

# **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 proficiency; (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 on the Resource Sheet on page 66).

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**

### ➤ **Enzyme Immunoassay (EIA) Test for *Chlamydia trachomatis***

- **Manufacturers**

Walpole/Carter Wallace

- **Collection Sites**

Endocervical, urethral, conjunctival, male urine (pre approval required)

- **Specimen Handling**

Transport and store at 2-25°C; test within 7 days of collection. Urine requires refrigerated storage and transport.

- **Principle**

EIA tests detect soluble chlamydia lipopolysaccharide (LPS) antigen with an antibody that has been labeled with an enzyme. The enzyme converts a colorless substrate into a colored product. The intensity of the color is measured with a spectrophotometer, which provides a numerical readout.

- **Turn Around Time (TAT) in Lab**

1-3 working days

- **Sensitivity/Specificity**

Varies with study parameters, population and anatomical site.

- **Limitations**

Not approved for nasopharyngeal or rectal specimens; not acceptable for medical/legal cases; proper specimen collection is critical. Adequacy of specimen cannot be determined. Gross blood may interfere with sensitivity. A second test using another technology is necessary to confirm positives, per CDC recommendations.

- **Result Interpretation**

- ▶ **Positive**

Each positive EIA specimen is confirmed in the laboratory. Thus, when a test result is marked Positive, the confirmation test was also Positive.

To maximize test sensitivity, each negative EIA result that is within a designated "gray zone" (45% in Region X) will be confirmed from the original patient sample (when the confirmed test is positive, the test will be reported as positive).

▶ **Negative**

When the EIA test result is clearly below the manufacturers' cutoff for a negative, the confirmation test will not be performed and the test will be reported as Negative. When the EIA is within a designated "gray zone" (45% in Region X) and the confirmed test is negative, the test result will be reported as Negative.

▶ **Suspect**

When a Positive EIA does not confirm, results will be reported to clinics as SUSPECT. A SUSPECT result is one in which the EIA is Positive (using the manufacturers' approved cutoff for a positive) BUT the confirmation test is Negative.

• **Test Results - Follow up**

**Case Reports to the State of SUSPECT Test Results**

Since a Suspect result means the EIA was positive, a Case Report should be submitted to the state.

If a repeat test is submitted, the case report may be delayed pending the second test result. If this test is SUSPECT or POSITIVE, submit a case report to the state. If the test is negative, no case report is necessary.

➤ **Direct Fluorescent Antibody (DFA) test for *Chlamydia trachomatis***

- **Manufacturers**

Walpole/Carter Wallace

- **Collection Sites**

Endocervical, urethral, and for rectal, conjunctival, nasopharyngeal on symptoms only

- **Specimen Handling**

Transport/Store at room temperature (20-30°C).

- **Principle**

Fluorescein isothiocyanate labeled monoclonal antibodies against the chlamydia major outer membrane protein (MOMP) are used to stain specimens applied directly to slide. Positive smears contain apple-green elementary bodies.

- **Turn Around Time (TAT) in Lab**

1-3 working days.

- **Sensitivity/Specificity**

Varies with study parameter and population, and with the minimum elementary body cutoff used to determine a positive test.

- **Test Comments**

Only test in which the adequacy of specimen collection can be evaluated while performing the test.

- **Limitations**

Cannot be used for medical/legal cases; critical specimen collection technique: must remove excess mucous before preparing adequate slide. Columnar epithelial cells must be visible on slide for slide to be satisfactory.

- **Result Interpretation**

Used as a Screening Test:

- ▶ **Positive**

Region X uses two or more elementary bodies for a positive result.

- ▶ **Negative**

Absence of elementary bodies and there are 3 or more columnar cells present on the slide.

▶ **Suspect**

One elementary body seen and confirmed by a second analyst.

Used as a confirmatory test:

▶ **Positive**

Two or more elementary bodies will be reported as positive. Fewer than ten elementary bodies shall be reviewed by a second analyst.

▶ **Negative**

Absence of elementary bodies slides; may be reviewed by more than one analyst.

▶ **Suspect**

The initial screening test was positive but the DFA test is negative.

➤ **Ligase Chain Reaction (LCR) Test for *Chlamydia trachomatis***

- **Manufacturer**

Abbott Laboratories

- **Collection Sites**

Endocervical, urethral, male and female urine (first part of voided stream).

- **Specimen Handling**

Swab specimens must be transported and stored at 2-30°C until tested; urine samples 2-8°C. Test specimens within 4 days of collection, alternately, may be frozen until shipped.

- **Principle**

The assay uses Ligase Chain Reaction (LCR) amplification technology for the direct, quantitative detection of plasmid DNA of *C. trachomatis* in clinical specimens. After amplification, the target products are detected by MEIA (Microparticle Enzyme Immunoassay).

- **Turn Around Time (TAT) in Lab**

1-3 Working days.

- **Sensitivity/Specificity**

Varies with study parameter, population and anatomical sites.

- **Test Comments**

Can test for gonorrhea and chlamydia from a single swab.

- **Limitations**

Not approved for nasopharyngeal, eye or rectal specimens. Not acceptable for medical/legal purposes. Adequacy of specimen cannot be determined. Therapeutic success or failure cannot be determined as chlamydial DNA and antigen may persist following appropriate antimicrobial therapy; however, tests are usually negative 3-4 weeks after treatment. Grossly bloody specimens may interfere with test performance.

- **Result Interpretation**
  - ▶ **Positive**  
Per manufacturer's published cutoff.
  - ▶ **Negative**  
Per manufacturer's published cutoff.
  - ▶ **Suspect**  
Not applicable.

➤ ***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.

- **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; however, tests are usually negative 3-4 weeks after 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). Combination tests for GC and CT does not differentiate between chlamydia and gonorrhea; therefore, secondary testing must be done for definitive diagnosis.

- **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.

- ▶ **Suspect**

Anything that falls within the negative gray-zone\* on a repeat test. The negative gray-zone is considered to be the difference greater than or equal to 200/RLU plus the mean of the negative reference but less than the cut off.

\* refers to Public Health Region X protocol.

➤ ***Nucleic Acid Probe (TMA) Test for Chlamydia Trachomatis***

- **Manufacturer**

Gen-Probe, Inc.

- **Collection Sites:**

Endocervical, male urethral, urine, and conjunctival.

- **Specimen Handling**

- ▶ **Swab** - Transport and storage are 2-25°C. Test specimens within 7 days of collection, alternately, may be frozen until shipped.
- ▶ **Urine** - Transport and storage are 24 hours at room temperature or 7 days at 2-25°C.

- **Principle**

TMA refers to upfront Transcription Mediated Amplification. 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.

- **Limitations**

Not approved for nasopharyngeal 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; however, tests are usually negative 3-4 weeks after treatment. Adequacy of specimen cannot be determined. Grossly bloody specimens may interfere with test performance.

- **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.

- ▶ **Suspect**

TMA – Equivocal Zone, package insert defines this as 40,000 to the 500,000 RLU to be retested.

➤ **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\* and has been the “gold standard” for comparison of other methods. Also, it is the only method recommended for specimen sites for which non-culture 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.

- ▶ **Suspect**

Not applicable.

\*Contact lab for chain of custody procedures.

## ***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 of 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, 3<sup>rd</sup> 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.

# REGION X CHLAMYDIA PROJECT SPECIMEN ADEQUACY PROGRAM

## Registration Form and Procedure Information

**Purpose:** This is a Quality Assurance process to help assess the quality of Chlamydia (CT) specimen collection. It is not meant as an immediate correlation of your current testing method results with the specimen adequacy result. It is offered to facilities participating in the Region X Project.

**Registration:**

1. Complete the following information and send it to your CT testing laboratory.
2. **Provide** - Clinician identifier that will be used throughout the specimen adequacy process.

Name \_\_\_\_\_  
*(name, initials or clinician number or other)*

Facility \_\_\_\_\_

Address \_\_\_\_\_

Telephone \_\_\_\_\_

Contact person to receive results

\_\_\_\_\_  
*(supervisor, clinician, other)*

Date request submitted \_\_\_\_\_

3. **Copy** this completed page and mail/FAX it to your CT testing Lab. The original is for your reference. If you choose to call the Lab, they will fill out the form from the information you give over the phone and send a copy to the contact person.

## Procedure Information:

1. The Lab will send out a set of 10 routine CT sample collection swabs **AND** 10 specimen adequacy slides/slide holder/swab units.
2. The clinician is to collect all other specimens first **then** collect 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/slider holder set, collect the sample as done for the routine CT, then roll the swab over the **circled area only** on the slide. NOTE - roll the swab slowly over the circled area - 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. \* Use identifier as indicated on the Specimen Adequacy Registration Form (page 1 of this packet).
4. Label the routine CT sample by your normal method **plus** add the clinician identifier to the bottom left area of the label.

**Both the routine CT sample tube and the SLIDE must be labeled with the patient's and clinician's identifiers. 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. The routine CT sample result and corresponding slide result along with adequacy criteria will be sent to the contact indicated on the registration form (page 1 of this packet). A training fact/reference sheet will be included with results that do not meet the stated acceptable performance. A blank result page and training page are attached for your information.

- 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 Project Coordinator.
- For statistical purposes, non-identifiable data will be shared with the Region X Project Coordinator at the Center for Health Training.

For questions or concerns regarding outcomes please call your testing laboratory.

## 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 Kelly Culbert at 206-685-9850, [seaptc@u.washington.edu](mailto: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](mailto:joness@health.state.ak.us)

#### MUNICIPALITY OF ANCHORAGE:

Cathy Feaster  
907-343-4789  
907-343-4633 – fax  
[feasterec@ci.anchorage.ak.us](mailto:feasterec@ci.anchorage.ak.us)

#### IDAHO:

Anne Williamson (STD)  
208-334-6526  
208-332-7346 - fax  
[willia25@idhw.state.id.us](mailto:willia25@idhw.state.id.us)

Susan Ault (FP)  
208-334-5959  
208-332-7346 - fax  
[aults@idhw.state.id.us](mailto:aults@idhw.state.id.us)

#### OREGON:

Doug Harger (STD)  
503-731-4026  
503-731-4082 - fax  
[DOUGLAS.R.HARGER@state.or.us](mailto:DOUGLAS.R.HARGER@state.or.us)

Carol Elliott (FP)  
503-731-4363  
503-731-4083 - fax  
[carol.j.elliott@state.or.us](mailto:carol.j.elliott@state.or.us)

#### WASHINGTON:

Katherine Gudgel (CT Coordinator)  
360-236-3450  
360-236-3470 - fax  
[katherine.gudgel@doh.wa.gov](mailto:katherine.gudgel@doh.wa.gov)

Chris Knutson (FP)  
360-236-3469  
360-236-3400 - fax  
[chris.knutson@doh.wa.gov](mailto:chris.knutson@doh.wa.gov)



## **Public Health Laboratories**

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

### **ALASKA**

Alaska Public Health Laboratory  
527 E 4th Ave  
Anchorage, AK 99519  
(907) 274-1602  
Contact: Rose Tanaka

### **IDAHO**

Bureau of Laboratories  
Virology and Serology Section  
2220 Old Penitentiary Road  
Boise, ID 83712  
(208) 334-2235  
Roy Moulton

### **PLUS**

3 District Health Laboratories  
Coeur d'Alene  
Idaho Falls  
Pocatello

### **OREGON**

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

### **WASHINGTON**

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

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

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