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:	04/25/2016
LATEST EFFECTIVE DATE:	03/12/2018
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 Health Department 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 Health Department Laboratory (CCHDL) 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

CCHDL 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

CCHDL – Canton City Health Department Laboratory
CLIA - Clinical Laboratory Improvement Amendments of 1988.
EMR - Electronic Medical Record System
ODH – Ohio Department of Health

E. PROCEDURES & STANDARD OPERATING GUIDELINES

Table of Contents

The CCHDL 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 CCHDL 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).*

INSTRUCTIONS FOR SPECIMEN COLLECTION

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

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 patients name is hyphenated, use only the first initial of the first part of the last name: FL010292. If the patient's last name is two parts without a hyphen, use both initials from the last name: FLL010292. Note that use of the patient name alone on the specimen is inadequate to uniquely identify the specimen. Note that microscope slides submitted for identification purposes are considered primary specimen containers and must be labeled appropriately.

TEST REQUESTS

All patient specimens submitted to the CCHLD for testing must be accompanied by an appropriately completed test requisition. Tests requisitions will be submitted electronically using the Canton City Electronic Medical Record System (EMR) unless the system is not in use during specimen collection. If a test is requested when the EMR is not in use, Submitters should use a test requisition form.

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 unique 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 exactly those provided on the specimen container/tube/slide; any discordance will result in the specimen being held prior to testing in order 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 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 CCHDL 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.

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 CCHDL in a satisfactory state for testing. For detailed information on appropriate pre-processing, storage, packaging and shipping of samples for submission to the CCHDL, 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 or Ziploc bags) 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.

COURIER SERVICE

The CCHDL utilizes department staff to transport specimens to local reference laboratories and the Ohio Department of Health's courier for specimens sent to the Ohio Department of Health Laboratory. 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 in order 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)

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., QNS);
- No or illegible patient name or other 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. 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 CCHDL, as appropriate:

- DOB or age, if appropriate;
- DOC;
- Sex;
- Address of submitter;
- Test requested;
- Source of specimen, if appropriate.

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 CCHDL, as appropriate. Changes will be documented in the Lab Orders and Results Log in the Quality Assessment and Assurance Policy binder.

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) in order 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 CCHDL 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 performed at the CCHDL 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.

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

GRAM STAIN

1. MATERIALS REQUIRED

Glass microscope slides (one end frosted)
Gram stains: Crystal Violet, Gram Iodine, Safranin
Staining tray or rack
Decolorizer (acetone, or acetone/alcohol mixture)
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 CCHD STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CCHD 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:

PERFORMING THE GRAM STAIN

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); 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 Coplan 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 Coplan 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 the alcohol/acetone solution, 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 Coplan 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

5. 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.
6. 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.

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

4. 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 a 50/50 mixture of 95% Denatured Ethanol and Acetone; 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.

5. CALIBRATION PROCEDURES

There are no calibrations performed for this procedure.
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:

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.

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:

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 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 over time trends or concerns in the test system performance.

8. 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 of E. coli and S. epidermidis 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.

9. 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.

10. 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 anything else is that clinically significant.

11. PANIC OR ALERT VALUES

There is no Panic or Alert Value.

12. 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 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. Send a "To Do" to the Lab Results group when the final test of the clinic day is completed so that the clinician or Medical Director through the use of 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.

13. 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. GC culture confirmation must be modified until full capability is regained.

14. 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 CCHD STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CCHD 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 so 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:

PERFORMING THE VAGINAL WET PREPARATION

1. The tube is swirled in order 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 CCHD clinical staff observed
WBCs: the average number of White Blood Cells observed in 10 High Power Fields; $\leq$ 30 OR > 30.

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 Medical Director and Director of Nursing must be informed at once so that STI clinic operations may be adjusted to allow for the suspension of testing; and
3. The Medical 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): $\leq$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 and the STAT Lab 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. 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. 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 Medical Director and Director of Nursing must be informed so that STI Clinic practice can be modified to accommodate this situation. An 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)

1. MATERIALS REQUIRED

   hCG test kit (Sure-Vue Urine hCG), which includes
   - Test strips
   - Package insert

   Timer (at minimum capable of a 3-minute interval)
   Urine collection containers – screw-top
   hCG Urine control kit which includes
   - 2 levels of activity
   - Package insert

   Appropriate PPE

2. PATIENT PREPARATION, SAMPLE COLLECTION, AND RELATED REQUIREMENTS

   The patient will be taken into a CCHD STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CCHD 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 may 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.

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

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 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.

12. DATA ENTRY AND REPORTING

The results of hCG Control runs are entered into the hCG 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 are entered into the hCG 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. 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. 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.
Proficiency Testing specimens are recorded in the patient hCG 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 hCG Pregnancy tests cannot be performed the Medical Director and/or Director of Nursing 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_SureVue Urine hcg strip pkg insert
- 400-001-03-02-A_Urine Controls pkg insert
- 400-001-03-03-F_hCG Patient Log
- 400-001-03-04-F_hCG QC Log

HIV-1/2 ANTIBODY PROCEDURE

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 CCHD STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CCHD 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. 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.
   Samples for confirmatory testing must have a minimum of 0.5 ml of serum or plasma (usually collected in a serum separator yellow top tube). Specimen must arrive at the reference laboratory within 7 days of collection. (http://www.odh.ohio.gov/pdf/idcm/sect4toc.pdf)

3. MICROSCOPIC EXAMINATION AND PROCEDURE

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

PROCEDURE

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).

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

Follow Control Package Insert (400-001-04-06-A) and record all quality results in the HIV QC log (400-001-04-04-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 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. The HIV Patient Log form includes an OPSCAN line which necessitates first confirming that the OPSCAN number entered into 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. 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 HIV 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.

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
    - 400-001-04-02-F_HIV Confirmatory 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 Product insert Version (3001-1215 rev 03/16B)
- 400-001-04-06-A_OraQuick Controls product insert

SYPHILIS-RAPID PLASMA REGAIN (RPR)

1. MATERIALS:

   RPR antigen suspension, in dispensing bottle w/ 20ga. unbeveled siliconized needle
   RPR Dispenstirs 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 CCHD STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CCHD 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"). The tube will be labeled with the patient's unique ID and clinic number. It will be brought back 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.
   Once the specimen has been received into the STAT lab all pertinent information is entered into the STAT lab logbook. 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 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. 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 5-10 minutes at a speed setting of 8 to separate the serum or plasma from the cells.
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, unless otherwise specified by the Medical Director or clinic staff.

QUALITATIVE PROCEDURE

4. Place 50 µl of serum or plasma onto an 18-mm circle of the RPR test card, using a disposable Dispenstir held vertically.
5. Using the inverted Dispenstir, 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 these 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.
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 µl of 0.9% saline in circles numbered 2 through 5. Do not spread the saline.
2. Again, using the pipettor place 50 µl of serum in circle 1 and 50 µl 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 µl from circle 2 (1:2) to circle 3, and mix as above.
5. Transfer 50 µl from circle 3 (1:4) to circle 4, and mix.
6. Transfer 50 µl from circle 4 (1:8) to circle 5 (1:16), mix; discard the last 50 µl.
7. Using the flat end of a clean Dispenstir spread the diluted serum to fill the entire surface of the circle, starting with the highest dilution (circle 5, 1:16). Using the same Dispenstir, 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 µL) 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:

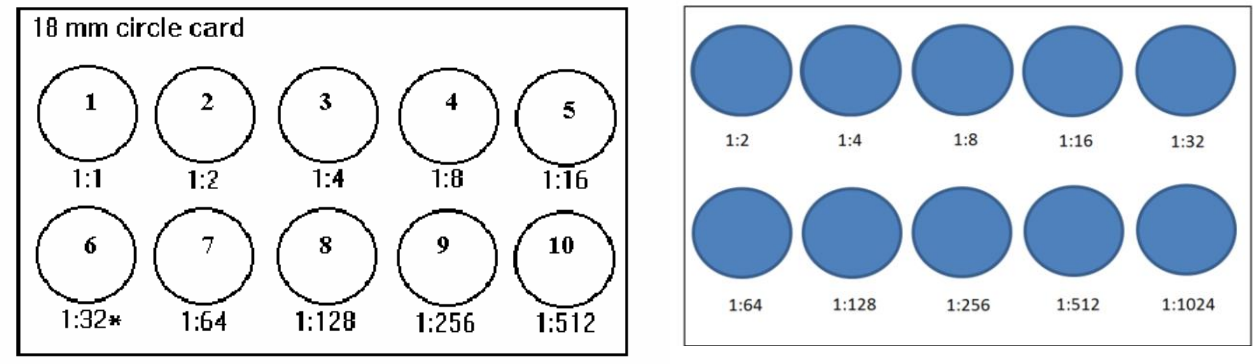
1:32 through 1:1024

13. 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.
14. Prepare a 1:16 dilution of the test specimen by adding 0.1ml of serum to 1.5ml of 0.9% saline. Mix thoroughly.
15. Place 50 µl of the 1:50 nonreactive serum diluent in labeled circles on the RPR card with the pipettor.
16. Using the pipettor place 50 µL of the 1:16 test specimen dilution into circle 6.
17. 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.
18. 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

19. 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.
20. Using a pipettor place 50 µl of 0.9% saline in circles numbered 2 through 5. Do not spread the saline.
21. Place 50 µl of the 1:50 nonreactive serum diluent in circles 6 through 10 of the RPR card with the pipettor.
22. Again, using the pipettor place 50 µl of serum in circle 1 and 50 µl of serum into the saline in circle 2.
23. 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.
24. Transfer 50 µl from circle 2 (1:2) to circle 3, and mix as above.
25. Transfer 50 µl from circle 3 (1:4) to circle 4, and mix.
26. Transfer 50 µl from circle 4 (1:8) to circle 5 (1:16), and mix.
27. Transfer 50 µl from circle 5 (1:16) to circle 6 (1:32), and mix
28. 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.

29. Using the flat end of a clean Dispenstir, starting with the highest dilution spread the diluted serum to fill the entire surface of the circle, (circle 10, 1:512). Using the same Dispenstir, 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.
30. Gently agitate the dispensing bottle to re-suspend the antigen particles.
31. 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.
32. Place the card on the rotator under the humidifying cover and rotate the card for 8 minutes at 100 ± 2 rpm.
33. 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.
34. 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, Dispenstirs, 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.

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 ± 1 drops per 0.5 ml; 17 µl 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 ± 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 ± 15 seconds of the set time.
(4) 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. This is verified every 12 to 18 months.

6. 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.

7. CONTROL PROCEDURES

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 for specific instructions on use and interpretation.

A Reactive control, capable of a minimum of a 1:4 reaction, must be used when a Reactive patient specimen is encountered and requires quantitation.
Refrigerator temperatures must be check and recorded daily.
Ambient (room) temperatures must be check and recorded each day of testing.
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.

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 ± 1 drops per 0.5 mL; 17 µL per drop). Replace the needle if it does not meet this specification. Be sure to test the calibration of the replacement needle.

When changing lots of antigen:

Each new lot number of antigen is run in parallel with old lot numbers and results recorded side by side in Q.C. worksheet.

The RPR reagents from the new and the reference (previous) lots are tested utilizing materials capable of providing reactive and nonreactive results, 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 in parallel, using new and reference (old) reagents.
2. Read and record test results.
3. Compare the results obtained with reference and new reagents. Determine whether new RPR reagents meet the criteria of acceptability.
4. If results between reagent lots are discordant, additional testing may be necessary.
5. If the new antigen gives the established reactivity, further testing can continue.

8. 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.

9. 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. 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. 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 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.

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) based on the three possibilities below. In all cases the ODRS case ID should be included in a "To Do" to the DIS or their supervisor, whomever has access to the EMR. This does not have to be done during clinic time, but must be done as soon after clinic as is reasonably possible.

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

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

GC CULTURE AND CONFIRMATION

1. MATERIALS REQUIRED

Plates of prepared culture media:
- a. Modified Thayer Martin (MTM) agar or Martin-Lewis (ML) agar
- b. Chocolate agar

Sterile inoculating devices:
- a. Disposable cotton swab and/or
- b. Disposable inoculating loop/needle

Oxidase reagent

Gram Staining materials (see also Gram Stain Procedure):
- a. Gram stains
- b. Decolorizing solution
- c. Rinse water
- d. Glass microscope slides
- e. Bibulous paper
- f. Alcohol lamp or other heat source

API NH system for the confirmation of Neisseria and Haemophilus
Supplemental ZYM B Reagent

Candle jars with candles

Incubator maintained at 35-37° C

Control cultures as appropriate for media and oxidase quality control *(N. gonorrhea, H. influenzae, H. paraphrophilus, E. coli, S. epidermidis,* etc.)

Biohazard waste container

Appropriate Personal Protective Equipment

2. PATIENT PREPARATION, SAMPLE COLLECTION, AND RELATED REQUIREMENTS

The patient will be taken into a CCHD STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CCHD STI Clinic protocol. Cervical (C), Oral/pharyngeal (O), Rectal (R), or Urethral (U) specimens will be collected by the clinician and inoculated onto a prepared plate of selective culture media (Martin-Lewis (M-L) or Modified Thayer Martin (MTM). The plate must be minimally labeled with a patient unique ID, patient number and collection site and have visible evidence of being swabbed. If neither of these items are present, then grounds for rejecting the sample exist. 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

The plates are then placed in a candle jar and the candle lit to decrease the oxygen content and increase the carbon dioxide content of the jar. The jar is maintained in the Nursing Preparation area of the STI clinic until it is transported to the CCHD Laboratory. Other culture plates may be added to the jar as specimens are obtained from patients, re-candling after each addition. There should not be more than 10 plates per jar to minimize the possibility of prematurely extinguishing the candle. When a jar is transported to the CCHD Laboratory the culture plates must be able to be matched with the corresponding orders for each plated patient specimen.

Once the culture plates are in the Laboratory ensure all pertinent information is entered into the GC Culture logbook. In the instance that the CCHD Laboratory is not able to perform N. gonorrhea cultural procedures the specimens may be referred to an outside laboratory (e.g. Aultman Laboratory Services) provided sample acceptance criteria are met.

Culture viability can be maintained for 48 hours after inoculation; viability after that time can be more problematic. Re-subbing the original culture onto a new plate of prepared culture media (non-selective or selective) can extend culture viability in cases where additional examination and analysis is indicated. In cases such as these the subbed culture plates need to be marked with patient identifiers, original culture site, and date of re-sub.

Long term storage of cultures or isolates of patient cultures are beyond the capabilities of the Canton City Health Department laboratory.

3. MICROSCOPIC EXAMINATION AND PROCEDURE

A Gram stained microscope slide prepared from a patient's specimen, properly examined by the laboratory staff, will assist in determining what course should be pursued in the analysis of the culture. The desired result is the observation of a pure culture of Gram Negative Diplococci (small, red-staining, pairs of spherical bacteria with the opposing sides slightly flattened – like "paired kidney beans") isolated from a patient's culture plate. This indicates that the analysis can proceed to the Confirmatory phase. An observation of a mixed flora from the patient's culture plate would require that additional passages on selective media be performed to arrive at a suitably pure culture so that the analysis can proceed.

NEISERRIA GONORRHEA CULTURAL PROCEDURE

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

Initial incubation:

Inoculated culture will be received from the STI clinic in candle jars.

1. Record all required information in the GC Culture logbook for each plate submitted.
2. Place no more than 10 culture plates in the candle jar(s), re-light the candle, place the lid on

the jar(s), and allow the candle(s) to burn out.

3. Incubate the jar(s) at 35-37° C for approximately 24 hours.

Initial Examination

Presumptive Identification:

1. Remove the jar(s) from the incubator, remove the lid(s), and examine the culture plates for Acceptance: properly labeled; accompanied by a completed lab slip or other documentation; not cracked, damaged, or broken; no evidence of previous freezing, dehydration, or prior microbial contamination; and evident marks on the agar surface indicating that a specimen was actually inoculated onto the plate. If specimen does not meet these criteria Reject sample. Rejection- the specimen must be re-collected. Rejected samples will be documented in the patient specimen log along with the reason for rejection.
2. Examine the plates for growth and Record initial observations in the GC Culture logbook. Utilize the description guide on the bottom of the log for a documentation key.
3. Apply drops of Oxidase Reagent to representative colonies of each type observed. Colonies exhibiting a red/violet color development which progresses to black are Oxidase Positive; no color development demonstrates that the colonies are Oxidase Negative- return these to incubation along with plates showing No Growth. Set any Oxidase Positive culture plates aside by themselves. Record your observations in the GC Culture logbook.
4. Make Gram stains of the Oxidase Positive colonies (refer to Gram Stain Procedure elsewhere in this manual). The colored colonies may be used as the Oxidase reagent does not affect bacterial morphology or Gram reaction. Culture plates supporting colonies which contain Gram Negative Diplococci, either pure or mixed with other flora, should be set aside. These specimens are considered presumptive at this point. Record your observations in the GC Culture logbook. All other cultures may be returned to incubation.
   NOTE: Steps 3 and 4 may not be able to be completed due to insufficient growth, continue incubation as described in step 6 below.
5. Sub representative colonies of any Oxidase Positive, Gram Negative Diplococci organisms onto fresh plates of media. Do not use the colonies that were treated with Oxidase reagent, as it is toxic to GC. Apparent pure colonies may be subbed onto Chocolate agar (non-selective); M-L or MTM (selective) should be used for mixed colonies. Streak the plates so as to produce isolated colonies. These will be used for Confirmatory testing of the suspect colonies. If lush growth is present at 24 hours, after subbing as described in this step, the initial plate may be used for immediate Confirmatory testing.
6. Place the culture plates into candle jars, re-light the candles, and incubate all culture plates at 35-37° C for an additional 24 hours. Subbed plates for confirmation testing only require 18 to 24 hours of growth.

Subsequent examination*:*

1. Remove the candle jars from the incubator and examine the culture plates. Those plates which were previously No Growth, Oxidase Negative, or Oxidase Positive-Not Gram Negative Diplococci should be re-examined according to the Initial examination-Presumptive Identification procedure above. If any presumptive colonies have developed during this incubation they should be re-subbed to the appropriate media and incubated for an additional 24 hours prior to Confirmatory testing.
2. If growth is suspected, but colonies are not visible to the naked eye the following step may be used. Flood the plates with Oxidase reagent; color development will reveal the presence of pinpoint colonies which may not have been apparent to the unaided eye. Gram stain any which appear. While these organisms are now dead and cannot be used for Confirmatory testing, any Oxidase Positive- Gram Negative Diplococci organisms thus revealed may be reported as Presumptive for N. gonorrhea. Any growth giving Presumptive results, or indefinite results on the presumptive tests, needs to be brought to the attention of the clinical personnel for a possible re-call and re-testing of the patient. Record all observations in the GC Culture logbook and dispose of the cultures as biohazardous waste.

CONFIRMATORY TESTING:

1. Examine the culture plates which were developed by subbing suspected presumptive colonies. If the growth appears to be uniform, unmixed colonies it may be used for confirmatory testing. Apply a drop of Oxidase reagent to ensure that it is still Oxidase positive. If the growth appears mixed it will next to be re-subbed onto a new plate of selective agar to attempt to come up with a pure culture.
   Note: When more than one culture plate for the same patient during the same clinic are processed and two or more are found to have gram negative diplococci follow the testing protocol below:
   1. First perform sugar confirmation on only one of the cultures, utilizing the one that morphologically is most consistent with GC.
   2. If the sugar confirmation is positive do Not perform a sugar confirmation on the other culture/s. The other samples will be reported out as oxidase positive and gram negative diplococci presumptively positive for N gonorrhea. And the confirmed specimen will be reported per the normal reporting protocol.
   3. If the first sugar confirmation is negative, then the second specimen will need to be processed for confirmatory testing. Report and record accordingly.
2. Assemble the components of the API NH test system in the work area and allow them to come to room temperature:
   a. API NH test strip

b. Supplemental ZYM B if necessary, depending on expiration date of opened container
c. 2ml. ampoule of 0.85% NaCl suspension medium
d. Incubation box (tray and lid)
e. Results sheet
f. Sterile disposable swab or loop
g. 4.0 McFarland standard
h. Disposable transfer pipet
i. Mineral oil
j. Timer

3. Follow manufacturer's instructions as described in the API NH Test Kit.
4. Enter this onto the Results Sheet along with all other pertinent data. Also, make all appropriate entries in the GC Culture Logbook.

4. MATERIAL PREPARATION

A. Oxidase test and reagent

All species of Neisseria produce an enzyme which oxidizes aromatic amines. The presence of this enzyme can be a presumptive indicator of Neisseria. To perform the test, prepare a 1% solution of *N*, *N-Dimethyl-p-phenylenediamine oxalate* (note: about 10ml. is usually sufficient for a week's work load). Dissolve 0.1g of the dried reagent in 10.0ml of Deionized water. Several minutes and some moderate agitation may be required for complete dissolution. A drop of the reconstituted solution is placed on each of several selected representative colonies which are then observed over several minutes for the typical red-to-violet-to-black color change of an oxidase-positive organism.

The reagent bottle should be labeled as to contents, lot number, date of preparation, date of expiration, storage conditions, and any other pertinent information. Oxidase reagent may be kept for one week after preparation if it is kept under refrigeration (2-8°C) between uses and in the dark. If the reagent develops excessive color or precipitate discard it and prepare new. Do not use the reagent past its expiration date.

Warning: N, N-Dimethyl-p-phenylenediamine oxalate causes skin and eye irritation. It may be fatal if swallowed. In case of skin contact flush with plenty of water for at least 15 minutes while removing contaminated clothing. In case of eye contact flush eyes with plenty of water for at least 15 minutes, occasionally lifting upper and lower eyelids. Get medical attention immediately. In case of ingestion, give 2-4 cupsful of milk or water. Get medical attention immediately.

B. JAMES and ZYM B reagents

1. James reagent is reconstituted by following the manufacturer's instructions. The reagent can be used for 30 days (or until expiration date if that comes first) after

reconstitution. It must be stored at 2-8°C between uses. James reagent is very sensitive to light: store in the dark, and wrap aluminum foil around bottles of reconstituted reagent. Approximately 1 hour of exposure to laboratory lighting can damage the reagent.

Label reconstituted bottles with date of reconstitution or expiration date. James reagent is normally pale yellow; do not use if it is any other color.

Warning: In case of contact with eyes, rinse immediately with plenty of water for at least 10 minutes. In case of contact with skin, wash with soap and plenty of water.

2. Zym B reagent is reconstituted by following the manufacturer's instructions. Zym B reagent is very sensitive to light; store it in the dark, at 2-8°C between uses. Approximately 1 hour of exposure to laboratory lighting can damage the reagent. Label reconstituted bottles with date of reconstitution and expiration date. Zym B reagent is normally yellow to amber in color; do not use if any tint of pink is observed. Zym B is stable for 2 weeks after opening. Obtain a new bottle if current bottle has been opened for greater than 2 weeks. Zym B that has been ordered separately from the kits can be used with the existing kits. Each new lot/shipment of Zym B must have quality control (QC) testing for positive and negative reactivity. This can be done in parallel with patient testing. If the lot/shipment of Zym B has already been QC'd, no further QC is required.

C. Gram Stains and reagents
    1. Instructions for the preparation Gram Stains and associated materials can be found in the Gram Stain Procedure located in this manual.

D. Control organisms

Neiserria gonorrhea, Haemophilus influenzae, and Aggregatibacter aphrophilus are used in the quality control of the API NH test kits. Other cultures are used for Media QC. Cultures of the organisms are obtained by rehydrating commercially available systems (Microbiologics Kwik-Stik or Lyfo Disks) and streaking for isolation on non-selective media.

1. Kwik-Stiks are self-contained systems wherein an enclosed ampoule of suspension media is broken, allowed to migrate down the hollow shaft of an enclosed swab to the pellet of lyophilized organisms, and the rehydrated material is then transferred to chocolate agar. The swab is rolled over approximately one-third of the plate; a sterile loop is used to streak the remainder of the plate for isolation. The plates are then incubated at 35°C in 5-10% CO_2 (candle jar) for 48 hours. Do not open the jar prior to that time.
2. Lyfo Disks are dehydrated pellets of lyophilized organisms which are rehydrated after aseptic transfer to 0.5ml of sterile 0.9% NaCl or Tryptic Soy Broth (TSB). Once the pellet has been mixed into the suspension fluid with a sterile swab, the procedure is the same as for Kwik-Stiks.

Note: These procedures utilize potentially infectious organisms. Gloves, eye protection, and protective clothing are required.

E. Non-Selective and Selective prepared media
The plates of prepared media will come from the manufacturer with an expiration date. They may be held at refrigerator temperature (2-8° C) until this time. Plates can be inoculated on the date of expiration. Care should be taken to avoid dehydration during storage. All media will be inspected visually for defects before use as a part of the initial Media QC procedure. Any observed defects noted during normal use will be brought to the attention of the Laboratory Manager.

F. 4.0 McFarland Standard
The #4 McFarland Standard used in developing a microbial suspension for use with the NH test system has an expiration date provided by the manufacturer. It may be stored at room temperature in the dark.

G. API NH test system
API NH test system components are stored under refrigeration (2-8° C). All components of the kit except the Zym B are good until the manufacturer's expiration date, or 30 days after opening reagents, whichever occurs first. Zym B is only stable for 2 weeks. Additional, Zym B can be purchased and used to extend the kit life to the intended 30 days.

5. CALIBRATION PROCEDURES

There are no calibrations performed for this procedure. Function checks are conducted daily for the incubator, refrigerator and room temperature and annually or as needed for the timer and thermometers.

6. REPORTABLE RANGE

The ranges are reported for the Collection Site:
C = cervical, O = oralpharyngeal, R = rectal, and U = urethral and for the observed results of culture incubation.
Refer to the Stat Lab Reference Range and Results Interpretation Guide for the most current reporting requirements.

7. CONTROL PROCEDURES

- Oxidase Reagent is checked for reaction and color development each day of use, or whenever laboratory personnel determine there is a need to check its performance. Stock cultures of known oxidase reaction – N. gonorrhea (oxidase positive), E. coli (gram negative, oxidase negative), and S. epidermidis (gram positive, oxidase negative) are maintained for this purpose.

A drop of the Oxidase reagent in use is applied to the control cultures each day of testing and observed for appropriate color development.

- Non-selective and Selective culture media (Chocolate agar; MTM and/or ML agars) are checked for Sterility and Ability to Support Growth with each new shipment, each new lot, or whenever laboratory personnel determine there is a need (i.e., power outage, suspected temperature excursion). More detailed information on this procedure may be found in the Media QC Procedure.
- API NH test system and reagents are checked for appropriate performance by testing the response of each reagent and test well through the use of control cultures. Suspensions of *Neisseria gonorrhoeae, Haemophilus influenzae,* and *Aggregatibacter aphrophilus* are prepared as if they were patient specimens, inoculated into API NH test strips, incubated, and read. Reactions should match those noted on the table under "Quality Control" on page 4 of the Product Insert. This should be done with each new lot, each new shipment, or whenever laboratory personnel determine there is a need to check system performance.
- Zym B reagent is checked for appropriate performance by testing the response of the reagent through the use of control cultures. Suspensions of *Neisseria gonorrhoea* and *Aggregatibacter aphrophilus* are prepared as if they were patient specimens, inoculated into API NH test strips, incubated, and read. Reactions should match those noted on the table under "Quality Control" on page 4 of the Product Insert. This should be done with each new lot, each new shipment, or whenever laboratory personnel determine there is a need to check system performance.

8. CORRECTIVE ACTION

- If the oxidase reagent fails to provide an appropriate color reaction, make a new solution and check its performance. If the situation is not corrected, make new control cultures and check reagent performance. If appropriate response is not obtained suspend patient testing until a new lot of reagent can be obtained.
- If Non-selective and/or Selective media do not meet specifications for sterility repeat QC testing with new plates. If inhibition of growth does not take place repeat QC testing with new plates. If support of growth is not acceptable repeat testing with fresh cultures. If the problem is not corrected suspend patient testing until new lots of media can be obtained.
- If the API NH test system and reagents do not provide the proper responses repeat QC testing with fresh cultures of the QC organisms. If the problem is not corrected suspend patient testing until a new lot of API NH test system can be obtained.

9. LIMITATIONS OF THE METHOD

1. Only pure subcultures 18-24 hours old should be used for confirmation. Colonies older than 48

hours may give false-negative results.

2. The API NH system is intended uniquely for the identification of those species included in the database.
3. Certain species of the genus Moraxella, Oligella may be wrongly identified as N. gonorrhea since their biochemical profiles on the NH strip are very similar.

10. NORMAL VALUES

Normal Values of CG culture for healthy patients are:
No Growth; Growth-Oxidase Negative-Not. N. gonorrhea; Growth-Oxidase Positive - NOT Gram Negative Diplococci- Not N. gonorrhea; and Growth-Oxidase Positive - Gram Negative Diplococci – Not N. gonorrhea.

11. ALERT OR PANIC VALUES

There are no Alert or Panic Values for the confirmation of N gonorrhea procedure.

12. DATA ENTRY AND REPORTING

The results of Oxidase Reagent daily tests are entered into the Daily QC Log, as well as other pertinent information such as date of new reagent preparation. Chocolate and MTM/ML agar sterility, growth, and inhibition are noted and recorded in the Media QC Log-Choc and Media QC LOG-MTM/ML. API NH test system QC tests are recorded in the API NH QC log. Patient specimen observations are entered into the GC Log and GC Confirmatory Log as the analyst performs the examination and initialed by the analyst upon completion.
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. 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.
Note: if the sample is positive for N gonorrhae 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 case ID and include the ID in the comments section of the EMR lab result entry. Additional “To Do’s” must be sent separately to the STI Nurse and treating authority and include the ODRS record ID.
Proficiency Testing specimens are recorded in the patient GC Log and GC Confirmatory 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 GC Culture Confirmation – or any component of the GC Culture procedure - 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. GC Culture testing must be suspended until capability is regained; patient specimens may need to be referred to an outside laboratory (e.g. Aultman Laboratory Services) if directed by the Medical Director.

14. GC CULTURE APPENDIX

- 400-001-06-01-F_GC Culture Patient Log
- 400-001-06-02-F_Oxidase Daily QC Log
- 400-001-06-03-F_API NH QC Log
- 400-001-06-04-F_Media QC Logs
- 400-001-06-05-P_Media QC Procedure
- 400-001-06-06-A_BD BBL Chocolate II Agar product insert
- 400-001-06-07-A_B D BBL Martin-Lewis Agar product insert
- 400-002-06-08-A_B D BBL Modified Thayer Martin product insert
- 400-001-06-09-A_BD letter dated August 2015
- 400-001-06-10-A_Microbiologics Instructions for use Lyfo Disk Kwik-Stik
- 400-001-06-11-A_Microbiologics Recommended Growth Requirements
- 400-001-06-12-A_Microgiologis Maintenance of Quality Control Strains
- 400-001-06-13-A_API NH System BioMerieux product insert
- 400-001-06-14-F_GC Confirmation Patient Log
- 400-001-06-15-A_ZYM B product insert

NUCLEIC ACID AMPLIFICATION TEST (NAAT) FOR CHLAMYDIA AND GONORRHEA: HOLOGIC APTIMA COMBO 2® DTS SYSTEM

1. MATERIALS REQUIRED

APTIMA COMBO 2 Assay materials as described in the product insert.
2 Eppendorf Repeater Plus pipettors
Repeat pipettor tips, 2.5 mL
Repeat pipettor tips, 5.0 mL
Repeat pipettor tips, 25.0 mL
1000 µL pipettor
Pipette tips, P1000 Style
20 µL to 200 µL pipettor
Pipette tips 20 µL to 200 µL
Ten Tube Units (TTU)
Ten Tip Cassettes (TTC)
APTIMA Urine Specimen Transport Tubes for Male and Female Urine Specimens
Bleach, 5% to 7% (0.7 M to 1.0 M) sodium hypochlorite solution
Biohazard waste container w/ biobags
Timers
Lab markers
Appropriate PPE

2. PATIENT PREPARATION, SAMPLE COLLECTION, AND RELATED REQUIREMENTS

The patient will be taken into a CCHD STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CCHD STI Clinic protocol. The clinician will have asked the patient to collect the specimen (urine) in a provided sample container, labeled it with the patient's unique ID and the patient's clinic number, and transported it to the STAT lab. The specimen should be maintained at room temperature (15-30° C) unless testing is to be delayed, in which case specimens may be held up to 30 days at 2-30° C.
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 NAAT (Aptima Combo 2) tests-recollection is the only recourse for unacceptable specimens. There must be sufficient sample to allow testing – 2.0 ml at minimum. 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.

Transfer the urine sample into the APTIMA urine specimen transport tube within 24 hours of collection. Store processed specimens at 2° to 30°C and test within 30 days of collection. Processed urine specimens should be assayed with the APTIMA COMBO 2 Assay within 30 days of collection. If longer storage is needed, freeze at -20°C to -70°C for up to 12 months after collection.

3. MICROSCOPIC EXAMINATION AND PROCDURE

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

PROCEDURE

Note: This procedure utilizes potentially infectious patient specimens. Gloves, eye protection, and protective clothing are required.
APTIMA COMBO 2 has very detailed instructions provided in the package insert and training manual. Follow all instructions specifically as stated in these resources.
Prior to starting the assay, the work surfaces and pipettors should have been wiped down with 2.5% to 3.5% sodium hypochlorite solution (50/50 bleach/water). Allow the sodium hypochlorite solution to contact surfaces and pipettors for at least 1 minute and then follow with a water rinse. Do not allow the sodium hypochlorite solution to dry. Cover the bench surface on which the test will be performed with clean, plastic-backed absorbent laboratory bench covers.

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. The test kits themselves are stored at varying temperatures. 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 Aptima DTS system components. The water baths and the LEADER HC+ are checked for conformance to manufacturer's specifications by Hologic Technical Support personnel every six months. Vortex mixers, vacuum pumps, and computer hardware and software are checked for functionality at the same time.
The timers used for the test should have performance verification annually and meet manufacturer's specifications.
The pipettors used for the test should be checked for delivery volume annually and as needed and correction factors noted.

6. REPORTABLE RANGE

The reportable ranges for both Chlamydia trachomatis and Neiserria gonorrhea are Negative and Positive.

Negative means that the genetic material of either Chlamydia trachomatis, or Neiserria gonorrhea, or both was not detected.
Positive means that the genetic material of 1 Inclusion Forming Unit ("Elementary Body") of Chlamydia trachomatis, or 50 bacterial cells of Neiserria gonorrhea, or both, was detected.

7. CONTROL PROCEDURES

The Positive Control, CT / Negative Control, GC; and the Positive Control, GC / Negative Control, CT act as controls for the target capture, amplification, and detection steps of the assay. The Positive Control, CT / Negative Control, GC serves as the negative control for the GC test results. The Positive Control, GC / Negative Control, CT serves as the negative control for the CT test results. A dual negative control furnished by the user (a "Blank") is added to monitor assay background. The Positive Controls must produce the following test results:

Positive Control, CT/Negative Control, GC	≥100 and <3,000 RLU (Relative Light Units)
Positive Control, GC/Negative Control, CT	≥150 and <3,000 RLU (Relative Light Units)

The APTIMA Assay software automatically evaluates the controls according to the above criteria and will report the Run Status as PASS if the run control criteria are met, and FAIL if the run control criteria are not met. If the Run Status is FAIL, all test results in the same run are invalid and must not be reported

8. CORRECTIVE ACTION

If a test run fails to meet Control Criteria for acceptability – the run is flagged as Fail by the Aptima DTS System 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 Medical Director and the Nursing Director (or appropriate clinical personnel), and contact the manufacturer.
If a specimen has an equivocal result, contact the ordering entity in order to determine if they would like the specimen rerun.
If the blank control that is run at the end of the specimens is positive, despite a "Pass" result the system has an evident error. Consult with the Laboratory Director or Technical Consultant to determine how to proceed.
A rerun may be necessary for other reasons, particularly after reviewing the initial results. The most likely scenario is when background counts are not consistent with prior runs. Consult with the Laboratory Director or Technical Consultant to determine if a rerun is needed. A rerun could lead to differing patient results which may lead to challenges in patient result interpretation.

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

The results of Aptima Combo 2 runs (NAATs) are printed out by the Aptima software after each run and entered into the Aptima Run logbook. Other pertinent information such as date of run, lot numbers and expirations of test kits and controls, and analyst performing the run are found on these printouts. Patient results, run date and analyst initials are entered into the Aptima log book after the analyst has performed the run.

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. 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.

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 case ID and include the ID in the comments section of the EMR lab result entry. Additional “To Do’s” must be sent separately to the STI Nurse and treating authority which include the ODRS ID.

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 Aptima Combo 2 tests (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. GC/CH GENE AMP APPENDIX

- 400-001-07-01-F_APTIMA Daily Run QC
- 400-001-07-02-F_APTIMA Patient Log
- 400-001-07-03-A_APTIMA Urine Transport Tubes Package Insert
- 400-001-07-04-A_APTIMA Combo II Package Insert

BLOOD LEAD

1. MATERIALS REQUIRED

Alcohol wipes
Gauze pads
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. Use the blood within 24 hours of collection. Store at 50°- 90°F (10°- 32°C).
- 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 equipment (PPE) when using the LeadCare II System. Consult the established policy of your organization for proper laboratory protection.

3. MICROSCOPIC EXAMINATION AND PROCEDURE

No microscopic examination is required for this testing procedure.

BLOOD LEAD TEST 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 this 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 of 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.
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 µg/dL
2. “High” = >65 µg/dL
3. The reportable range 3.3 to 65 µg/dL

7. CONTROL PROCEDURES

In order to ensure the accuracy of your LeadCare II results. 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.
- 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 either 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-imercaptopropanol) (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.*

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 of the following information:

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; and
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.

ORAQUICK HCV RAPID ANTIBODY TEST PROCEDURE

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 CCHD STI Clinic examination room by a clinician, examined and evaluated, and the procedure explained as per CCHD 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 stored at 2-8° C for up to 7 days or at 15 - 30° C for up to 3 days. 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 HCV Rapid 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. 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 HCV Rapid Antibody test procedure; it is a macroscopic technique.

PROCEDURE

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

   Follow Package Insert.

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 direction.

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 specimen observations are entered into the HCV 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. 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 referral. 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.

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

F. CITATIONS & REFERENCES

- Centers for Medicare & Medicaid Services, 42 Code of Federal Regulations Part 493 Clinical Laboratory Improvement Amendments of 1988 the "Sexually Transmitted Diseases Treatment Guidelines, 2010" created by the U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and the "Program Operations Guidelines for STI Prevention, Medical and Laboratory Services"
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- *Gen-Probe Aptima Combo 2 Assay product insert,* 501798 Rev. D/2012-05; Gen-Probe Inc., 2001-2012.
- *Gen-Probe Aptima Combo 2 Assay Procedural Notes (online: wwwGenprobe.com),* IN0037-06 Rev. A.
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- OAC 3701-30-05 Record-keeping and reporting requirements.
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G. CONTRIBUTORS

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4. Julie Carman, Laboratory Technician

H. APPENDICIES & ATTACHMENTS

1. 400-0001-01 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_SureVue Urine hcg strip pkg insert
    - 400-001-03-02-A_Urine Controls pkg insert
4. 400-001-04-P_HIV-ORAQUICK ADVANCE HIV ½
    - 400-001-04-05-A_OraQuick Advance Rapid HIV ½ Product insert
    - 400-001-04-06-A_OraQuick Controls product insert
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
    - 400-001-05-07-A_RPR Card Tests Product Insert
6. 400-001-06-P_GONORRHEA CULTURE
    - 400-001-06-05-P_Media QC Procedure
    - 400-001-06-06-A_BD BBL Chocolate II Agar product insert
    - 400-001-06-07-A_B D BBL Martin-Lewis Agar product insert
    - 400-002-06-08-A_B D BBL Modified Thayer Martin product insert
    - 400-001-06-09-A_BD letter dated August 2015
    - 400-001-06-10-A_Microbiologics Instructions for use Lyfo Disk Kwik-Stik
    - 400-001-06-11-A_Microbiologics Recommended Growth Requirements
    - 400-001-06-12-A_Microgiologis Maintenance of Quality Control Strains
    - 400-001-06-13-A_API NH System BioMerieux product insert
7. 400-001-07-P_GENETIC AMPLIFICATION GC AND CH
    - 400-001-07-03-A_APTIMA Urine Transport Tubes Package Insert
    - 400-001-07-04-A_APTIMA Combo II Package Insert
8. 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

9. 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

10. 400-001-10-P_HCV RAPID ANTIBODY TEST

- 400-001-10-02-A_OraQuick HCV Rapid Antibody Test Product insert

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
- 400-001-04-02-F_HIV Confirmatory 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
- 400-001-05-02-P_RPR Reagent Preparation
- 400-001-05-03-F_RPR Patient Log

6. 400-001-06-P_GONORRHEA CULTURE

    - 400-001-06-01-F_GC Culture Patient Log
    - 400-001-06-02-F_Oxidase Daily QC Log
    - 400-001-06-03-F_API NH QC Log
    - 400-001-06-04-F_Media QC Logs
    - 400-001-06-14-F_GC Confirmation Patient Log

7. 400-001-07-P_GENETIC AMPLIFICATION GC AND CH

    - 400-001-07-01-F_APTIMA Daily Run QC
    - 400-001-07-02-F_APTIMA Patient Log

8. 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
    - 400-001-09-A_General Forms

9. 400-001-09-GENERAL FORMS

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

10. 400-001-10-HCV RAPID ANTIBODY

    - 400-001-10-01-F_ HCV Patient 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.