

Section IV:

Laboratory Testing, Quality Assurance & Improvement Program

INTRODUCTION

The laboratory committee of the Region X IPP has developed extensive measures which assist clinicians and laboratorians in monitoring quality assurance. These measures include (1) descriptions of the various diagnostic tests used; (2) a description of cell types found on slide samples; (3) a process for specimen collection evaluation; (4) a method for notifying all project labs as well as the national laboratory coordinator of any problems related to laboratory testing products or methodology. In collaboration with the training committee, a resource guide has been developed to assist any provider whose specimens do not meet the regional standard.

Clinicians are encouraged to participate in the specimen adequacy program. The Centers for Disease Control and Prevention (CDC) have established quality assurance indicators for this project. One such indicator is the monitoring of specimen collection proficiency by new clinicians as well as those who are seasoned clinicians. For additional information on this, please contact your state FP or CT Coordinator (listed in the Resource Section).

Laboratorians also have a process for evaluating each other through the sharing of specimens. Labs within Region X take turns preparing known (positive and negative) samples and send them to the other Region X labs that use the same test method for blind analysis. Results are compiled, shared and evaluated for consistency.

DESCRIPTION OF CHLAMYDIA TESTING TECHNOLOGIES

Nucleic Acid Probe (PACE II) Test for *Chlamydia trachomatis*

- Manufacturer
 - Gen-Probe, Inc.
- Collection Sites
 - Endocervical, male urethral and conjunctival.
- Specimen Handling
 - Transport and storage are 2-25° C. Test specimens within 7 days of collection, alternately, may be frozen until shipped.
- Principle
 - A direct specimen test where copies of a chemiluminescent labeled, single-stranded DNA probe combine with target organism's ribosomal RNA to form stable DNA:RNA hybrids. The labeled hybrids are separated from non-hybridized probe and are measured in a luminometer. The test results are calculated as the difference between the response of the specimen and the mean response of the negative reference.
- Turn Around Time (TAT) in Lab
 - 1-3 working days
- Sensitivity/Specificity
 - Varies with study parameters, population and anatomical site
- Test Comments
 - Can test for gonorrhea and chlamydia from a single swab, but not in a single/same run.

- Limitations

- Not approved for nasopharyngeal, urine, or rectal specimens.
- Not acceptable for medical/legal purposes.

Therapeutic success or failure cannot be determined as chlamydial DNA and antigen may persist following appropriate antimicrobial therapy. Follow up tests to determine success of treatment for pregnant women should not be collected until four (4) weeks following completion of treatment. Residual nucleic acid from dead organisms may persist, leading to false positive results for specimens collected sooner than four weeks post-treatment.

Adequacy of specimen cannot be determined. Grossly bloody specimens may interfere with test performance. Low-level (borderline) positives are currently repeated using the Probe Competition Assay (PCA). Both Ct and GC can be detected in the initial test and cannot be differentiated without a second test on the same specimen to determine which organism(s) is/are present.

- Result Interpretation

- *Positive*: A positive is reported as the difference greater than or equal to 350 RLU plus the mean of the negative reference. When the test result is marked positive, the PCA confirmation test is also positive.
- *Negative*: A negative is reported as the difference less than 350 RLU plus the mean of the negative reference.
- *Equivocal*: Anything that falls between 200 RLU and the positive cut off for the screening test.

Target Capture, Transcription-Mediated Amplification (TC-TMA)

Test for *Chlamydia trachomatis* – (APTIMA)

- Manufacturer
 - Gen-Probe Incorporated
- Collection Sites
 - Endocervical, urethral, male and female urine (first part of stream, 20 – 30 ml)
- Specimen Handling
 - *Swab*: Transport and store at 2° to 30°C in the APTIMA Combo 2 Swab Specimen Transport Tube until tested. Test swabs within 60 days of collection.
 - *Urine*: Transport and store at 2° to 30°C in the APTIMA Combo 2 Urine Specimen Transport Tube. (Urines must be transferred from the primary container to the urine transport tube within 24 hours of collection, however do not delay; transfer ASAP.) Test urines within 30 days of collection.
 - Swabs and urines may be frozen at -20° to -70°C for up to 90 days after collection.
- Principle
 - The assay combines the technologies of target capture (isolates the target nucleic acid strands), Transcription-Mediated Amplification (TMA), and Dual Kinetic Assay (DKA) for the amplified detection of rRNA molecules. The rRNA amplification product, amplicon, combines with labeled DNA probes to form stable RNA:DNA hybrids. During the chemiluminescent detection reaction, the hybrids emit light (Relative Light Units – RLU) measured as photon signals in a luminometer.
- Turn Around Time (TAT) in Laboratory
 - 1 – 3 working days

- Sensitivity/Specificity
 - Varies with study parameter, population and anatomical site
- Test comments
 - Can test for chlamydia and gonorrhea from a single swab or urine specimen in a single test run.
 - Swabs are not affected by blood, gynecological lubricants and spermicides. Urines are not affected by blood, vitamins, minerals and over-the-counter pain relievers.
- Limitations
 - Not approved for nasopharyngeal, eye or rectal specimens.
 - Not acceptable for medical/legal purposes in adults unless confirmed by a second NAAT assay OR culture. Not acceptable for medical/legal purposes in pediatric cases.
 - Adequacy of specimen cannot be determined directly from the test.
 - Therapeutic success or failure cannot be determined as chlamydial nucleic acid may persist following appropriate antimicrobial therapy. Follow up tests to determine success of treatment for pregnant women should not be collected until four (4) weeks following completion of treatment. Residual nucleic acid from dead organisms may persist, leading to false positive results for specimens collected sooner than four weeks post-treatment.
- Result Interpretation
 - *Positive*: 100 to < 3,000 RLU (x1000)
 - *Negative*: 1 to <25 RLU (x1000)
 - *Equivocal*: 25 to <100 RLU (x1000)

Digene Hybrid 2 Capture

- Manufacturer
 - Digene
- Collection Sites
 - Endocervical and male urethral specimens
- Specimen Handling

Specimens may be held for up to two weeks at room temperature and shipped without refrigeration to the testing laboratory. Specimens should be shipped in an insulated container using either an overnight or 2-day delivery vendor. At the testing lab, specimens should be stored at 2-8° C if the assay is to be performed within one week. If the assay will be performed later than one week, store specimens at 20°C for up to 3 months.

- Principle

The hc2 CT_ID DNA Test using Hybrid Capture 2 technology is a nucleic acid hybridization assay with signal amplification that utilizes microplate chemiluminescent detection. Specimens containing the target DNA hybridize with a specific Chlamydia RNA probe cocktail. The resultant RNA-DNA hybrids are captured onto the surface of a microplate well coated with antibodies specific for RNA-DNA hybrids. Immobilized hybrids are then reacted with alkaline phosphatase-conjugated antibodies specific for RNA-DNA hybrids, and detected with a chemiluminescent substrate. Several alkaline phosphatase molecules are conjugated to each antibody. Multiple conjugated antibodies bind to each captured hybrid resulting in substantial signal amplification. As the substrate is cleaved by the bound alkaline phosphatase, light is emitted, which is measured as relative light units (RLUs) on a luminometer. The intensity of the light emitted denotes the presence or absence of target DNA in the specimen.

- Turn Around Time (TAT) in Lab
 - Within three working days
- Sensitivity/Specificity
 - Varies with study parameters, population and anatomical site
- Test Comments
 - Can test for gonorrhea and chlamydia from a single swab, but not in a single/same run.
- Limitations
 - Not FDA approved for nasopharyngeal, urine, or rectal specimens.
 - Not acceptable for medical/legal purposes.
 - The hc2CT-ID DNA Test is not intended to determine therapeutic success.
- Result Interpretation
 - *Positive*: Specimens with RLU/Cutoff Value ratios ≥ 2.50 are considered positive for Chlamydia trachomatis DNA.
 - *Negative*: Specimens with RLU/Cutoff Value ratios < 1.00 do not contain Chlamydia trachomatis DNA or contain DNA below the detection limit of the assay.
 - *Equivocal*: Specimens with RLU/Cutoff Value ratios ≥ 1.00 and < 2.50 are considered equivocal. Repeat

Cell Culture Test for Chlamydia trachomatis

- Manufacturers
 - In house
- Collection Sites
 - Endocervical, urethral, conjunctival, nasopharyngeal, rectal, tissue biopsy, endometrial, tubal.
- Specimen handling
 - Transport and store at 4° C; test within 4 days of collection.
- Principle
 - Specimens are inoculated and centrifuged into a medium containing cycloheximide treated McCoy (mouse strain) cells. Specimens are incubated for 40-48 hours. Cells are fixed and stained with monoclonal fluorescent antibody (FA). FA stained cells viewed through a fluorescence microscope exhibit a fluorescent green inclusion if infected with the organism. Non-infected cells appear red.
- Turn Around Time (TAT) in Lab
 - 2-3 Working days
- Sensitivity/Specificity
 - Varies with study parameters, population and laboratory performing test.
- Test Comments
 - Culture is still considered the preferred test for medical/legal cases* Also, it is the only method recommended for specimen sites for which nonculture methods have not been developed or evaluated. (Prior to submission, consult lab about options on unlisted collection sites.)

- Limitations
 - Specimen transport and storage times and temperatures are critical; technically difficult procedure requiring expertise in tissue culture techniques.
- Result Interpretation
 - *Positive*: A positive is reported as greater than or equal to 1 inclusion forming unit. All positives are confirmed by a second analyst.
 - *Negative*: A negative is only considered negative in the absence of significant cell cytotoxicity.
 - *Equivocal*: Two readers can not agree upon results or suspect a nonviable organism.

*Contact lab for chain of custody procedures. Harborview Research & Training Lab at 206-341-5300

DEFINITION OF CELL TYPES FOUND ON SLIDE SAMPLES:

Columnar Epithelial Cells:

- A slide with these cells in the majority is IDEAL.
- Host Cells to Chlamydia trachomatis.
- Line the endocervical canal in a single layer.
- Have a basally located (eccentric or off-center) nucleus which is round to oval and may look “frothy” or “lacey”.
- When looking at columnar cells from above, the cytoplasm is seen as a narrow rim around the nucleus.
- May be seen in strips of parallel-arranged cells or in tight sheets (honeycomb pattern).

Atypical OR Metaplastic Columnar Epithelial Cells:

- It is not established that these cells can host an infection with chlamydial infectious particles. However, their presence on a slide indicates swab sample site is correct since these cells are found in the correct area of interest.
- Demonstrate changes from normal columnar epithelium; cells are extremely enlarged and nucleus contains excessive pigmentation (due to injury, repair).

Superficial/Intermediate Squamous Cells:

- Not a good slide if these cells are the majority.
- Not known to be host cells for chlamydial infectious particles.
- Line the vagina and the outer portion of the uterine cervix (ectocervix).
- Are large, flat, platelike cells with a small central nucleus.

Metaplastic Squamous Epithelial Cells:

- Not host cells for chlamydia, however their presence indicates correct area for swab collection.
- Lower organizational order than the mature cell.
- Are transformed squamous epithelial cells (due to noxious agents or processes) which are rounder than normal squamous cells, have dense cytoplasm and large nuclei with fine granular chromatin.

Erythrocytes (Red Blood Cells):

A slide with red blood cells as major cell type is acceptable for assessing specimen adequacy only if ten or more columnar epithelial or metaplastic cells are also found on the slide.

References

Reith, EDW. J., Ph.D, Michael H. Ross Ph.D., Atlas of Descriptive Histology, 3rd Edition, 1997.

Bibbo, Marluce, M.D., Sc.D., F.I.A.C., Comprehensive Cytopathology, Second Edition, 1997.

Acknowledgments

Cindy Fennel, STD Prevention/Training, Sue Szabo, STD Clinic, Harborview; Debbie Vernon, Cytology Lab, Harborview.

SPECIMEN ADEQUACY – PROCEDURE INFORMATION:

1. Enclosed are a set of 10 routine CT sample collection swabs AND 10 specimen adequacy swab/slides/slide-holder units.
2. The clinician collects all other specimens first then the CT samples as follows:
 - Routine CT swab: Collect sample and place the swab in the tube as indicated on the swab package.
 - Specimen Adequacy swab: Using the swab from the swab/slide/slide-holder set, collect the sample as done for the routine CT, then roll the swab over the circled area only on the slide. Do not drag or push the swab. Ensure that all surfaces of the swab come into contact within the circle and that the entire circled area is covered evenly with specimen. The result should be a thin even smear.
3. Label the slide. Using a pencil, write the patient's and clinician's identifier on the frosted area of the slide. Allow the sample on the slide to air dry completely before placing it inoculated side up into the slide holder.
4. Label the routine CT sample by your normal method plus add the clinician identifier to the bottom left area of the label.
 - *The collector's (clinician's) identifier needs to be consistent for all 10 patients.
5. Rubber band the CT swab tube and the slide-holder (containing the slide) together. Ship these along with the regular Region X Chlamydia form to your testing lab as normally done.
6. The routine CT sample will be tested and the result returned to your facility in the normal manner.
7. A "Chlamydia Specimen Adequacy Report" will be sent to your supervisor. A training fact/reference sheet will be included with results that do not meet the stated acceptable performance.
 - For training follow up, please call the contacts noted on the Training Fact/Reference Sheet.
 - To evaluate training needs, a copy of the clinician's results will be sent to your State's CT Infertility Prevention Project Coordinator.
 - For statistical purposes, non-identifiable data will be shared with the Region X Project Office at the Center of Health Training.

For questions or concerns regarding outcomes please call your testing laboratory at _____ (phone).

RESOURCES FOR CHLAMYDIA SPECIMEN ADEQUACY

Collection for the State Of _____

Your state offers the following resources to assist you in reaching the regional standard for specimen collection:

- Review “Specimen Collection for Chlamydia” video. If you do not have a copy within your agency, please contact your state FP or STD manager listed below.
- When feasible, ask a senior clinician who has met the proficiency standard to observe your collection technique.
- Attend a training offered by Seattle STD-HIV Prevention Training Center. Contact Anne Meegan at 206-685-9850, seaptc@u.washington.edu, or visit the website at <http://weber.u.washington.edu/~seaptc>.
- Review resources for improving specimen collection provided by your state Family Planning or CT/STD manager listed below.

ALASKA:

Susan Jones (CT Coordinator)
907-269-8061
907-561-4239 – fax
joness@health.state.ak.us

Municipality of Anchorage:

Cathy Feaster
907-343-4789
907-343-4633 – fax
feasterec@ci.anchorage.ak.us

IDAHO:

Anne Williamson (STD)
208-334-6526
208-332-7346 – fax
willia25@idhw.state.id.us

Susan Ault (FP)
208-334-5959
208-332-7346 – fax
aults@idhw.state.id.us

OREGON:

Doug Harger (STD)
503-731-4026
503-731-4082 – fax
DOUGLAS.R.HARGER@state.or.us

Carol Elliott (FP)
503-731-4363
503-731-4083 – fax
carol.j.elliott@state.or.us

WASHINGTON:

Katherine Gudgel (CT Coordinator)
253-395-6734
katherine.gudgel@doh.wa.gov
– OR –
Ellen Gish
360-236-3450
360-236-3470 – fax
Ellen.Gish@DOH.WA.GOV

Jane Wilson (FP)
360-236-3469
360-236-3400 – fax
jane.wilson@doh.wa.gov

PUBLIC HEALTH LABORATORIES

Public Health Laboratories approved to participate in the project include the following:

ALASKA

Alaska Public Health Laboratory
4500 Boniface Pkwy
Anchorage, AK 99507
907-334-2111
907-334-2161 Fax
Contact: Gregg Herriford

WASHINGTON

Washington State Public
Health Laboratories
1610 NE 150th ST
Shoreline, WA 98155-9701
(206) 361-2884
Contact: Mike McDowell

IDAHO

Bureau of Laboratories
Virology and Serology Section
2220 Old Penitentiary Road
Boise, ID 83712
(208) 334-2235
Contact: Colleen Greenwalt

Infectious Disease Laboratory

University of Washington
300 Ninth Ave., Rm. 627
Seattle, WA 98195
(206) 341-5304
Contact: Linda Cles

OREGON

Oregon Public Health Laboratory
1717 SW 10TH
Portland, OR 97201
(503) 229-5882
Contact: Chris Biggs

Spokane Regional Health Laboratory
1101 W. College, RM 210
Spokane, WA 99201
(509) 324-1440
Contact: Karen Crouse