free-falling drop (17 µL) of antigen suspension in each circle. DO NOT MIX.
31. Place the card on the rotator under the humidifying cover and rotate the card for 8 minutes at 100 ± 2 rpm.
32. Immediately remove the card from the rotator; briefly rotate and tilt the card by hand (three or four to-and-fro motions) under an incandescent light to aid in differentiating nonreactive from minimally reactive results.
33. Record the results of testing in the Stat Lab RPR Logbook and on the Patient Laboratory Slip or EMR.



Examples of 18mm ten-spot RPR test card

4. MATERIAL PREPARATION

The antigen suspension is packaged in ampules. Store unopened ampules at 2 to 8°C; do not store the antigen in bright sunlight or in temperatures above 29°C; do not freeze. An unopened ampule of antigen is stable up to the expiration date.

To prepare antigen for testing, attach the hub of the dispensing needle to the fitting on the plastic dispensing bottle. Gently agitate the antigen ampule to re-suspend the particles. Open the ampule; use a wipe to shield your fingers from possible glass shards. Squeeze the dispensing bottle to

collapse it. Insert the needle into the ampule and withdraw all the antigen suspension into the dispensing bottle. Alternatively, utilize a sterile pipet to transfer contents into the plastic dispensing bottle.

Opened antigen suspension is good for 90 days if refrigerated and capped to prevent evaporation. A 0.9% Saline is prepared by diluting 0.9 grams of sodium chloride in 100 mL of deionized water. Diluent (serum) is prepared by diluting human serum 1:50 in 0.9% Saline solution. It is used to dilute serum specimens at dilutions of 1:32 and above. Refer to 400-001-05-06-A, Syphilis Dilution Sheet for an aid.

Utilize a minimum of a 1:4 reactivity sample (can be from PT or patient) for control serum sample, which must be brought to room temperature prior to use.

The solid components of the RPR test system (cards, Dispensstirs, bottles, and needles) may be stored at room temperature. Control cards and antigen suspension must be stored under refrigeration (2-8°C) until expiration and brought to room temperature prior to use.

See 400-001-05-02-P_RPR Reagent Preparation for instructions to prepare a 0.9% Saline Solution and a 1:50 nonreactive patient diluent for RPR Quantitation.

7. 5. CALIBRATION/VERIFICATION

(1) A reactive control is used to determine level of reactivity of the test for lot to lot comparison, when a patient specimen is reactive and requires quantitation, or when desired as an indication of whether reagents are deteriorating.

(2) Check the needle calibration each time a new needle is used, when the needle has been dropped or wiped, or when the control pattern is not met, to ensure the delivery of the correct volume of antigen suspension (30 drops \pm 1 drops per 0.5 ml; 17 μ L per drop).

Place the needle on a 1-mL syringe or on a 2-mL pipette. Fill the syringe or pipette with RPR antigen suspension. Alternatively dispense 0.5 ml of antigen into a clean, dry dispensing bottle. Holding the assembly in a vertical position, count the number of drops delivered in 0.5 ml. The needle is correctly calibrated if 30 drops \pm 1 drop is delivered in 0.5 ml. Replace the needle if it does not meet this specification. Be sure to test the calibration of the replacement needle.

(3) Time. The rotator's timer should be checked against another laboratory timer or stop watch. The rotator's timer should be within \pm 15 seconds of the set time.

(4) The Zip IQ centrifuge is set to 3200 rpm which is approximately 1,000 x g of centrifugal force. The back-up centrifuge is set at an 8 to provide 3,200 to 4,500 rpm which translate to 1,000 - 2,000 x g of centrifugal force. The rpm is verified every 12 to 18 months.



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15. REPORTABLE RANGE

The reportable range of the RPR procedure is from Nonreactive to Reactive with a dilution of 1:1 progressing through a dilution of 1:1024. Dilutions exceeding 1:1024 are reported as >1:1024. At the discretion of the Medical Director additional dilutions may be added.

16. CONTROL PROCEDURES

In order to:

- (1) Detect immediate errors that occur due to test system failure, adverse environmental conditions, and operator performance, and
- (2) Monitor over time the accuracy and precision of test performance that may be influenced by changes in test system performance and environmental conditions, and variance in operator performance.

The following control procedure will be used for syphilis. Document 1-5 below on the RPR QC Log (400-001-05-01-F) and all others as noted:

1. An RPR control card, with Reactive, Minimally Reactive, and Negative controls must be run every day that samples are tested, and each control area must show the appropriate reaction. Follow control card package insert (400-001-05-05-A) for specific instructions on use and interpretation.
 2. A graded Reactive control, capable of a minimum of a 1:4 reaction, must be used when a Reactive patient specimen is encountered and requires quantitation.
 3. Ambient (room) temperatures must be check and recorded each day of testing.
 4. The Zip IQ centrifuge must be inspected before use to ensure that the rotor is secure on the rotor shaft. If the rotor is loose, tighten using the hex wrench which is located near the centrifuge.
 5. Rotator speed must be checked each day of testing. The speed of the rotator can be determined by counting the number of rotations made per minute. To count the rotations, hold a pencil or pen where the table of the rotator can contact it, and count the number of times the rotator touches it in 15 seconds. If the rotator is properly adjusted the count should be 25. The rotator's speed should be verified each day it is used.
- Refrigerator temperatures must be checked and recorded daily on clinic refrigerator log.
 - Size and uniformity of antigen droplets produced by the dispensing needles must be observed every day of testing. Check the calibration of a needle each time a new needle is used, when the needle has been dropped or wiped, or when the control pattern is not met; to ensure the delivery of the correct volume of antigen suspension (30 drops \pm 1 drops per 0.5 mL; 17 μ L per drop). Replace the needle if it does not meet this specification. Be sure to test the calibration of the replacement needle. Refer to the Maintenance manual for needle verification procedure.

- RPR reagents from new lot/shipment are tested utilizing materials capable of providing reactive and nonreactive result, this may include any of the following: reactive and nonreactive control serum samples, commercially supplied controls, graded proficiency testing samples, or the test control card which provides positive and negative reactivity.
 1. Test all serum or control card specimens.
 2. Read and record test results on the RPR QC Log (400-001-05-01-F).
 3. If the new antigen gives the established reactivity, further testing can continue.

17. CORRECTIVE ACTION

If any component of the RPR testing system is not performing to specification, testing must be suspended until the cause is determined and corrections are performed. This may include but is not limited to:

- Replacement or repair of equipment.
- Replacement of bottles, needles, or cards.
- Replacement of antigen, control, diluents, and other reagents.

Specimens may be referred for testing to outside facilities (e.g., Aultman Lab Services) at the direction of the Medical Director. These specimens need to be packaged according to Biological Substance – Category 'B' specifications by trained personnel for ground shipment via Canton City Health Department personnel and vehicles or by the Aultman Courier Service.

18. LIMITATIONS OF THE PROCEDURE

This test is to be used with serum or plasma – other fluids are not acceptable.

Serum that is excessively lipemic, hemolyzed, or contaminated may interfere with the reaction. Serum that has been repeatedly frozen and thawed may be falsely negative in the test. Serum or reagents that have not reached room temperature before performing the test may cause false negative reactions. Improperly diluting the serum samples will cause erroneous results. If the sample is diluted too much, it may be falsely negative. If not diluted enough, a false-positive result may occur. There may be false positive reactions among people who abuse drugs, have autoimmune diseases such as lupus or mononucleosis, have been vaccinated, or have been exposed to other treponemal diseases such as yaws or pinta. See the product insert for more information.

A prozone reaction may be encountered occasionally. In a prozone reaction, complete or partial inhibition of reactivity occurs with undiluted serum (maximum reactivity is obtained only with diluted serum). The prozone phenomenon may be so pronounced that only a rough reading is produced in the qualitative test by a serum that will be strongly reactive when diluted. All test specimens producing any degree of roughness or reactivity with the RPR card test antigen in the qualitative test should be retested by using the quantitative procedure.

10. EXPECTED RESULTS AND NORMAL VALUES

Normal Values

The normal value for a patient specimen is Non-reactive.

11. ALERT OR PANIC VALUES

There are no Alert or Panic Values for the RPR procedure, however reactive results must immediately be brought to the attention of the clinician.

12. DATA ENTRY AND REPORTING

The results of Control Card runs are entered into the Daily QC Log, as well as other pertinent information such as room temperature and rotator speed. Patient specimen observations are entered into the RPR log book as the analyst performs the examination and initialed by the analyst upon completion.

Results are provided to the Nursing Division by transcribing the results from the log into the EMR. To enter a result into the EMR, the scheduler page on the left toolbar is selected. The date of the clinic is then selected from the calendar. The patients' names for the current clinic are displayed and the correct patient name can be selected by clicking on the name and scrolling down to the "Facesheet" option. Once the "Facesheet" page opens, the current laboratory tests can be found in the "Pending Services" section. To result a test, the "Pending Services" section can be expanded by clicking on the "Expand to Detail View" icon or the test can be result directly from the "Pending Service" section by clicking on the flask icon which is the "Add Result" button. In the expanded view, the "Add Result" option must be selected for the desired test. The first field on the resulting page is the "Observation Identifier" and the patient's unique ID is entered into this field. The next field to be entered is the "Observation Date." The "Observation Date" field will automatically populate with the current date and will only need to be modified if the date of testing is different from the current date. The following field is the "Reference Range" and Non-Reactive will be selected for the RPR test. The "Result Value" field requires data entry and a dash (-) is sufficient for Non-Reactive samples. For Reactive samples, the result of the titer will be entered in the "Result Value" field. The "Result Flag" field will be entered with the result of the test, as either Non-Reactive or Reactive. Note: if the sample is Reactive, then the patient information must be reported to the Ohio Department of Health utilizing the Ohio Disease Reporting System (ODRS), see instructions below. The "Acknowledge Result" will always have "yes" selected and the "Units" field can be left blank. The last field is the "Comments" section and it will usually remain empty unless a specific comment for the sample is necessary. Once the required fields are filled in, the "Add" button can be selected. After results have been entered, routine RPRs require a status change to "Completed." This is done by selecting the "Expand to Detailed View" icon on the "Result" section on the "Facesheet" page and using the + icon for each individual test. Once the test details can be



seen, the “Status” can be changed to “Completed” and the “Save” button is selected.

Alternatively, if the EMR is not available, transcribe the results onto the Lab Requisition Form and hand carry the results to the appropriate nursing staff.

If the sample is reactive follow 400-001-05-15-A_Syphilis Special Reporting Requirements. In addition, reactive tests must also be reported to the Ohio Department of Health utilizing the Ohio Disease Reporting System (ODRS) based on the three possibilities below. In all cases, the ODRS case ID needs to be sent to the DIS and their supervisor **and** indicated on the patient log. This does not have to be done during clinic time but must be done as soon after clinic as is reasonably possible and should also include if an FTA was sent out. For RPRs, the ODRS case ID does not need to be entered into the EMR. ODRS should be entered:

1. Enter a new case into ODRS,
2. Update an existing case with all available information including demographics as needed,
3. An existing case is in ODRS, but the record is unable to be edited, send information to DIS to reflect inability to update record and include the ODRS record number.

Proficiency Testing specimens are recorded in the patient RPR Log as if they were an actual patient. Results are then transcribed into the Proficiency Testing Providers Electronic Reporting System at the earliest convenience and prior to the testing close date.

13. SYSTEM FAILURE OR INOPERABILITY

If RPR tests cannot be performed the Medical Director and/or Director of Nursing must be immediately informed so that STI Clinic practice can be modified to accommodate this situation. RPR testing must be suspended until capability is regained; patient specimens may need to be referred to an outside laboratory if directed by the Medical Director.

14. SYPHILIS APPENDIX

- 400-001-05-01-F_RPR QC Log
- 400-001-05-02-P_RPR Reagent Preparation
- 400-001-05-03-F_RPR Patient Log
- 400-001-05-04-A_RPR Card Test Controls Package Insert
- 400-001-05-05-A_RPR Antigen Package insert
- 400-001-05-06-A_Syphilis Dilution Sheet
- 400-001-05-07-A_RPR Card Tests Product Insert
- 400-001-05-13-P_Procedure for Spinning Blood Samples
- 400-001-05-14-F_Syphilis Innovative Grant Patient Log
- 400-001-05-15-A_Syphilis Special Reporting Requirements



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BLOOD LEAD (WAIVED)

1. MATERIALS REQUIRED

Alcohol wipes
Gauze pads
Kim wipes
Powder-free gloves
Personal Protective Equipment as needed (goggles/shield/lab coat)
Sensors
Treatment Reagent Tubes
Heparinized Capillary Tubes/Plungers
Transfer Droppers
Calibration Button
Lead Control Level 1
Lead Control Level 2
LeadCare II Analyzer (using 4AA batteries or the AC Adapter)

2. PATIENT PREPARATION

Specimen Collection and Handling

- The LeadCare II test kit includes capillary tubes for the collection of a whole blood sample directly from the patient's finger.
- Proper preparation of the puncture area is important. Refer to CDC guidelines, "Steps for Collecting Fingerstick Blood Samples in Micro-Vials for Lead Testing".

For samples collected in micro-vials or vacuum collection tubes:

- Use only heparin or EDTA as anticoagulants. If you use EDTA collection tubes, they must be at least one half full otherwise you could obtain falsely lower blood lead results.
- Use only fresh whole blood. Add blood within 24 hours of collection to reagent. Store whole blood at 50°- 90°F (10°- 32°C). Store blood/reagent mixture for up to 7 days refrigerated and 48 hours at room temperature.
- Do NOT use plasma or serum.
- Do NOT refrigerate the whole blood prior to mixing with treatment reagent.
- Make sure to invert the specimen container multiple times to thoroughly mix the blood before filling the capillary tube.
- Make sure the blood sample does not contain clots. Blood clots can lead to erroneous blood lead results.
- Use the capillary tube and plunger provided with the test kit to remove 50 µl of blood from the collection tube and dispense it into the treatment reagent tube.

- REJECT samples that contain less than 50% of intended fill volume and are not within the guidelines stated above. REJECT specimens that are not clearly labeled. Do not discard the specimen prior to consulting with the clinician for the sample status. This permits the clinician to clarify any potential concerns as well as make possible exceptions which must be clearly documented.

PRECAUTIONS:

Handle all products and objects containing human blood as if capable of transmitting diseases. Follow established recommendations for prevention of blood-borne transmissible diseases. For example, consult the "Universal Precautions" issued by the U.S. Public Health Service, Centers for Disease Control. Review your internal protocol for preventing transmission of blood-borne pathogens and your biohazardous waste disposal procedures prior to implementing the LeadCare II Blood Lead Testing System.⁴

CAUTION – contains 0.34 M Hydrochloric Acid which may cause eye, skin, and respiratory system irritation. Avoid contact with skin, eyes, and clothing. In case of accidental contact immediately flush skin and eyes with running water for up to 15 minutes and move to fresh air. Seek medical assistance in situations where eye contact, skin irritation or burn; or difficulty breathing occurs. You MUST wear appropriate laboratory personal protective (and safety) equipment (PPE) when using the LeadCare II System.

3. MICROSCOPIC EXAMINATION AND PROCEDURE

No microscopic examination is required for this testing procedure.

Refer to your LeadCare II Quick Reference Guide and User's Guide for detailed test instructions.

NOTE: Properly calibrate the analyzer with the calibration button that comes with each test kit.

1. Turn on analyzer

- Wait for SELF TEST to finish.
- Make sure the sensor lot number (printed on the sensor container) matches the number displayed on the screen.

2. Refer to the Quality Control section of the package insert for important information about when and how to perform quality control testing to ensure the accuracy of your lead results. Perform quality control testing if necessary.

3. Obtain a *whole* blood sample

- Label a treatment reagent tube with the patient ID.
- Use the heparinized capillary tube provided. Holding it almost horizontally, fill to the 50 µL black line with fresh whole blood.
- Use a clean gauze pad to remove any excess blood from the outside of the capillary tube.

NOTE: The accuracy of the test depends on filling the capillary tube properly. Make sure the blood reaches the 50 µL black line without gaps or bubbles.



4. Mix the whole blood sample with treatment reagent

- Remove the cap from the treatment reagent tube and place it *top down* on a clean surface. Do not allow the inside part of the cap to touch anything.
- Place the capillary tube into the treatment reagent tube. Insert a plunger into the top of the capillary tube. Dispense all the blood into the treatment reagent.
- Remove the empty capillary tube and replace the cap on the treatment reagent tube.
- Invert the tube 8 to 10 times to mix the sample completely. The sample is ready when the mixture turns brown. Caution: Any visual impairment, such as color blindness may affect the operator's ability to detect the sample color change. Operators with vision deficiencies should invert the tube 8 to 10 times to ensure that the sample is properly mixed.

NOTE: The accuracy of the blood lead test depends on properly transferring 50 µL of blood into the treatment reagent. Use the capillary tubes and plungers provided with the test kit as instructed to ensure the accuracy of your results. A capillary tube should also be used to prepare controls, proficiency samples, and venous samples for analysis.

NOTE: Store blood/reagent mixture for up to 7 days refrigerated and 48 hours at room temperature.

5. Apply treatment reagent and blood mixture to a sensor

- Open a sensor container, remove a sensor, and re-close the container.
- Insert the sensor into the analyzer until you hear a "beep" and the screen displays the message, "ADD 1 DROP OF SAMPLE TO X ON SENSOR".
- Make sure the sensor lot number matches the number on the display.
- Make sure the sample is thoroughly mixed.
- Remove the cap from the tube. Insert a transfer dropper into the tube. Squeeze the dropper and insert into the sample. Release the pressure to draw the sample into the dropper.
- Place the dropper on the "X" of the sensor and squeeze to dispense the sample onto the sensor. When the sample is added, the analyzer beeps, and begins the test automatically.

6. Read the blood lead result

- After 3 minutes, the analyzer beeps and displays the blood lead result on the screen. Read and record the result in µg/dL on the sheet provided.

7. Discard used materials

- After the test is completed, remove the sensor. Discard used materials in appropriate containers.

4. MATERIAL PREPARATION

Sensor Composition: The active electrode area in each sensor contains a small amount of gold particles in an inert matrix.

Treatment Reagent Composition: The treatment reagent contains 250 µL of a dilute hydrochloric acid solution in water (0.34 M).

Blood Lead Control Composition: Lead salt in buffered aqueous solution with bovine serum albumin (BSA). Two levels of quality control material are provided with the test kit, designated “Level 1” and “Level 2”. The actual target values are specified on the labels.

STORAGE AND HANDLING:

The test kit has an expiration date assigned. It is printed on the exterior of the box. Do NOT use the test kit past the expiration date. NOTE: The treatment reagent, blood lead controls and the sensors have separate expiration dates. The earliest expiring component is used to set the test kit’s expiration date.

To keep the LeadCare II Blood Lead Test Kit fresh, observe the following:

- Store in a cool, dry place. Storage temperature should be between 60°- 80°F (15°- 27°C). Do NOT freeze or refrigerate.
- Store away from direct sunlight.
- Keep sensors sealed in their container until the sample is prepared and you are ready to perform the test. The container is lined with desiccant to keep the sensors fresh.
- Use the treatment reagent immediately after opening the tube.
- Do NOT place any object in the treatment reagent tube other than the capillary and dropper provided with this test kit. Contamination could occur.
- Do NOT use sensors, blood lead controls and treatment reagent past their expiration dates.
- Precautions: SDS sheet can be found in the User’s Guide.
- Treatment reagent may be harmful if swallowed. Keep out of reach of children. If swallowed, consult a physician. If there is contact with skin or eyes, flush with water.
- Blood lead control material may be harmful if it comes in contact with the eyes or an open wound. Practice universal precautions when handling. If there is contact with skin or eyes, flush with water.

5. CALIBRATION PROCEDURES

The LeadCare II Analyzer **MUST** be calibrated for the test kit lot in use. Use only the calibration button that comes with the test kit. Make sure that the calibration code on the calibration button matches the lot number on the sensor container, and on the controls.

1. Turn on analyzer

- Wait for SELF TEST to finish. The analyzer is ready when the PREPARE SAMPLE message appears.

2. Calibrate analyzer

- Remove the calibration button from the test kit.
- Touch calibration button to the calibration button reader on the analyzer.

- Hold calibration button to the reader until analyzer "beeps". "CALIBRATION SUCCESSFUL" will appear briefly on the screen.
- The new calibration code ("sensor lot") will be displayed on the screen.
- Make sure the code matches the calibration button and the lot number of the test kit being used.
- Analyzer is now calibrated and ready for a blood lead test.

6. REPORTABLE RANGE

Refer to the most recent version of LeadCare II Blood Lead Test Kit for Test Result

In general, observations can be reported as:

1. "Low" = $<3.3 \mu\text{g/dL}$
2. "High" = $>65 \mu\text{g/dL}$
3. The reportable range 3.3 to 65 $\mu\text{g/dL}$

7. CONTROL PROCEDURES

According to CLIA guidelines for Waived Laboratories, controls should be run according to the manufacturer's instructions, which are:

- Each new lot.
- Each new shipment of materials, even if it's the same lot previously received.
- Each new operator (i.e., operator who has not performed the test recently).
- Monthly as a check on continued storage conditions (do not break seal of new test kit for monthly check-once opened monthly check required).
- When problems (storage, operator, instrument, or other) are suspected or identified.

The blood lead level that appears on the analyzer display should be within the acceptable range provided for that control. If the blood lead levels displayed are within the range listed for the control, your LeadCare II system is working properly. If the reported blood lead levels are *not* within the listed range, refer to the Troubleshooting section of the User's Guide. If, after following the instructions, the controls are still out of range, call LeadCare Product Support at 1-800-275-0102, and notify the Supervisor.

Procedure for Testing the Blood Lead Controls

1. Prepare the sample

- Label a treatment reagent tube "Level 1".
- Gently swirl the Level 1 control vial. Remove the cap from the vial and place it *top down* on a clean surface.
- Fill one of the capillary tubes with the control you are testing. To accomplish this, tilt the control vial, insert the capillary tube into the liquid while holding the green end of the capillary tube almost horizontally. Capillary action will fill the tube to the 50 μL black line.



- Use a clean wipe to remove excess control material from the outside of the capillary tube.
2. Mix the control material with treatment reagent
 - Remove the cap from the treatment reagent tube and place it *top down* on a clean surface. Do not allow the inside part of the cap to touch anything.
 - Place the capillary tube into the treatment reagent tube. Insert a plunger into the top of the capillary tube. Dispense all of the control into the treatment reagent.
 - Remove the empty capillary tube and replace the cap on the treatment reagent tube.
 3. Invert the tube 8 to 10 times to mix the sample completely. The resulting mixture will be red. Apply treatment reagent and control mixture to a sensor.
 - Open a sensor container, remove a sensor, and re-close the container.
 - Insert the sensor into the analyzer until you hear a “beep” and the screen displays the message, “ADD 1 DROP OF SAMPLE TO X ON SENSOR”.
 - Make sure the sensor lot number matches the number on the display.
 - Make sure the sample is thoroughly mixed.
 - Remove the cap from the tube. Insert a transfer dropper into the tube. Squeeze the dropper and insert into the sample. Release the pressure to draw the sample into the dropper.
 - Place the dropper on the “X” of the sensor and squeeze to dispense the sample onto the sensor. When the sample is added, the analyzer beeps, and begins the test automatically.
 4. Read the blood lead result
 - After 3 minutes, the analyzer beeps and displays the blood lead result on the screen. Read and record the result in µg/dL on the sheet provided.
 5. Discard used materials
 - After the test is completed, remove the sensor. Discard used materials in appropriate containers.
 6. Repeat this process for the Level 2 control.

In addition to regular controls LeadCare II requires Proficiency Testing in accordance with the Clinical Lead Licensure rules. Copies of the results must be provided to the Ohio Department of Health, Division of Quality Assurance (DQA) Lead Poisoning Prevention Program within five (5) days of receiving the results.

The Canton City Health Department will follow existing Proficiency Testing Protocols and apply the following information as applicable.

“Facilities using the LeadCare II instrument will be permitted to use the standard CLIA regulatory three (3) sets of five (5) annual samples testing protocol. Or one of the alternative QA options of: two (2) sets of three (3) annual samples, or (3) sets of two (2) annual samples, testing protocols for proficiency testing. The alternate QA test protocols are the absolute minimum proficiency testing protocols for LeadCare II instruments used

by approved Clinical Lead Laboratories for Ohio. If one of the less costly options is utilized, each Clinical Laboratory shall maintain proficiency testing result of three (3) out of three (3) correct samples, or (2) out of (2) correct samples for each testing round. If a laboratory fails to meet acceptable test results, then the laboratory must provide an explanation of corrective actions to maintain satisfactory performance.” (ODH Letter to LeadCare II users. From David Holston Environmental Abatement Section Chief, re Clinical Lead Laboratory Approval Requirements, Dated February 12, 2013)

8. CORRECTIVE ACTION

Corrective action to take when calibration or control results fail to meet the laboratory's criteria for acceptability: If the reported blood lead levels are *not* within the listed range, refer to the Troubleshooting section of the User's Guide. If, after following the instructions, the controls are still out of range, call LeadCare Product Support at 1-800-275-0102 and consult a supervisor immediately. Testing should be suspended until the issue is resolved. IMPORTANT: Do NOT proceed to patient samples unless both the Level 1 and Level 2 control results are within the acceptable ranges.

9. LIMITATIONS OF THE PROCEDURE

- For blood collected in the capillary tubes provided with the test kit: Dispense the blood from the capillary tube into a treatment reagent tube within 10 minutes of collection and mix well.
- For blood collected in other collection devices: Use only fresh, unrefrigerated whole blood within 24 hours stored at 50°- 90°F (10°-32°C) with the LeadCare II System. Do NOT use plasma or serum. Use the capillary tubes and plungers provided with the test kit to transfer 50 µL of blood from the collection device into the treatment reagent tube.
- After mixing the blood with the treatment reagent, analyze it in less than 48 hours if stored at room temperature. If stored refrigerated analyze within 7 days. NOTE: Allow mixture to reach room temperature before analyzing.
- Extremes in humidity may affect the blood lead results. Performance has been validated from 12% to 80% RH (non-condensing). Use of the LeadCare II system outside of this range is not recommended.
- Do NOT use the LeadCare II System in drafts. This could lead to inaccurate results.
- Keep the LeadCare II System out of direct sunlight.
- The analyzer will only function in the temperature range of 54°- 97°F (12°- 36°C). Otherwise, the analyzer will display a temperature error code. Refer to analyzer display messages in the User's Guide (Chapter 5).
- Allow all of the LeadCare II System components to reach a steady temperature before using.
- Clinical testing demonstrates that altitudes up to 8,000 feet (2,440 meters) above sea level do not affect results obtained with the LeadCare II System.

- Use the sensors, the treatment reagent tubes, capillary tubes and transfer droppers only once. Do NOT reuse. Reuse could lead to erroneous results.
- Do NOT use damaged (bent, scratched, cut, etc.) sensors.
- The following substances (at the concentrations listed) do NOT affect the results of the LeadCare II system: copper (90 µmol/L), zinc (54 µmol/L), arsenic (0.78 µmol/L), cadmium (0.27 µmol/L), aluminum (0.45 µmol/L), ascorbic acid (0.30 mmol/L), uric acid (1.5 mmol/L).
- The LeadCare II system was also tested in the presence of 37 drugs commonly found in pediatric blood samples. The following concentrations do NOT affect the results of the LeadCare II system: acetaminophen (396 µmol/L), acetylsalicylic acid (6.0 mmol/L), ibuprofen (396 µmol/L), heparin (80,000 units/L), calcium sodium EDTA (6.7 mmol/L), succimer (DMSA) (78 µmol/L), DMPS (2,3-dimercapto-1-propane sulfonic acid) (78 µmol/L), D-penicillamine (0.17 mmol/L), BAL (2,3-mercaptopropanol) (0.97 µmol/L). Refer to the User's Guide for a complete list of drugs tested.

10. EXPECTED RESULTS AND NORMAL VALUES

Refer to the most recent version of LeadCare II Blood Lead Test Kit for Test Result information.

In general, observations can be reported as:

"Low" = <3.3 µg/dL

"High" = >65 µg/dL

The reportable range 3.3 to 65 µg/dL

The normal range is <3.3 µg/dL

11. PANIC OR ALERT VALUES

Follow the Ohio Department of Health recommendations for Repeat Testing Guidelines.

12. DATA ENTRY AND REPORTING

The results of the Controls, Proficiency Testing and Patient specimen observations are entered onto the Blood Lead Testing System Data Log. The Patient results are then recorded on the Lead Clinic Requisition and Reporting Form (400-001-009-F_Lead Reporting Form). These forms are then provided to the Nursing Division. Report Alert/Panic levels the same day of testing to the Nursing Division, otherwise within seven calendar days of sample collection of sample collection.

Additional reporting requirements must also be followed as described in OAC 3701-30-05 Record-keeping and reporting requirements.

The records must be transmitted electronically to the director within seven calendar days of obtaining the result.

Instructions to complete the electronic reporting process can be found in the *Canton City's LeadCare Reporting Software User's Guide*, 400-001-08-03-P.



Any clinical laboratory that performs any analysis of human blood on a child under sixteen years of age and residing in Ohio to detect or determine levels of lead shall collect and report to the director all the following information that they have available:

1. Child's name and parent's or guardian's name.
2. Child's street and mailing address, including the city, state, county, and zip code.
3. Child's social security number, date of birth, gender, race and ethnicity.
4. Telephone number, with area code, where the parents or guardians can be reached.
5. Specimen matrix (blood).
6. Analyte (lead).
7. Procedure used to obtain the specimen and the date it was obtained.
8. Physician's or healthcare provider's first name, last name, address, telephone number, and national provider identifier, if applicable.
9. Child's Medicaid number, if any.
10. Clinical laboratory improvement amendments of 1998 (CLIA) number of the laboratory performing the analysis.
11. The accession number, the date the sample was analyzed, and the test result in micrograms per deciliter.

13. SYSTEM FAILURE OR INOPERABILITY

Only proceed to patient samples if both the Level 1 and Level 2 control results are within the acceptable ranges and the LeadCare II unit is operable.

14. LEAD APPENDIX

- 400-001-08-01-A_LeadCare II Blood Lead Test Kit package insert
- 400-001-08-02-A_Steps for Collecting Fingerstick Blood Samples in Micro-Vials for Lead Testing
- 400-001-08-03-P Canton City LeadCare Reporting Software Users Guide
- 400-001-08-04-F_Blood Lead Testing System Data Entry Log
- 400-001-08-05-F_Blood Lead Testing Requisition and Reporting Form
- 400-001-08-06-A_Blood Lead II Users Guide

ORAQUICK HCV RAPID ANTIBODY TEST PROCEDURE (WAIVED)

1. MATERIALS REQUIRED:

HCV test kit (OraQuick HCV Rapid Antibody Test), which includes:

- HCV test device
- Developer Solution
- Package Insert

HCV test stand (reusable)

HCV specimen collection loop

Timer capable of measuring 20 to 40 minutes

Biohazard waste container

Appropriate PPE

2. PATIENT PREPARATION, SAMPLE COLLECTION, AND RELATED REQUIREMENTS

The patient will be taken into a CCPH STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CCPH STI Clinic protocol. The clinician will collect a fingerstick whole blood sample in a Specimen Collection Loop and perform the test procedure in the exam room following the OraQuick HCV package insert instructions. The clinician must examine the sample for suitability for Acceptance, as there is no referral for HCV Rapid Antibody tests-recollection is the only recourse for unacceptable specimens. The specimen should be maintained at room temperature (15-30° C) and there must be sufficient sample to completely fill the Specimen Collection Loop. Lack of any of these is a basis for sample Rejection, which will be noted in the logbook along with an explanation as to why. The clinician will fill out a lab slip with the patient's results and bring it to the STAT lab. Once the lab slip is in the STAT lab all pertinent information is entered into the STAT lab logbook.

3. MICROSCOPIC EXAMINATION AND PROCEDURE

There is no microscopic examination for the HCV Rapid Antibody test procedure; it is a macroscopic technique.

Note: This procedure utilizes potentially infectious patient specimens. Gloves, eye protection, and protective clothing are required.

Follow the Package Insert, 400-001-10-02-A_OraQuick HCV Rapid Antibody Test Product insert, for complete directions for kit use.

4. MATERIAL PREPARATION

All necessary materials should be assembled prior to testing. The test kits and testing related materials and equipment should all be at operating temperature (15-37° C) prior to performing the test. The test kits themselves should be stored at room temperature. Ensure that all testing materials are within their expiration dates. Check the timer for a readable display (adequate battery).

5. CALIBRATION

The timer used for the test should be calibrated annually and meet manufacturer's specifications. There is no calibration required for the test device (Waived test).

6. REPORTABLE RANGE

This is a qualitative test; there are three (3) possible results: Non-Reactive, Reactive, or Invalid. Refer to the Package Insert for additional reporting information.

7. CONTROL PROCEDURES

In order to:

- (1) Detect immediate errors that occur due to test system failure, adverse environmental conditions, and operator performance, and
- (2) Monitor over time the accuracy and precision of test performance that may be influenced by changes in test system performance and environmental conditions, and variance in operator performance, the following control procedure will be used:

Follow Package Insert to complete the following:

Run the Kit Controls under the following circumstances:

- Each new operator prior to performing testing on patient specimens,
- When opening a new test kit lot,
- Whenever a new shipment of test kits is received,
- If the temperature of the test kit storage area falls outside of 2°- 30°C (36°- 86°F),
- If the temperature of the testing area falls outside of 15°- 37°C (59°- 99°F), and
- At intervals as requested by the HIV Coordinator.

8. CORRECTIVE ACTION

If a test fails to meet Control Criteria for acceptability (a red line in the control region of the device viewing window with a clear background – white to light pink) the test procedure should be reviewed and the test re-run with a new device. If the problem persists discontinue testing immediately, notify the Medical Director and/or the Nursing Director (or appropriate clinical personnel), and contact the manufacturer's Customer Service for further directions.

9. LIMITATIONS OF PROCEDURE

Follow Package Insert.

10. EXPECTED RESULTS AND NORMAL VALUES

Follow Package Insert.

11. PANIC OR ALERT VALUES

There is no Panic or Alert Values.

12. DATA ENTRY AND REPORTING

Patient results are entered into the HCV log book

13. SYSTEM FAILURE OR INOPERABILITY

If HCV tests cannot be performed the Clinic Director must be immediately informed so that STI Clinic practice can be modified to accommodate this situation. Patients suspected of being exposed to or infected with the HCV virus may need to be referred to other providers for appropriate testing and treatment.

14. HCV RAPID ANTIBODY APPENDIX

- 400-001-10-01-F_HCV Patient Log
- 400-001-10-02-A_OraQuick HCV Rapid Antibody Test Product insert



Public Health
Prevent. Promote. Protect.

Canton City Health District

Canton City Health District
Laboratory
FINAL

XPert® CT/NG NUCLEIC ACID AMPLIFICATION TEST (NAAT) FOR CHLAMYDIA AND GONORRHEA

Below is information excerpted from the Xpert CT-NG Product Insert (400-001-11-01-A_Xpert CT-NG Product Insert) and the Cepheid Xpert Operational Manual (400-001-11-07-A_Operator Manual). Refer to these and other cited documents for reference and further details. The excerpt is not intended to be all inclusive, but rather to provide the basic information necessary to run the Cepheid Xpert CT/NG Assay.

1. MATERIALS REQUIRED

Xpert CT/NG Assay materials as described in the product insert.

Xpert CT/NG cartridges with integrated reaction tubes and 1mL pipette stored at 2 - 28°C.

CD

- Assay Definition Files (ADF)
- Instructions to import ADF into GeneXpert software
- Instructions for Use (Package Insert)

Primary samples must be collected with the appropriate kit:

- Xpert Swab Specimen Collection Kit
- Xpert Urine Specimen Collection Kit

Bleach, 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution used to make a 1:10 cleaning solution

70% alcohol at a minimum for quarterly probe cleaning

70% denatured ethanol for quarterly cleaning and/or 70% pure alcohol wipes for daily cleaning

Biohazard waste container w/ biobags

Lab markers

Appropriate PPE

2. PATIENT PREPARATION, SAMPLE COLLECTION, AND RELATED REQUIREMENTS

The patient will be taken into a CCPH STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CCPH STI Clinic protocol. For urine samples the clinician will have asked the patient to collect the specimen (urine) in a provided sample container, for pharyngeal swab specimens and rectal swab specimens, the clinician will use the Xpert Swab Specimen Collection Kit following manufacturer's specimen collection procedures. Samples will be labeled with the patient's unique ID and the patient's clinic number and transported to the STAT lab. The specimen should be maintained at room temperature (15-30° C) unless testing is to be delayed. Swab samples in Xpert Swab Transport Reagent tubes and urine specimen that is transferred to the Xpert Urine Transport Reagent tube can be stored up to 45 days at 2 °C to 15 °C, or up to 3 days at 2 °C to 30 °C before testing with the Xpert CT/NG test.

Once the specimen is in the STAT lab all pertinent information is entered into the STAT lab logbook. It is examined for suitability and acceptance, as there is no referral for NAAT (Xpert CT/NG) tests- recollection is the only recourse for unacceptable specimens. For Urine samples there must be sufficient sample to allow testing – 7.0 ml is required to be transferred into specimen tube.

Alternatively, the urine sample can be tested directly by filling the transfer pipette that comes with the cartridges, to the fill line (approximately 1ml) and adding it directly Xpert CT/NG cartridge. Lack of any of these is a basis for sample Rejection, which will be noted in the logbook along with an explanation as to why. Do not discard the specimen prior to consulting with the clinician for the sample status. This permits the clinician to clarify any potential concerns as well as make possible exceptions which must be clearly documented.

3. MICROSCOPIC EXAMINATION AND PROCEDURE

There is no microscopic examination for the NAAT test procedure; it is a macroscopic technique.

Note: This procedure utilizes potentially infectious patient specimens. Gloves, eye protection, and protective clothing are required.

Before starting these procedures, make sure that the instrument is running 6-color GeneXpert Software Version 4.3 or higher.

Important: Start the test within 30 minutes of adding the sample to the cartridge.

Preparing the urine transport tube

1. Ensure urine is well mixed in the urine cup before transferring aliquot to the Xpert Urine Transport Reagent tube.
2. Obtain one Xpert Urine Specimen Collection Kit.
3. Open the packaging of a disposable transfer pipette provided in the collection kit. Note: do not use the pipette from the assay kit.
4. Insert the transfer pipette into the bottom of the urine cup.
5. Transfer approximately 7 mL of urine so that the volume reaches the black dashed line on the tube label.
6. Replace the cap on the tube and tighten. Label with patient unique ID and patient number (date required if sample is not tested on the same day as collection).

Note: urine samples can be added directly to the cartridge and therefore, do not need to be added to the urine transport tubes in order to be tested.

Preparing the cartridge

To add the sample to the Xpert CT/NG Assay cartridge:

1. Open the cartridge lid. **Note:** Do not open a cartridge until ready to test. Use cartridges within 30 minutes after opening the lid.
2. Gently invert the transport tube 3 to 4 times to ensure adequate mixing of sample and transport matrix.
3. Unwrap the transfer pipette.
4. Open the transport tube lid, compress the bulb of the transfer pipette, insert the pipette into the transport tube, and release the bulb to fill the transfer pipette above the mark on the pipette shaft Figure 1: Transfer pipette and fill mark (Figure 1). Ensure the pipette is filled with no air bubbles present.

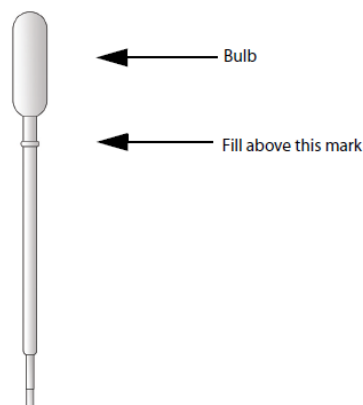
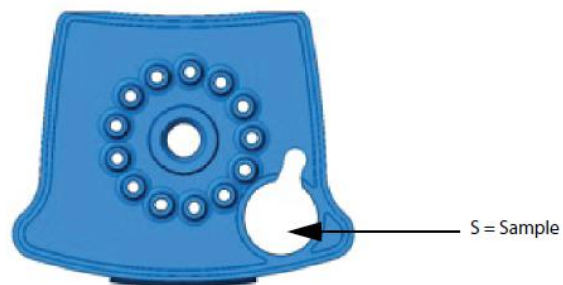


Figure 1: Transfer pipette and fill mark

5. Empty the pipette's content into the Sample chamber of the cartridge (See Figure 2)

Figure 2: Chambers shown from top of cartridge



6. Close the cartridge lid.

Starting the Test

Note: Before you start the test, make sure that the system is running 6-color GeneXpert 4.3 software or higher and that the Xpert CT/NG Assay Definition File is imported into the software.

NOTE: Do not load a GeneXpert cartridge that has been dropped or shaken after the lid of the cartridge has been opened. Dropping or shaking the cartridge after it has been opened can cause invalid results. Bent or broken reaction tubes can also produce invalid results. Do not re-use spent cartridges.

NOTE: Always pick up the cartridge by its body. Do not pick up the cartridge by the protruding reaction tube (See Figure 3)



Figure 3: Cartridge Showing Body and Reaction Tube

This section lists the default steps to operate the GeneXpert System. For detailed instructions, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*, depending on the model that is being used.

1. Turn on the GeneXpert Instrument System:

Using the GeneXpert Dx instrument, first turn on the instrument and then turn on the computer. The GeneXpert software will launch automatically.

2. Log on to the GeneXpert System software using the user name and password.

3. In the GeneXpert System window, click **Create Test**. The **Create Test** window appears.

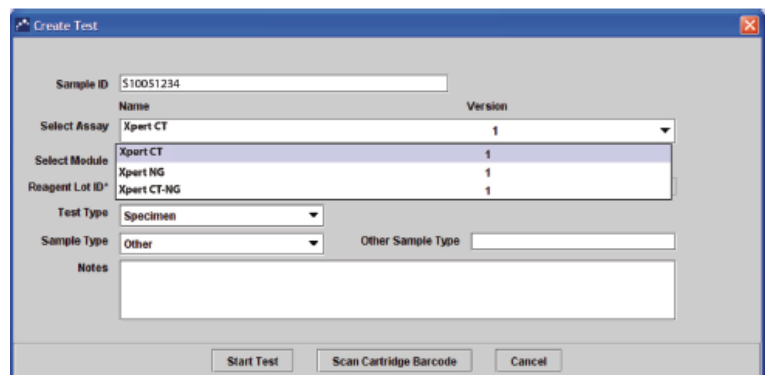


Figure 4: Create Test Window



4. Manually type the Sample ID, make sure the ID is typed correctly. The ID is associated with the test results and is shown in the **View Results** window.
5. Scan the barcode on the Xpert CT/NG Assay cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, and Expiration Date.

Note: If the barcode on the Xpert CT/NG cartridge does not scan, then repeat the test with a new cartridge and provide cartridge to supervisor.

6. Verify the **Select Assay** field shows Xpert CT_NG

Note: Only the test result for the assay selected at this step will be collected once the test is started. Both CT and NG results will only be collected if the Xpert CT-NG option is chosen.

7. Click **Start Test**. In the dialog box that appears and
 - i. Open the instrument module door with the blinking green light and load the cartridge.
 - ii. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.
 - iii. Wait until the system releases the door lock before opening the module door and removing the cartridge.
 - iv. Dispose of used cartridges in the appropriate biohazard specimen waste containers.
8. Viewing and Printing Results. This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.
 1. Click the **View Results** icon to view results.
 2. Upon completion of the test, click the **Report** button of the View Results window to view and/or generate a PDF report file.
9. Retests-Reasons to Repeat the Assay

If any of the following test results occur, repeat the test according to instructions in the Retest Procedure.

- An **INVALID** result indicates that the SPC and/or the SAC failed. The sample was not properly processed, PCR was inhibited, or the sample was inadequate.
- An **ERROR** result indicates that the PCC failed and the assay was aborted possibly because the reaction tube was filled improperly, a reagent probe integrity problem

was detected, pressure limits were exceeded, or a valve positioning error was detected.

- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

10. Retest Procedure

Obtain the leftover treated sample from the CT/NG Swab Transport Reagent, Swab Transport Reagent, CT/NG Urine Transport Reagent or Urine Transport Reagent tube. Repeat the test with a new cartridge. If the leftover treated sample volume is insufficient, or the retest continues to return an INVALID, ERROR, or NO RESULT, collect a new sample and repeat the test with a new cartridge.

4. MATERIAL PREPARATION

All necessary materials should be assembled before testing. The test kits and testing related materials and equipment should all at room temperature (15-30° C) prior to performing the test. Follow the package insert and training material for all material preparation handling and storage procedures.

5. CALIBRATION

There are no user calibrations that can be performed on the system components.

6. REPORTABLE RANGE

The reportable ranges for both Chlamydia trachomatis and Neisseria gonorrhea are Not Detected (Negative) and Detected (Positive).

Negative means that the genetic material of select organism was not detected.

Positive means that the genetic material was detected.

The results are interpolated by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and will be shown in the View Results window.

Additional information can be found in the package insert.

7. CONTROL PROCEDURES

In order to:

- (1) Detect immediate errors that occur due to test system failure, adverse environmental conditions, and operator performance, and
- (2) Monitor over time the accuracy and precision of test performance that may be influenced by changes in test system performance and environmental conditions, and variance in operator performance, the following control procedure will be used:

Each test includes a Sample Processing Control (SPC), a Sample Adequacy Control (SAC) and a Probe Check Control (PCC).

- **Sample Processing Control (SPC)**—Ensures the sample was correctly processed. The SPC contains genomic DNA of *Bacillus globigii* that is included in each cartridge. The SPC verifies that binding and elution of target DNA have occurred if the organisms are present and verifies that sample processing is adequate. Additionally, this control detects sample associated inhibition of the real-time PCR assay. The SPC should be positive in an analyte negative sample and can be negative or positive in an analyte positive sample. The SPC passes if it meets the validated acceptance criteria.
- **Sample Adequacy Control (SAC)**—Ensures that the sample contains human cells or human DNA. This multiplex assay includes primers and probes for the detection of a single copy human gene. The SAC signal is only to be considered in an analyte negative sample. A negative SAC indicates that no human cells are present in the sample due to insufficient mixing of the sample or because of an inadequately taken sample.
- **Probe Check Control (PCC)**—Before the PCR reaction starts, the GeneXpert instrument measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. PCC passes if it meets the validated acceptance criteria.
- **External Controls**— External controls (positive and negative) are required by the manufacturer. Based on an Individualized Quality Control Plan Risk Assessment the following control external quality control procedure is in place. Perform external controls on each new lot/shipment and monthly following manufacturer's instructions. See GeneXpert 400-001-11-08-P_Individualized Quality Control Plan for details.

8. CORRECTIVE ACTION

See procedure Step 9 for Retest information.

If a test run fails – the run is flagged by the software- the test procedure should be reviewed, all testing equipment checked for proper functioning, all reagents checked for acceptability, and the testing area examined as to environmental conditions and apparent cleaning and decontamination. If all appears correct the test should be re-run. If the problem persists discontinue testing immediately, notify the Supervisor and the Clinical Manager.

9. LIMITATIONS OF PROCEDURE

See package insert and training manual for lengthy list of limitations.

10. EXPECTED RESULTS AND NORMAL VALUES

Negative results for both *C. trachomatis* and *N. gonorrhea* are expected in healthy individuals who have not been exposed to either of those two organisms.

11. PANIC OR ALERT VALUES

There are no Panic or Alert Values.

12. DATA ENTRY AND REPORTING

Patient results are obtained from the Cepheid Xpert result log and documented onto the patient result log (400-001-11-04-F_Xpert Patient Log). Patient results are provided to the Nursing Division by transcribing the results from the log into the EMR as indicated on the Reference Range and Results Interpretation Form. To enter a result into the EMR, the scheduler page on the left toolbar is selected. The date of the clinic is then selected from the calendar. The patients' names for the current clinic are displayed and the correct patient name can be selected by clicking on the name and scrolling down to the "Facesheet" option. Once the "Facesheet" page opens, the current laboratory tests can be found in the "Pending Services" section. To result a test, the "Pending Services" section can be expanded by clicking on the "Expand to Detail View" icon or the test can be result directly from the "Pending Service" section by clicking on the flask icon which is the "Add Result" button. In the expanded view, the "Add Result" option must be selected for the desired test. The first field on the resulting page is the "Observation Identifier" and the patient's unique ID is entered into this field. The next field to be entered is the "Observation Date." The "Observation Date" field will automatically populate with the current date and will only need to be modified if the date of testing is different from the current date. The following field is the "Reference Range" and Negative will be selected for the CT/NG NAAT test. The "Result Value" field requires data entry and a dash (-) is sufficient. The "Result Flag" field will be entered with the result of the test, as either Negative or Positive. If error/invalid/No result is obtained on initial and re-test, document error code or message in the Lab Orders and Results Log (400-004-03-F). If error code or message only occurs on initial test, documenting on patient log may be sufficient. The "Acknowledge Result" will always have "yes" selected and the "Units" field can be left blank. Once the required fields are filled in, the "Add" button can be selected. Note: if the sample is positive for N gonorrhea or Chlamydia then the patient information must be reported to the Ohio Department of Health utilizing the Ohio Disease Reporting System (ODRS). When entering a case into ODRS note the ODRS ID on the result log. "To Do's" are required for all positive results and must be sent separately to the STI Nurse and treating authority which include the ODRS ID. After results have been entered, CT/NG NAAT tests require a status change to "Completed." This is done by selecting the "Expand to Detailed View" icon on the "Result" section on the "Facesheet" page and using the + icon for each individual test. Once the test details can be seen, the "Status" can be changed to "Completed" and the "Save" button is selected. If the EMR is not available, transcribe the results onto the Lab Requisition Form and hand carry the results to the appropriate nursing staff.

If error/invalid/no result is obtained on initial or retest enter the error code in the result area of the log book and document corrective action in the Lab Orders and Results Log (400-004-03-F).

Proficiency Testing specimens are recorded in the patient log as if they were an actual patient.

Results are then transcribed into the Proficiency Testing Providers Electronic Reporting System at the earliest convenience and prior to the testing close date.

New lots of test kits or controls are entered into the Reagent Log.

13. SYSTEM FAILURE OR INOPERABILITY

If Xpert NG/CT (NAATs) cannot be performed the Medical Director and Director of Nursing must be immediately informed so that STI Clinic practice can be modified to accommodate this situation.

Patients suspected of being exposed to or infected with Chlamydia trachomatis or Neisseria gonorrhea may need to be referred to other providers for appropriate testing and treatment.

14. XPERT NG/CT APPENDIX-REFERENCES

- 400-001-11-01-A_Xpert CT-NG Product Insert
- 400-001-11-02-A_Xpert Swab Specimen Collection Kit Product Insert
- 400-001-11-03-A_Xpert Urine Specimen Collection Kit Product Insert
- 400-001-11-04-F_Xpert Patient Log
- 400-001-11-05-F_Xpert Quality Control Log
- 400-001-11-06-F_Xpert Monthly Log
- 400-001-11-07-A_Operator Manual (Reference)
- 400-001-11-08-P_Individualized Quality Control Plan (Reference)
- 400-001-11-09-A_Xpert Unique ID



XPert® TV NUCLEIC ACID AMPLIFICATION TEST (NAAT) FOR TRICHOMONAS VAGINALIS (TV)

Below is information excerpted from the Xpert TV Product Insert (400-001-12-01-A_Xpert TV Product Insert) and the Cepheid Xpert Operational Manual (400-001-11-07-A_Operator Manual). Refer to these and other cited documents for reference and further details. The excerpt is not intended to be all inclusive, but rather to provide the basic information necessary to run the Cepheid Xpert TV Assay.

4. MATERIALS REQUIRED

Xpert TV Assay materials as described in the product insert.

Xpert TV cartridges with integrated reaction tubes and 1mL pipette stored at 2 - 28°C.

CD

- Assay Definition Files (ADF)
- Instructions to import ADF into GeneXpert software
- Instructions for Use (Package Insert)

Primary samples must be collected with the appropriate kit:

- Xpert Urine Specimen Collection Kit

Bleach, 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution used to make a 1:10 cleaning solution

70% alcohol at a minimum for quarterly probe cleaning

70% denatured ethanol for quarterly cleaning and/or 70% pure alcohol wipes for daily cleaning

Biohazard waste container w/ biobags

Lab markers

Appropriate PPE

5. PATIENT PREPARATION, SAMPLE COLLECTION, AND RELATED REQUIREMENTS

- The patient will be examined and evaluated, and the procedure explained as per CCPH protocol. The clinician will ask for urine samples to be collected in a provided sample container. Samples will be labeled with the patient's unique ID and date of birth and transported to the lab. (see 400-001-11-09-A_Xpert Unique ID for further info on labeling) Urine specimen that is transferred to the Xpert Urine Transport Reagent tube can be stored up to 28 days at 2 °C to 8 °C, or up to 14 days at 15 °C to 30 °C before testing with the Xpert TV test. Urine that is not in a transport tube can be stored for 4 days at 2 °C to 8 °C or 4 hours at 15 °C to 30 °C. Storage of samples is not affected by patient gender.

Once the specimen is in the lab all pertinent information is entered into the patient TV lab logbook. It is examined for suitability and acceptance, as there is no referral for NAAT tests-recollection is the only recourse for unacceptable specimens. Urine samples must be of sufficient sample volume

to allow testing – 7.0 ml is required to be transferred into specimen tube. (Note: Urine must be in a transport tube for testing) Lack of any of these is a basis for sample Rejection, which will be noted in the logbook along with an explanation as to why. Do not discard the specimen prior to consulting with the clinician for the sample status. This permits the clinician to clarify any potential concerns as well as make possible exceptions which must be clearly documented.

6. MICROSCOPIC EXAMINATION AND PROCEDURE

There is no microscopic examination for the NAAT test procedure; it is a macroscopic technique.

Note: This procedure utilizes potentially infectious patient specimens. Gloves, eye protection, and protective clothing are required.

Before starting these procedures, make sure that the instrument is running 6-color GeneXpert Software Version 4.3 or higher.

Important: Start the test within 30 minutes of adding the sample to the cartridge.

Preparing the urine transport tube

1. Ensure urine is well mixed in the urine cup before transferring aliquot to the Xpert Urine Transport Reagent tube.
2. Obtain one Xpert Urine Specimen Collection Kit.
3. Open the packaging of a disposable transfer pipette provided in the collection kit. Note: do not use the pipette from the assay kit.
4. Insert the transfer pipette into the bottom of the urine cup.
5. Transfer approximately 7 mL of urine so that the volume reaches the black dashed line on the tube label.
6. Replace the cap on the tube and tighten. Label with patient unique ID and patient number (date required if sample is not tested on the same day as collection).

Preparing the cartridge

To add the sample to the Xpert TV Assay cartridge:

1. Inspect the test cartridge for damage. If damaged do not use.
2. Open the cartridge lid. **Note:** Do not open a cartridge until ready to test. Use cartridges within 30 minutes after opening the lid.
3. Gently invert the transport tube 3 to 4 times to ensure adequate mixing of sample and transport matrix.

4. Unwrap the transfer pipette.
5. Remove the transport tube cap, compress the bulb of the transfer pipette, insert the pipette into the transport tube and release the bulb to fill the transfer pipette up to the mark (500 μ l) on the pipette shaft. See Figure 1. Ensure the pipette is filled with no air bubbles present.

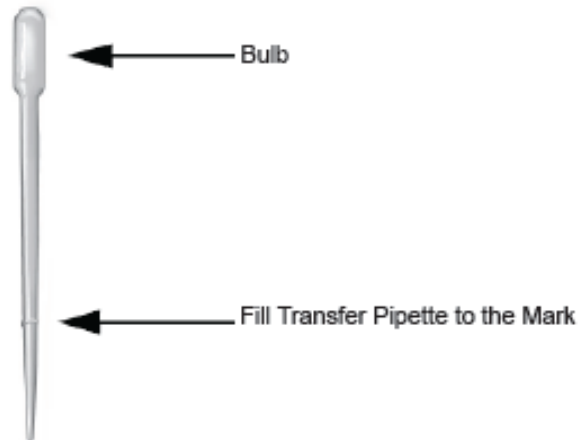


Figure 1: Transfer pipette and fill mark

6. Empty the pipette's content into the Sample chamber of the cartridge (See Figure 2)

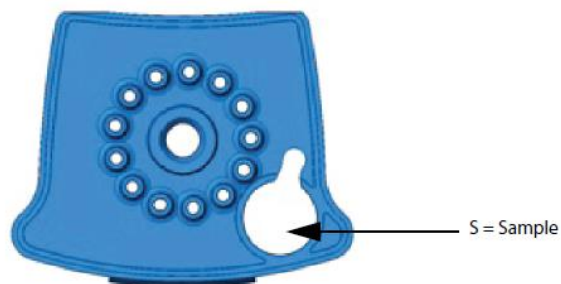


Figure 2: Chambers shown from top of cartridge.

7. Close the cartridge lid and retain the sample until testing is completed.

Starting the Test

Note: Before you start the test, make sure that the system is running GeneXpert 4.3 software or higher and that the Xpert TV Assay Definition File is imported into the software.

NOTE: Do not load a GeneXpert cartridge that has been dropped or shaken after the lid of the cartridge has been opened. Dropping or shaking the cartridge after it has been opened can cause invalid results. Bent or broken reaction tubes can also produce invalid results. Do not re-use spent cartridges.

NOTE: Always pick up the cartridge by its body. Do not pick up the cartridge by the protruding reaction tube (See Figure 3)



Figure 3: Cartridge Showing Body and Reaction Tube

This section lists the default steps to operate the GeneXpert System. For detailed instructions, see the *GeneXpert Dx System Operator Manual*.

11. Turn on the GeneXpert Instrument System:

Using the GeneXpert Dx instrument, first turn on the instrument and then turn on the computer. The GeneXpert software will launch automatically.

12. Log on to the GeneXpert System software using the username and password.

13. In the GeneXpert System window, click **Create Test**. The **Create Test** window appears.

14. Manually type the Sample ID, make sure the ID is typed correctly. The ID is associated with the test results and is shown in the **View Results** window.

15. Scan the barcode on the Xpert TV Assay cartridge. Using the barcode information, the software automatically fills the boxes for the following fields: Reagent Lot ID, Cartridge SN, and Expiration Date.

Note: If the barcode on the Xpert TV cartridge does not scan, then repeat the test with a new cartridge and provide cartridge to supervisor.

16. Click **Start Test**.

- i. Open the instrument module door with the blinking green light and load the cartridge.
- ii. Close the door. The test starts and the green light stops blinking. When the test is finished, the light turns off.

- iii. Wait until the system releases the door lock before opening the module door and removing the cartridge.
- iv. Dispose of used cartridges in the appropriate biohazard specimen waste containers.

17. Viewing and Printing Results. This section lists the basic steps for viewing and printing results. For more detailed instructions on how to view and print the results, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

1. Click the **View Results** icon to view results.
2. Upon completion of the test, click the **Report** button of the View Results window to view and/or generate a PDF report file.

18. Retests-Reasons to Repeat the Assay

If any of the following test results occur, repeat the test according to instructions in the Retest Procedure.

- An **INVALID** result indicates that the SPC and/or the SAC failed. The sample was not properly processed, PCR was inhibited, or the sample was not properly collected.
- An **ERROR** result indicates that the test failed possibly because the reaction tube was filled improperly, a reagent probe integrity problem was detected, pressure limits were exceeded, or a valve positioning error was detected.
- A **NO RESULT** indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

19. Retest Procedure

Obtain the leftover treated sample from the Urine Transport Reagent tube. Repeat the test with a new cartridge. If the leftover treated sample volume is insufficient, or the retest continues to return an INVALID, ERROR, or NO RESULT, collect a new sample and repeat the test with a new cartridge.

15. MATERIAL PREPARATION

All necessary materials should be assembled before testing. The test kits and testing related materials and equipment should all be at room temperature (15-30° C) prior to performing the test. Follow the package insert and training material for all material preparation handling and storage procedures.

16. CALIBRATION

There are no user calibrations that can be performed on the system components.

17. REPORTABLE RANGE

The reportable ranges for TV are Not Detected (Negative) and Detected (Positive).

Negative means that the genetic material of select organism was not detected.

Positive means that the genetic material was detected.

The results are interpolated by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms and will be shown in the View Results window.

Additional information can be found in the package insert.

18. CONTROL PROCEDURES

In order to:

- (1) Detect immediate errors that occur due to test system failure, adverse environmental conditions, and operator performance, and
- (2) Monitor over time the accuracy and precision of test performance that may be influenced by changes in test system performance and environmental conditions, and variance in operator performance, the following control procedure will be used:

Each test includes a Sample Processing Control (SPC), a Sample Adequacy Control (SAC) and a Probe Check Control (PCC).

- **Sample Processing Control (SPC)**—Ensures the sample was correctly processed. The SPC contains genomic DNA of *Bacillus globigii* that is included in each cartridge. The SPC verifies that binding and elution of target DNA have occurred if the organisms are present and verifies that sample processing is adequate. Additionally, this control detects sample associated inhibition of the real-time PCR assay. The SPC should be positive in an analyte negative sample and can be negative or positive in an analyte positive sample. The SPC passes if it meets the validated acceptance criteria.
- **Sample Adequacy Control (SAC)**—Ensures that the sample contains human cells or human DNA. This multiplex assay includes primers and probes for the detection of a single copy human gene. The SAC signal is only to be considered in an analyte negative sample. A negative SAC indicates that no human cells are present in the sample due to insufficient mixing of the sample or because of an inadequately taken sample.
- **Probe Check Control (PCC)**—Before the PCR reaction starts, the GeneXpert instrument measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. PCC passes if it meets the validated acceptance criteria.
- **External Controls**— The manufacturer states “positive and negative external controls should be used in accordance with local, state, and federal accrediting organizations’ requirements as applicable.” Per CLIA guidelines without an Individualized Quality Control Plan Risk Assessment (IQCP) controls must be completed on each new lot/shipment and each day of testing patient specimens. This requirement can be minimized based on an IQCP to using controls on each new lot/shipment and monthly following manufacturer’s instructions.

19. CORRECTIVE ACTION

See procedure Step 9 for Retest information.

If a test run fails – the run is flagged by the software- the test procedure should be reviewed, all testing equipment checked for proper functioning, all reagents checked for acceptability, and the testing area examined as to environmental conditions and apparent cleaning and decontamination. If all appears correct the test should be re-run. If the problem persists discontinue testing immediately, notify the Supervisor and the Clinical Manager.

20. LIMITATIONS OF PROCEDURE

See package insert and training manual for lengthy list of limitations.

21. EXPECTED RESULTS AND NORMAL VALUES

Negative results for TV are expected in healthy individuals who have not been exposed to the organism.

22. PANIC OR ALERT VALUES

There are no Panic or Alert Values.

23. DATA ENTRY AND REPORTING

- Patient results are obtained from the Cepheid Xpert result log and documented onto the patient result log (400-001-12-02-F_Xpert TV Patient Log). Patient results are provided to the Nursing Division by transcribing the results from the log onto the CCPH Lab Slip, ensuring all fields are completed.

Proficiency Testing specimens are recorded in the patient log as if they were an actual patient.

Results are then transcribed into the Proficiency Testing Providers Electronic Reporting System at the earliest convenience and prior to the testing close date.

New lots of test kits or controls are entered into the Reagent Log.

24. SYSTEM FAILURE OR INOPERABILITY

If Xpert TV (NAATs) cannot be performed the TV Program Coordinator must be immediately informed so that clinical practices can be modified to accommodate this situation.

25. XPERT TV APPENDIX

- 400-001-12-01-A_Xpert TV Instructions for Use
- 400-001-12-02-F_XpertTV Patient Log
- 400-001-12-03-F_Xpert TV Quality Control Log
- 400-001-11-07-A_Operator Manual
- 400-001-11-09-A_Xpert Unique ID

F CITATIONS AND REFERENCES

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G. CONTRIBUTORS

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3. Heather MacDonald, Laboratory Technician
4. Julie Carman, Laboratory Technician

H. APPENDICIES & ATTACHMENTS

1. 400-001-01 GRAM STAINS
 - 400-001-01-03-P_Gram Stain Reagent Preparation
 - 400-001-01-04-A_Product Insert Fisher Gram-Check control slides
2. 400-001-02-P_WET PREPARATIONS
 - 400-001-02-01-P_Wet Prep Reagent Preparation
3. 400-001-03-P_PREGNANCY (HCG)
 - 400-001-03-01-A Urine hCG strip pkg insert
4. 400-001-04-P_HIV-ORAUQUICK ADVANCE HIV ½
 - 400-001-04-05-A_OraQuick Advance Rapid HIV ½ Product insert
 - 400-001-04-06-A_OraQuick Controls product insert
 - 400-001-04-07-A_INSTI HIV-1/HIV-2 Antibody Test Kit Product insert Version (51-1080 (I) rev 02/19)
 - 400-001-04-08-A_INSTI Controls product insert



- 400-001-04-09-A_INSTI Quick Reference Guide
5. 400-001-05-P_RPR-SYPHILIS
- 400-001-05-02-P_RPR Reagent Preparation
 - 400-001-05-04-A_RPR Card Test Controls Package Insert
 - 400-001-05-05-A_RPR Antigen Package insert
 - 400-001-05-06-A_Syphilis Dilution Sheet
 - 400-001-05-09-A_Syphilis Health Check Package Insert
 - 400-001-05-10-A_HealthCheckQuickReference
 - 400-001-05-11-A_HealthCheckControl Package Insert
 - 400-001-05-07-A_RPR Card Tests Product Insert
 - 400-001-05-14-P_Procedure for serum separator tubes
 - 400-001-05-15-A_Syphilis Special Reporting Requirements
6. 400-001-08-P_BLOOD LEAD
- 400-001-08-01-A_LeadCare II Blood Lead Test Kit package insert
 - 400-001-08-02-A_Steps for Collecting Fingerstick Blood Samples in Micro-Vials for Lead Testing
 - 400-001-08-03-P Canton City LeadCare Reporting Software Users Guide
 - 400-001-08-06-A_Blood Lead II Users Guide
7. 400-001-09-A_GENERAL ATTACHMENTS
- 400-001-09-01-P_Electronic Laboratory Reporting Procedure
 - 400-001-09-02-A_Reference Range and Results Interpretation
 - 400-001-09-03-A_Lab Slip Legend
 - 400-001-09-05-A_Reference Lab CLIA Licenses
 - 400-001-09-06-P_Shipping Policy
8. 400-001-10-P_HCV RAPID ANTIBODY TEST
- 400-001-10-02-A_OraQuick HCV Rapid Antibody Test Product insert
9. 400-001-11-P_XPERT NG/CT
- 400-001-11-01-A_Xpert CT-NG Product Insert
 - 400-001-11-02-A_Xpert Swab Specimen Collection Kit Product Insert
 - 400-001-11-03-A_Xpert Urine Specimen Collection Kit Product Insert
 - 400-001-11-07-A_Operator Manual



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10. 400-001-12-P_XPERT TV

- 400-001-12-01-A_Xpert TV Instructions for Use
- 400-001-11-07-A_Operator Manual

I. REFERENCE FORMS

1. 400-0001-01 GRAM STAINS

- 400-001-01-01-F_Gram Stain Quality Control Log
- 400-001-01-02-F_Stat Lab Log Sheet

2. 400-001-02-P_WET PREPARATIONS

- 400-001-02-02-F_Wet Prep QC Log

3. 400-001-03-P_PREGNANCY (HCG)

- 400-001-03-03-F_hCG Patient Log
- 400-001-03-04-F_hCG QC Log

4. 400-001-04-P_HIV-ORAQUICK ADVANCE HIV ½

- 400-001-04-01-F_HIV Room Temp Log
- 400-001-04-03-F_HIV Patient Log
- 400-001-04-04-F_OraQuick QC Log

5. 400-001-05-P_RPR-SYPHILIS

- 400-001-05-01-F_RPR QC Log
- 400-001-05-03-F_RPR Patient Log
- 400-001-05-08-F_Syphilis Health Check Control Log

6. 400-001-08-P_BLOOD LEAD

- 400-001-08-04-F_Blood Lead Testing System Data Entry Log
- 400-001-08-05-F_Blood Lead Testing Requisition and Reporting Form

7. 400-001-09-GENERAL FORMS

- 400-001-09-04-F_Control and reagent log.doc

8. 400-001-10-HCV RAPID ANTIBODY

- 400-001-10-01-F_HCV Patient Log



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9. 400-001-11-P_XPERT NG/CT

- 400-001-11-04-F_Xpert Patient Log
- 400-001-11-05-F_Xpert Quality Control Log
- 400-001-11-06-F_Xpert Monthly Log

10. 400-001-12-P_XPERT TV

- 400-001-12-02-F_XpertTV Patient Log
- 400-001-12-03-F_Xpert TV Quality Control Log

K. APPROVAL

This document has been approved in accordance with the “800-001-P Standards for Writing and Approving PPSOGFs” procedure as of the effective date listed above.