Laboratory Testing and Quality Assurance

INTRODUCTION

A. RECOMMENDED TESTS

A variety of diagnostic assays are acceptable for chlamydia testing. In *Screening Tests to Detect Chlamydia trachomatis and Neisseria gonorrhoeae Infections* – MMWR 2002, CDC recommends use of amplified testing (e.g., APTIMA, (ProbeTec), polymerase chain reaction (PCR)) in lieu of other tests, which include enzyme and optical immunoassays (EIA, OIA), nucleic acid hybridization assays (e.g., Gen-Probe PACE 2 or 2C), and direct detection assays that use fluorescent monoclonal antibodies (DFA). Culture is generally reserved for medical-legal cases.

List of tests currently in use by Region VIII here (for a more detailed description of each test, please refer to Rick Steece's Testing Technologies document):
TMA: APTIMA: a target capture RNA amplification test
SDA: BD Probetek
PCR:
Culture

For the necessary laboratory requisition forms, collection devices, and specific instructions regarding the storage and transport of specimens, please contact the laboratory that serves your clinic. A list of participating laboratories is given below.

Colorado

Division of Laboratories
Colorado Department of Public Health and Environment
8100 Lowry Blvd.
Denver, CO 80220
303-692-3090

Branch Laboratory
Colorado Department of Public Health and Environment
1515 Patterson
Grand Junction, CO 81506
970-245-7800

El Paso County Health Department
719-578-3121

Denver Public Health
303-436-7365

Weld County Health Department
970-353-0635

Denver General Hospital
303-436-6929

The Children's Hospital
303-861-6215

Montana

Public Health Laboratory
Montana Department of Public Health and Human Services
P.O. Box 6489
Helena, MT 59604
406-444-3444

North Dakota

Division of Microbiology
North Dakota Department of Health
P.O. Box 5520
1205 Avenue A West
Bismarck, ND 58506-5520
701-328-5262

South Dakota

Public Health Laboratory
South Dakota Department of Health
615 E. 4th St.
c/o 500 E. Capitol
Pierre, SD 57501
605-773-4973

Utah

Utah Division of Epidemiology and Laboratory Services
46 North Medical Drive
Salt Lake City, UT 84113
801-584-8400

Wyoming

Public Health Laboratory Services
Wyoming Department of Health
517 Hathaway Building
Cheyenne, WY 82002
307-777-7431

II. LABORATORY SERVICES

Policy Statement: The Region VIII Infertility Prevention Project Advisory Committee recommends that high complexity CLIA approved laboratories be used to process sexually transmitted disease (STD) specimens to ensure that patients/clients receive accurate, timely, and cost effective tests. Public health laboratories have the following qualities that make them a particularly good choice:

- Experience and expertise
- Strong communicable disease control mission
- Community-based focus
- Experience in public health data reporting
- Reliability and flexibility

CRITERIA FOR LABORATORY PARTICIPATION

All laboratories participating in this project must meet the following criteria:

1. Have a current CLIA certificate.

2. Be enrolled and show evidence of satisfactory performance in a recognized (by CMS) proficiency testing program.

3. Be performing confirmatory[1], supplemental[2], or amplification testing in LOW PREVALENCE POPULATIONS (see **Use of Repeat Testing in Low Prevalence Populations** below).
   Note: Low prevalence is considered to be less than 5% by current CDC guidelines.

4. Employ test method/s including FDA-licensed confirmatory tests or provide scientific evidence for the validity of the methods according to CLIA criteria.

5. Provide required data to the regional project and be reporting test results as required by state regulations.

6. Provide a turnaround time from laboratory receipt to report of 1-3 days.

Use of Repeat Testing in Low Prevalence Populations
Routine additional testing to improve the predictive value of a positive screening test should be considered when the prevalence of either *C. trachomatis* or *N. gonorrhoeae* infection is low. According to 2002 CDC Laboratory Guidelines: *Screening Tests to Detect Chlamydia trachomatis and Neisseria gonorrhoeae Infections*, approaches to additional testing, in order by theoretical consideration, after a positive screening test are:

- Test a second specimen with a different test that uses a different target, antigen or phenotype and a different format
- Test the original specimen with a different test that uses a different target, antigen, or phenotype and a different format
- Repeat the original test on the original specimen with a blocking antibody or competitive probe
- Repeat the original test on the original specimen

Laboratories participating in the Region VIII IPP Project, who test populations with low prevalence (<5% positivity), should consider routinely performing an additional test after a positive screening test, using one of the approaches listed above.

In practice, most clinical laboratories would not be able to provide testing using a different target or format. It may be difficult for clinicians to obtain a second specimen, and for the laboratory to track the repeat specimen for additional testing. In the case of NAATs, repeating the original test on the original specimen remains the most viable option.

Laboratory Request Forms
In general, the following minimal data should be included on a laboratory request form:
• Patient/Client Full Name
• Age or Date of Birth
• Gender
• Specimen Collection Date
• Source of Specimen
• Provider Location

Additional important information to be recorded on the form includes: patient/client zip code; race/ethnicity of patient/client; patient/client ID number; name of clinician; risk information; signs and symptoms; presumptive treatment; billing information; and other data as required by individual states. It is important to note that these data are for the laboratory request form and may differ from the data required by law when laboratories (or providers) report a suspected or confirmed case of CT.

Public Health Reporting Regulations
All laboratories within Region VIII must report positive chlamydia test results to their appropriate health department. Please note that state reporting requirements for positive test results vary and providers and laboratorians must be familiar with state laws and regulations.
• Colorado: Laboratories are required to report any result diagnostic of or highly correlated with clinical illness for Chlamydia trachomatis and Neisseria gonorrhoeae within seven days. These positive results may be reported to either the state health department or local health department. Similarly, physicians and hospitals are required to report gonorrhea cases within seven days of diagnosis. No physician reports of chlamydia are required.

• Montana: submitted within seven calendar days after it is received by the local health officer
• North Dakota: report to North Dakota Department of Health within 7 days
• South Dakota: reported within three working days from the time of identification.
• Utah: reported within three working days from the time of identification.
• Wyoming: report required within 7 days from both the attending healthcare provider/hospital and the laboratory performing the test.

Healthcare providers are also required by law to report documented or suspected cases of chlamydia to their local health departments.

Laboratory Reporting to Providers
Policy Statement: The laboratory results should be reported within three days of specimen receipt in the laboratory. This is the Region VIII standard for laboratory turn around time.

Interpretation Guide to *Chlamydia trachomatis* testing

F. INTERPRETATION OF LABORATORY RESULTS*

NON-CULTURE AMPLIFIED TEST		
Reported Result	**Lab Testing Performed**	**Interpretation**
NEGATIVE or NOT DETECTED	Initial test was negative and no additional testing was required.	This result indicates absence of *C. trachomatis* nucleic acid
POSITIVE or DETECTED	Initial test was positive and, if performed, the repeat test was positive.	This result indicates presence of *C. trachomatis* nucleic acid
BORDERLINE/ EQUIVOCAL	Initial test was in a signal range that requires a repeat test and the repeat test also produced a signal that was not positive, but may be indicative of the presence of low numbers of organisms in the specimen.	The result is SUSPICIOUS for the presence of *C. trachomatis* nucleic acid. Another sample should be collected and submitted.

CULTURE		
Reported Result	**Lab Testing Performed**	**Interpretation**
NEGATIVE or NOT ISOLATED	No typical fluorescence seen after incubation	This result indicates absence of viable *C. trachomatis*
POSITIVE or ISOLATED	Typical fluorescence seen after incubation	This result indicates presence of viable *C. trachomatis*

Unsatisfactory Specimens
The laboratory will not process certain specimens that are possibly compromised in ways that will affect the lab test. Such specimens will be reported back to the clinic as **UNSATISFACTORY**

SPECIMEN. Examples include:

- Specimen submitted from an unacceptable anatomical site for the test methodology. *Note: Rectal and pharyngeal specimens have been deemed acceptable for off-label use in laboratories that have fufilled the requirements for validation.*
- Inadequate specimen identification.
- Specimen has no identification on the tube or specimen and lab requisition identification do not match.
- Broken tube or specimen has leaked.
- Wrong swab; cytology brush; wrong collection kit used.
- Inappropriate urine specimen volume.
- Specimen transport deviates from manufacturer's recommended time and temperature.

Test of Cure

- Non-pregnant patients/clients do not need to be retested for chlamydia after completing treatment with azithromycin, doxycycline, ofloxacin or levofloxacin unless symptoms persist or re-infection is suspected because these therapies, if taken correctly, are highly efficacious.
- Non-pregnant patients/clients should be retested 3-4 weeks after completion of treatment with erythromycin because it is less efficacious and side effects discourage compliance.
- Test of cure is recommended for pregnant women regardless of the treatment regimen because of efficacy concerns in pregnancy.

The validity of chlamydial culture testing performed at less than 3 weeks following completion of therapy among patients/clients failing therapy has not been established. Thus, a repeat culture test should not be obtained until after 3 weeks following completion of treatment.

Non-culture tests conducted at less than 3-4 weeks following completion of therapy for patients/clients successfully treated may sometimes produce a false positive result because of the continued excretion of dead organisms. For a non-culture test, a repeat test should not be conducted until after 4 weeks.

Quality Assurance

Quality assurance (QA) refers to planned, step-by-step activities that let one know that testing is being carried out correctly, results are accurate, and mistakes are found and corrected to avoid adverse outcomes. Quality assurance is an ongoing set of activities that help to ensure that the test results provided are as accurate and reliable as possible for all persons being tested. Quality assurance activities should be in place during the entire testing process; pre-analytical, analytical, and post-analytical.

The purpose of a Quality Assurance Plan is to provide a periodic review of the laboratories systems and operations on a regular basis using defined monitors to assure quality for policies, procedures, and performance within the limits of acceptable standards.

An accurate chlamydia diagnosis is dependent on good laboratory practice for which quality assurance is integral. Recommended components of quality assurance include but are not limited to an effective quality assurance plan that includes:

- Use of positive and negative control specimens in each testing batch per manufacturer's recommendation.
- Adequate and continued technical staff training and competency. For more information about training opportunities, please refer to Appendix ##.
- Participation in a recognized proficiency testing program for the particular test method used.
- A well-defined, written test protocol.
- Maintenance of testing equipment following manufacturer's recommendations
- A process for performing a root cause analysis of identified issues or problems, determining a corrective action and monitoring the outcomes.

As part of the Quality Assurance process, the Region VIII Laboratory Subcommittee has developed a group e-mail that allows members to rapidly exchange information. The National Chlamydia Laboratory Coordinator, Dr. Richard Steece, is also a member of this group e-mail. This group e-mail is a method for notifying all project labs as well as the national laboratory coordinator of any problems related to laboratory testing products or methodology.

Choosing a Quality Laboratory

Factors to consider:

- Chlamydia technology offered.
- Experience/expertise in STD testing with sufficient volume to maintain proficiency.
- Adequate number of appropriately trained (and licensed if applicable) personnel performing the test.
- Caliber of testing personnel.
- Timely laboratory to clinic turnaround time.
- Consistently passes proficiency testing for chlamydia.
- Laboratory accreditation.
- Availability of technical consultation.
- Appropriate pick-up, handling and storage of specimens.
- Repeat testing of questionable results at no charge.
- Willingness to provide summary prevalence or other program reports.
- Electronic reporting capabilities.
- Test price is dependent on many factors, e.g., transportation, volume purchase, cost of reagents, personnel cost, packaging of tests, overhead, accessory services, etc. It is advisable to carefully determine what is included in the price and, to the extent possible, how it is determined. Price alone should not determine the choice of test.

Note: A visit to the laboratory is appropriate in order to review their quality assurance documents, see the physical facility and meet the managers, technical supervisor and staff.

E. SEXUAL ASSAULT AND ABUSE CASES

STD Testing in Cases of Sexual Assault or Abuse

Examination of victims is required for two purposes

1) to determine if an infection is present so that it can be successfully treated and
2) to acquire evidence for potential use in a legal investigation.

Testing to satisfy the first purpose requires a method that is highly sensitive, whereas satisfying the second purpose requires a method that is highly specific. NAAT tests are considered to be the most sensitive assays available, and culture the most specific.

Sexual Assault in Adults and Adolescents

Among sexually active adults, the identification of sexually transmitted infection after an assault might be more important for the psychological and medical management of the patient than for legal purposes because the infection could have been acquired before the assault.

Initial Examination

" Testing for N. gonorrhoeae and C. trachomatis from specimens collected from any sites of penetration or attempted penetration.

" Culture or FDA-cleared nucleic acid amplification tests for either N. gonorrhoeae or C. trachomatis. NAAT offer the advantage of increased sensitivity in detection of C. trachomatis.

Follow-Up Examinations

" Examination for STDs should be repeated within 1-2 weeks of the assault unless prophylactic treatment was provided.

" After the initial postassault examination, follow-up examinations provide an opportunity to detect new infections acquired during or after the assault

Sexual Assault or Abuse of Children

Because of the legal and psychosocial consequences of a false-positive diagnosis, only tests with high specificities should be used.

Initial and 2-Week Follow-Up Examinations

" Specimen collection for culture for *N. gonorrhoeae* from the pharynx and anus in both boys and girls, the vagina in girls, and the urethra in boys. For boys with a urethral discharge, a meatal specimen discharge is an adequate substitute for an intraurethral swab specimen. Isolates and specimens should be retained or preserved in case additional or repeated testing is needed. Gram stains are inadequate.

"Cultures for *C. trachomatis* from specimens collected from the anus in both boys and girls and from the vagina in girls. However, a meatal specimen should be obtained if urethral discharge is present. Pharyngeal specimens for C. trachomatis are not recommended for children of either sex because the yield is low. Data are insufficient to adequately assess the utility of nucleic acid amplification tests in the evaluation of children who might have been sexually abused, but these tests might be an alternative if confirmation is available and culture systems for C. trachomatis are unavailable."

Guidelines excerpted from:

Centers for Disease Control and Prevention. Sexually Transmitted Disease Treatment Guidelines 2006. MMWR 2006;55(No. RR-11)
http://www.cdc.gov/STD/treatment/2006/sexual-assault.htm

Centers for Disease Control and Prevention. Screening Tests To Detect Chlamydia trachomatis and Neisseria gonorrhoeae Infections -2002. MMWR 2002;51(No. RR-15)
http://www.cdc.gov/STD/LabGuidelines/4-LG.htm#Sexual%20Assault%20and%20Sexual%20Abuse

PUBLIC HEALTH LABORATORIES

Public Health Laboratories are a primary component of the national public health infrastructure. Public health laboratories provide data on which many public health programs rely. Public health laboratories provide essential services of disease surveillance and prevention as well as identification of new and re-emerging infectious diseases that could threaten the public's welfare. In fact, public health laboratories play a major role in the investigation of disease outbreaks and efforts to bring outbreaks under control.

Licensure and certification of medical and environmental laboratories is a state and/or federal mandate that public health laboratories have been responsible for overseeing for a long time. This legal responsibility continues to expand in order to protect the public's health as the demand to assure high quality laboratory services increases.

Other demands on public health laboratories increase as new public health problems arise, such HIV/AIDS and other STDs, tuberculosis, and drug resistant communicable diseases, such gonorrhea.

In addition to the daily services rendered to support state health and environmental programs, the public health laboratory must maintain expertise and flexibility to investigate disease outbreaks, to conduct special disease surveillance activities, to determine immunity levels for a variety of vaccine preventable diseases.

Clearly, the nation's public health laboratories play a vital role in disease prevention programs and are central to the national public health infrastructure. Public health laboratories represent a first line of defense in the rapid recognition and prevention of the spread of communicable disease. When properly supported and used, public health laboratories are a critical source of information.

Furthermore, Public Health Laboratories do the following:

1) Provide services which support and strengthen public health disease control and prevention programs, maternal and child health programs, and epidemiological programs;
2) Provide testing services of high quality at very low cost to the public;
3) Provide or facilitate training of laboratory personnel in both the public and private sectors;
4) Develop and provide oversight for state-wide quality assurance and laboratory improvement programs to ensure the reliability of laboratory data used for communicable disease control;
5) Identify the need for new laboratory methodology and develop new laboratory methods for use in the public sector for disease prevention;
6) Capture important laboratory data which are essential for public health analysis and policy decisions;
7) Provide reference diagnostic testing to private sector laboratories that may not have the capability to fully identify communicable diseases of public health importance;
8) Serve as a major data link with the U.S. Public Health Services Centers for Disease Control for surveillance of diseases on nation and international concern such as HIV/AIDS, tuberculosis, and sexually transmitted diseases.

Reference: Association of State and Territorial Public Health Laboratory Directors, Role of Public Health Laboratories, January 31, 1997.

APPENDIX A

CHLAMYDIA TESTING TECHNOLOGIES

(07/2003)

Richard Steece, Ph.D., D(ABMM)
National Chlamydia Laboratory Coordinator
Infertility Prevention Project

Note: The use of specific names, trademarks and products does not constitute an endorsement by the Association of Public Health Laboratories or the National Centers for Disease Control and Prevention.

CELL CULTURE METHOD

Reagent Manufacturer(s): Cells – Diagnostic Hybrids; ViroMed, Inc.; American Type Culture Collection (ATCC). **Fluorescent Antibody Reagents** – Wampole Laboratories MicroTrak (Syva), Bio-Rad Laboratories (Kallestad), VWR Scientific Products (Bartels).

Collection Sites: Endocervical, urethral (male and female), nasopharyngeal (infants), conjunctival, vaginal and rectal.

Specimen Handling: Use chlamydia specific transport media, e.g. 2SP, M4, etc. Refrigerate immediately after collection, transport at 2-8^0 C. If greater than 48 hours from collection to processing, store frozen at –70^0 C or colder (do not store at –20^0C C). Specimens can be stored for up to 6 months at –70^0 C.

Principle of Test: Specimens are inoculated and centrifuged into a medium containing cycloheximide-treated cells (McCoy, BGM, HeLa, etc.). The cells are grown in one-dram shell vials on glass cover slips or in multi-well plates. Specimens are incubated for 48-72 hours and stained with fluorescent labeled antibodies.

Turn-Around Time[1]: 2-4 days.

Advantages[2]: Culture is currently the preferred test for medico legal cases (very high specificity, 99-100%). Culture has been evaluated for several collection sites for which most non-culture methods have not been FDA cleared (approved). Culture preserves the organism for further studies such as "genetic fingerprinting" and antibiotic susceptibility testing.

Disadvantages: The turn around time is from 2-4 days for results (incubation period 48-72 hours). Culture is less sensitive (40-80%) compared to the newer Nucleic Acid Amplified Tests (90-96%). Culture requires expertise in cell culture and is technically more demanding than any of non-amplification tests. The specimen transport and storage times are critical. Culture is very labor intensive and expensive.

Confirmatory Testing: Not Done

Proficiency Testing Programs: Available. Laboratories performing cell culture for chlamydia are required to enroll in CMS (HCFA) approved proficiency testing programs.

Specimen Adequacy[3]: Proper specimen collection and transport is critical. Specific training on proper specimen collection and transport requirements is required for all clinicians collecting specimens. Training and periodic retraining should be a part of an ongoing quality assurance program. Laboratories are encouraged to periodically evaluate endocervical specimens for the presence of columnar and cuboidal epithelial cells, provide feedback, and as indicated, training to clinicians.

[1]Turn-Around Time: is defined as the time the specimen is received in the testing laboratory to the time the specimen is reported out by the testing laboratory within the normal work week (excluding weekends and holidays).

[2]Only highlights of the advantages and disadvantages are listed. For further information regarding the advantages and disadvantages check the literature and/or with the specific manufacturer.

[3]Screening Tests to Detect Chlamydia trachomatis and Neisseria gonorrhoeae Infections – 2002 MMWR October 18, 2002/Vol. 51/No. RR-15

ENZYME IMMUNOASSAY (EIA) TEST

<u>Reagent Manufacturers:</u> Wampole Laboratories MicroTrak (Syva), Bio-Rad Laboratories (Kallestad), and others.

<u>FDA Cleared (Approved) Collection Sites[1]:</u> Wampole Laboratories MicroTrak (Syva); Female endocervical, male urethral, male urine specimens, and ocular specimens from symptomatic patients. Bio-Rad Laboratories (Kallestad); male urethral and female endocervical specimens.

<u>Specimen Handling (Product Inserts)[1]:</u> <u>In General:</u> use the manufacturers specific transport system. Follow the specific manufacturers recommendations for transport conditions and times.

Wampole Laboratories MicroTrak (Syva); Endocervical, urethral and ocular specimens are transported and stored at 2^0 to 25^0C and tested within 7 days of collection. Urine specimens should be transported and stored at 2^0 to 8^0C and tested within 7 days of collection. Do not freeze specimens.

Bio-Rad Laboratories (Kallestad); Specimens are stable at room temperature for up to 7 days, although refrigeration is preferable.

<u>Principle of Test:</u> **(Bio-Rad Laboratories (Kallestad)** When treated specimens are added to a microplate well, chlamydia present (antigens) bind to antibody coating the microplate well. Next, an antibody specific for chlamydia is added which binds to chlamydia (antigen) captured by the antibody on the microplate well. A solution of an enzyme-conjugated antibody is added which then binds to the chlamydia antibody. Following a washing step to remove any unbound components in the microplate well an enzyme specific substrate is added to each well. If chlamydia (antigen) is present the substrate reacts with the enzyme and produces a color change that can be detected and measured in a spectrophotometer.

<u>Turn-Around Time[2]:</u> 1-3 days

<u>Advantages:</u> The Enzyme Immunassay Tests (EIA) are effective for large scale screening (large volume). Viable organisms are not required for the test. EIAs provide a rapid turn around time (3-5 hours) for results. In addition, they are relatively inexpensive and only require moderate technical skill.

<u>Disadvantages:</u> The EIA is less (60-70%) sensitive compared to the Nucleic Acid Amplified Test (90-96%). In addition, the specificity is less than with Cell Culture (specificity 95-99%). The EIA is not FDA cleared (approved) for rectal, respiratory, vaginal, etc. specimens. While the Wampole Laboratories MicroTrak (Syva) EIA test is FDA cleared (approved) for testing urine specimens, the sensitivity and specificity is significantly reduced and screening urines with the EIA is not currently recommended.
<u>Confirmatory Testing:</u> Recommended when screening populations with low prevalence (positivity), e.g. ≤5% positivity. Several methods are available to confirm positive results. A neutralization (blocking assay) and a Direct Fluorescent Antibody test are both routinely used for confirmatory testing of initial positive results.

<u>Proficiency Testing Programs:</u> Available. Laboratories performing EIA for chlamydia are required to enroll in CMS (HCFA) approved proficiency testing programs.

<u>Specimen Adequacy[3]</u> Proper specimen collection and transport is critical. Specific training on proper specimen collection and transport requirements is required for all clinicians collecting specimens. Training and periodic retraining should be a part of an ongoing quality assurance program. Laboratories are encouraged to periodically evaluate endocervical specimens for the presence of columnar and cuboidal epithelial cells, provide feedback, and as indicated, training to clinicians.

[1]Collection Sites and Specimen handling information is directly from product inserts.
[2]<u>Turn-Around Time:</u> is defined as the time the specimen is received in the testing laboratory to the time the specimen is reported out by the testing laboratory within the normal work week (excluding weekends and holidays).
[3]Screening Tests to Detect Chlamydia trachomatis and Neisseria gonorrhoeae Infections – 2002 MMWR October 18, 2002/Vol. 51/No. RR-15

DIRECT FLUORESCENT ANTIBODY TEST (DFA)

<u>Reagent Manufacturers:</u> Wampole Laboratories MicroTrak (Syva), Bio-Rad Laboratories (Kallestad), VWR Scientific Products (Bartels).

FDA Cleared (Approved) Collection Sites[1]: Wampole Laboratories MicroTrak (Syva); Urethral (males), cervical (females), rectal (symptomatic), conjunctival (symptomatic) and nasopharyngeal specimens (symptomatic). Bio-Rad Laboratories (Kallestad); Urogenital (adult), rectal (adult), ocular (pediatric) and nasopharyngeal (pediatric).

<u>Specimen Handling (Product Inserts)[1]:</u> <u>In General:</u> use the manufacturers specific transport system. Follow the specific manufacturers recommendations for transport conditions and times.

Wampole Laboratories MicroTrak (Syva); For best results, store/transport at 20^0 to 30^0C or refrigerated at 2^0 to 8^0C and stain within 7 days of collection. If not stained within 7 days, fixed specimens should be stored at -20^0C.

Bio-Rad Laboratories (Kallestad); Store the slide in the Pathfinder Collection Kit at room temperature. Process the slide within 7 days.

<u>Principle of Test:</u> The patient specimen is collected, the slide and mailing container are both marked with the patients name and/or ID. The swab is then lightly rolled onto the single welled-slide provided (within the encircled well area only) and the specimen is fixed immediately (methanol). After the specimen has air dried (2-3 minutes) it is placed in the mailing container and transported to the laboratory. At the laboratory, the slides are then stained with a fluorescent labeled antibody specific for chlamydia. The antibody conjugate binds specifically to any *Chlamydia trachomatis* present in the specimen. A rinse step removes unbound antibody. When slides are viewed under a fluorescence microscope, chlamydia positive specimens contain apple-green elementary bodies contrasted by the red background of the counterstained cells.

<u>Turn-Around Time[2]:</u> 1-2 days

<u>Advantages:</u> The DFA is FDA cleared (approved) for many different types of specimens. Viable organisms are not required for the test. The test provides a rapid turn around time (1-2 hours) for results. In addition, the DFA is relatively inexpensive, requires moderate technical skill, and the quality of the specimen can be visualized (columnar or cuboidal epithelial cells).

<u>Disadvantages:</u> The DFA is not suitable for large volume testing. The DFA is less (50-70%) sensitive compared to the Nucleic Acid Amplified Test (90-96%). In addition, the specificity is less than with Cell Culture (specificity 95-99%).

Confirmatory Testing: Not Available

Proficiency Testing Programs: Available. Laboratories performing the DFA test for chlamydia are required to enroll in CMS (HCFA) approved proficiency testing programs.

Specimen Adequacy[3] Proper specimen collection and transport is critical. Specific training on proper specimen collection and transport requirements is required for all clinicians collecting specimens. Training and periodic retraining should be a part of an ongoing quality assurance program. Laboratories are encouraged to periodically evaluate endocervical specimens for the presence of columnar and cuboidal epithelial cells, provide feedback, and as indicated, training to clinicians.

[1]Collection Sites and Specimen handling information is directly from product inserts.

[2]Turn-Around Time: is defined as the time the specimen is received in the testing laboratory to the time the specimen is reported out by the testing laboratory within the normal work week (excluding weekends and holidays).

[3]Screening Tests to Detect Chlamydia trachomatis and Neisseria gonorrhoeae Infections – 2002 MMWR October 18, 2002/Vol. 51/No. RR-15

GEN-PROBE PACE® 2 ASSAY – (NUCLEIC ACID PROBE [NAP] TEST)

Reagent Manufacturer(s): Gen-Probe, Incorporated (PACE® 2, *Chlamydia trachomatis* (CT) or *Neisseria gonorrhoeae* (GC), PACE® 2C, Combined CT/GC)

FDA Cleared (Approved) Collection Sites[1]: Gen-Probe, Incorporated, PACE® 2: Endocervical, male urethral and conjunctival specimens; PACE® 2C: Endocervical and male urethral specimens.

Specimen Handling (Product Inserts)[1]: In General: use the manufacturers specific transport system. Follow the specific manufacturers recommendations for transport conditions and times. Gen-Probe, Incorporated, PACE® 2, PACE® 2C: Transport the tubes to the laboratory at 2^0 to 25^0C and store at 2^0 to 25^0C until tested. Samples should be assayed within 7 days of collection. If longer storage is necessary, process the specimen as described in sample preparation and freeze at -20^0 to -70^0C.

Principle of Test: The Gen-Probe PACE system is a nucleic acid probe (NAP) that uses a chemiluminescent labeled, single-stranded DNA probe complementary to the ribosomal RNA of the target organism (CT or GC). After the ribosomal RNA is released from the organism by heating the labeled DNA probe combines with the target organism's ribosomal RNA to form a stable DNA:RNA hybrid. The labeled, DNA:RNA hybrid is separated from the non-hybridized probe and is measured in the Gen-Probe luminometer. The test results are calculated as the difference between the response of the specimen and the mean response of the negative reference (control). In the PACE® 2C test (combination assay), both CT and GC are detected simultaneously, positive test result must be rerun with CT and GC specific reagents.

Turn-Around Time[2]: 1-3 days

Advantages: The Gen-Probe PACE 2 assays are suitable for low, medium and high volume laboratories. Viable organisms are not required for the test. Nucleic Acid Probes (NAP) provide a rapid turn around time (3-5 hours) for results. Specificity is fairly high, and the assay is not susceptible to carry-over contamination that may occur in nucleic acid amplification tests. In addition, they are relatively inexpensive and only require moderate technical skill. With the Gen-Probe assay, it is also possible to test for CT and GC from the same specimen.

Disadvantages: The Gen-Probe Pace assays are less sensitive than Nucleic Acid Amplified Tests. The Gen-Probe PACE assays are not FDA cleared (approved) for rectal, respiratory, and vaginal specimens.

Confirmatory Testing: Available and recommended when screening populations with low prevalence (positivity), e.g. ≤5% positivity or specimens above the test cut off (CO) to ≤1,500 Relative Light Units (RLU). The Gen-Probe confirmatory test is the Probe Competition Assay (PCA), similar to the EIA neutralization confirmatory procedure.

Proficiency Testing Programs: Available. Laboratories performing NAP tests for CT and GC are required to enroll in CMS (HCFA) approved proficiency testing programs.

Specimen Adequacy[3] Proper specimen collection and transport is critical. Specific training on proper specimen collection and transport requirements is required for all clinicians collecting specimens. Training and periodic retraining should be a part of an ongoing quality assurance program. Laboratories are encouraged to periodically evaluate endocervical specimens for the presence of columnar and cuboidal epithelial cells, provide feedback, and as indicated, training to clinicians.

[1]Collection Sites and Specimen handling information is directly from product inserts.

[2]Turn-Around Time: is defined as the time the specimen is received in the testing laboratory to the time the specimen is reported out by the testing laboratory within the normal work week (excluding weekends and holidays).

[2]Screening Tests to Detect Chlamydia trachomatis and Neisseria gonorrhoeae Infections – 2002 MMWR October 18, 2002/Vol. 51/No. RR-15

NUCLEIC ACID PROBE/SIGNAL AMPLIFICATION (NAPSA) TEST

Reagent Manufacturer(s): Digene CT/GC Hybrid Capture II Test

FDA Cleared (Approved) Collection Sites[1]: Endocervical and male urethral specimens

Specimen Handling (Product Inserts)[1]: In General: use the manufacturers specific transport system. Follow the specific manufacturers recommendations for transport conditions and times. Specimens may be held for up to two weeks at room temperature and shipped without refrigeration to the testing laboratory. At the testing laboratory, specimens should be stored at 2^0 to 8^0C if the assay is to be performed within one week. If the assay will be performed later than one week, store specimens at –20^0C for up to 3 months. Specimens collected in Specimen Transport Medium can not be used for culture or other testing methods.

Principle of Test: Specimens are denatured to produce single strands of DNA. A specific single stranded RNA probe is added which recognizes and hybridizes with specific DNA sequences of the CT and GC genomes and plasmids. The resulting RNA/DNA hybrids are then captured on a microtiter plate by antibodies specific to the hybrids. The use of enzyme conjugated antibodies and a substrate produce a signal that is measured on a luminometer. Multiple antibodies, each conjugated to enzyme, bind to each captured hybrid, resulting in a substantial "signal amplification".

Turn-Around Time[2]: 1-3 days

Advantages: The Digene CT/GC Hybrid Capture II Test is effective for large scale screening (high volume). Viable organisms are not required for the test. The NAPSA test provides a rapid turn around time (5-6 hours) for results. In addition, it only requires moderate technical skill.

Disadvantages: Insufficient peer reviewed publications are available for the Digene CT/GC Hybrid Capture II Test to accurately predict test sensitivity and specificity. However, based on the few preliminary publications and the signal amplification technology, the sensitivity appears to be slightly higher that the current Gen-Probe Pace 2/2C Test and less than the Nucleic Acid Amplified Tests (NAAT). Test specificity appears to compare with the Gen-Probe Pace Test. The NAPSA is not FDA cleared (approved) for rectal, respiratory, and vaginal specimens.

Confirmatory Testing: Not Available

Proficiency Testing Programs: Available. Laboratories performing NAPSA for chlamydia are required to enroll in CMS (HCFA) approved proficiency testing programs.

Specimen Adequacy[3] Proper specimen collection and transport is critical. Specific training on proper specimen collection and transport requirements is required for all clinicians collecting specimens. Training and periodic retraining should be a part of an ongoing quality assurance program. Laboratories are encouraged to periodically evaluate endocervical specimens for the presence of columnar and cuboidal epithelial cells, provide feedback, and as indicated, training to clinicians.

[1]Collection Sites and Specimen handling information is directly from product inserts.

[2]Turn-Around Time: is defined as the time the specimen is received in the testing laboratory to the time the specimen is reported out by the testing laboratory within the normal work week (excluding weekends and holidays).

[3]Screening Tests to Detect Chlamydia trachomatis and Neisseria gonorrhoeae Infections – 2002 MMWR October 18, 2002/Vol. 51/No. RR-15

NUCLEIC ACID AMPLIFICATION TESTS

GENERAL COMMENTS:

ADVANTAGES:

Nucleic Acid Amplification Tests (NAATs) are the most sensitive and specific tests currently available for the detection of chlamydia. The sensitivity of NAATs are in the mid to high 90s, with specificity equal to cell culture (99-100%). While culture is the preferred test for medico legal cases, most experts have suggested that NAATs for chlamydia could be used as an alternative to culture if culture is not available. If a NAAT is used for medico legal purposes, it is currently recommended that an alternate NAAT be performed on all initial positive results[1,2]. Viable organisms are not required for the test. NAATs provide a rapid turn around time (3-5 hours) for results

DISADVANTAGES:

The NAATs are technically demanding and special areas[3] within laboratories need to be utilized in order to run these tests due the increased potential for cross contamination of specimens. The specimen transport and storage times with most NAATs are critical (excluding Gen-Probe APTIMA Combo 2). The NAATs are not FDA cleared (approved) for respiratory, conjunctival, vaginal or rectal specimens. The NAATs are still fairly expensive, running from 2-4 times the diagnostic reagent costs for Non-Amplified Tests. No confirmatory or supplemental tests are currently FDA cleared for the NAATs. However, Gen-Probe currently provides Analyte Specific Reagents[4] (ASR) for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* that may be used to "confirm" positive specimens. Laboratories initiating any Nucleic Acid Amplified Test (NAAT) should repeat all positive specimens during their initial verification process and evaluate the need to continue to "confirm" positive specimens on a routine basis. For additional information on "confirmation" of positive specimens refer to CDCs, "Screening Tests To Detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections – 2002", MMWR, October 18, 2002/Vol. 51/No. RR-15[2].

[1]CDC. Sexually Transmitted Diseases Treatment Guidelines. 2002. MMWR 2002;51:72-73.

[2]CDC. Screening Tests to Detect Chlamydia trachomatis and Neisseria gonorrhoeae Infections. 2002. MMWR 2002;51:23.

[3]Each manufacturer has specific laboratory facility and space requirements for their specific NAAT technology. Any laboratories initiating NAATs should check with the manufacturer on the facility and space requirements before making any test methodology decisions.

[4]See Appendix A for more information on Analyte Specific Reagents (ASR).

POLYMERASE CHAIN REACTION (PCR) TEST

Reagent Manufacturer: Roche Diagnostics; Roche (**COBAS** AMPLICOR CT/NG Tests) and Roche (**MICROTITER** AMPLICOR CT/NG Test)

FDA Cleared (Approved) Collection Sites[1]: Urine from males and females, endocervical swab specimens, male urethral swab specimens (symptomatic or asymptomatic).

Specimen Handling (Product Inserts)[1]: Urine specimens may be transported to the test site at 18^0 to 25^0C. Urine specimens are stable for 24 hours at 18^0 to 25^0C. Urine specimens that require shipment to off-site test centers must be shipped via overnight delivery with guaranteed arrival within 24 hours; shipment can be at 18^0 to 25^0C. If urine specimens are shipped at 18^0 to 25^0C, they should be stored at 2^0 to 8^0C until time of shipment to ensure that the period of 18^0 to 25^0C storage does not exceed 24 hours. Urine specimens that will not be processed within 24 hours of collection must be stored at 2^0 to 8^0C and must be processed within 7 days of collection. Urine specimens that cannot be processed within 7 days of collection can be stored at -20^0C or lower for up to 2 months.

Swab specimens may be transported to the test site at 18^0 to 25^0C provided that the total time of storage and transport at 18^0 to 25^0C is less than 1 hour. Refrigerate swab specimens if transport to the laboratory is delayed for more than one hour from the time of collection. If used for culture, specimens should be handled using recommendations for culture (-70^0C if not cultured within 24 hr). Swab specimens that require off-site laboratories should be shipped at refrigerated temperature as soon as possible after collection according to the laboratory's procedures for the transport of chlamydial culture specimens. Store swab specimens that are not tested upon receipt at the testing laboratory within 7 days of collection must be stored at -20^0C or colder and tested within 30 days of collection.

Principle of Test: Specimens are treated to release the chlamydia DNA and make it accessible for replication and detection. After treatment two short, labeled (biotinylated) nucleotides (primers) that are complementary to the chlamydia target DNA along with an enzyme (polymerase) and excess nucleotides are added to the reaction mixture. The primers bind to the specific chlamydia target DNA. The polymerase enzyme extends each DNA sequence utilizing the excess nucleotides to form a complementary DNA sequence (amplicon). This amplification process occurs when the reaction mixture is incubated in a DNA thermal cycler and alternately heated and cooled, multiple times (cycles). Each cycle exponentially increases the amount of target DNA. After amplification, the amplicons are hybridized by utilizing a specific DNA probe that is complementary to the amplicons. This probe is bound to a solid support (microparticles) and used to capture the amplicons. The last step in this process is the assay detection system that uses a standard enzyme/substrate reaction in order to detect color change and subsequent positive specimens.

Turn-Around Time[2]: 1-3 days

Advantages: The PCR NAAT is FDA cleared for both chlamydia and gonorrhea screening on the same specimen. In addition, the test is FDA cleared (approved) for urine specimens (sensitivity for urine specimens is slightly lower compared to swab specimens). The Roche COBAS AMPLICOR instrument is semi-automated. The Roche PCR test comes in two configurations, the COBAS AMPLICOR system for the smaller volume laboratories and the Roche Microtiter Plate test for larger volume laboratories. The PCR test has the ability to include an internal amplification control that enables the laboratory to determine the presence of inhibitors that may produce a false negative result. The PCR test has the ability to prevent false positive results (contamination) from any previously amplified products (amplicons) with AmpErase.

Disadvantages: Only limited number of specimens can be run with the Roche COBAS AMPLICOR system (similar to the Abbott LCx instrument).

Proficiency Testing Programs: Available. Laboratories performing NAATs for chlamydia are required to enroll in CMS (HCFA) approved proficiency testing programs.

Specimen Adequacy[3] Proper specimen collection and transport is critical. Specific training on proper specimen collection and transport requirements is required for all clinicians collecting specimens. Training and periodic retraining should be a part of an ongoing quality assurance program. Laboratories are encouraged to periodically evaluate endocervical specimens for the presence of columnar and cuboidal epithelial cells, provide feedback, and as indicated, training to clinicians.

[1]Collection Sites and Specimen handling information is directly from product inserts.

[2]Turn-Around Time: is defined as the time the specimen is received in the testing laboratory to the time the specimen is reported out by the testing laboratory within the normal work week (excluding weekends and holidays).

[3]Screening Tests to Detect Chlamydia trachomatis and Neisseria gonorrhoeae Infections – 2002 MMWR October 18, 2002/Vol. 51/No. RR-15

GEN-PROBE AMPLIFIED CHLAMYDIA TRACHOMATIS ASSAY (AMP CT) –(TRANSCRIPTION MEDIATED AMPLIFICATION [TMA] TEST)

Reagent Manufacturer: Gen-Probe, Incorporated

FDA Cleared (Approved) Collection Sites[1]: Endocervical and male urethral swab specimens and in female and male urine specimens.

Specimen Handling (Product Inserts)[1]: Transport swab specimens to the laboratory and store at 2^0 to 25C until tested. Specimens should be assayed with the AMP CT Assay within 7 days of collection. If longer storage is needed, freeze at -20^0C to -70^0C.

Transport urine specimens to the laboratory at ambient temperature within 24 hours of collection, or at 2^0C to 8^0C within 7 days of collection. Store urines at 2^0C to 8^0C within 7 days of collection. Urine specimens should be assayed with the Gen-Probe Amplified Chlamydia Trachomatis Assay within 7 days of collection. If longer storage is needed, mix the specimen, then aliquot into single-use portions and freeze at -70^0C. Once thawed, do not re-freeze urines.

Principle of Test: Specimens are processed (treated) to release rRNA from CT and amplified utilizing Transcription-Mediated Amplification (TMA). The TMA system amplifies specific rRNA target via DNA intermediates. After amplification the amplified products (amplicons) are detected by nucleic acid hybridization (probes). The chemiluminescent single-stranded DNA probe is complementary to the amplicons. The labeled DNA probe combines with the amplicons to form a stable RNA:DNA hybrid. The labeled RNA:DNA hybrids are measured in a luminometer. A positive result is a luminometer reading greater than or equal to the cut-off value. A value less than the cut-off is a negative result.

Turn-Around Time[2]: 1-3 days

Advantages: The AMP CT assay is FDA cleared for urine specimens (sensitivity for urine specimens is slightly lower compared to swab specimens). Because TMA is isothermal, no thermocycling equipment is needed. Since the amplification target is RNA and RNA is more labile than DNA the potential for cross contamination is reduced.

Disadvantages: The AMP CT assay is not FDA cleared for gonorrhea screening. The current configuration of the AMP CT requires multiple pipeting steps and is labor intensive. A large volume version is available which uses a semi-automated Tecan pipetting/diluting station.

Proficiency Testing Programs: Available. Laboratories performing NAATs for chlamydia are required to enroll in CMS (HCFA) approved proficiency testing programs.

Specimen Adequacy[3] Proper specimen collection and transport is critical. Specific training on proper specimen collection and transport requirements is required for all clinicians collecting specimens. Training and periodic retraining should be a part of an ongoing quality assurance program. Laboratories are encouraged to periodically evaluate endocervical specimens for the presence of columnar and cuboidal epithelial cells, provide feedback, and as indicated, training to clinicians.

[1]**Collection Sites and Specimen handling information is directly from product inserts.**

[2]**Turn-Around Time: is defined as the time the specimen is received in the testing laboratory to the time the specimen is reported out by the testing laboratory within the normal work week (excluding weekends and holidays).**

[2]**Screening Tests to Detect Chlamydia trachomatis and Neisseria gonorrhoeae Infections – 2002 MMWR October 18, 2002/Vol. 51/No. RR-15**

GEN-PROBE® APTIMA® Combo 2 Assay

Reagent Manufacturer: Gen-Probe, Incorporated

FDA Cleared (Approved) Collection Sites[1]**:** Endocervical and male urethral swab specimens and in female and male urine specimens. The assay may be used to test specimens from symptomatic and asymptomatic individuals.

Specimen Handling (Product Inserts)[1]**:** Swab specimens must be transported to the laboratory in the swab specimen transport medium and tube. Swab specimens must be transported to the laboratory at 2^0 to 30^0C and tested within 60 days of collection.

Urine specimens can be transported to the laboratory at 2^0 to 30^0C in either the primary collection device (urine cup) or in the urine specimen transport tube. Urine specimens must be transferred into the GEN-PROBE specimen transport tube within 24 hours of collection and before being assayed. After transfer, urine specimens can be stored at 2^0 to 30^0C for up to 30 days after collection.

The APTIMA Combo 2 Assay Unisex Swab Specimen Collection Kit for Endocervical and Urethral Swab Specimens and the APTIMA Combo 2 Assay Urine Specimens Collection Kit for Male and Female Urine Specimens are intended to be used only with the GEN-PROBE APTIMA Combo 2 Assay. Performance has not been established with other products.

Principle of Test: The GEN-PROBE APTIMA Combo 2 Assay combines the technologies of target capture, Transcription-Mediated Amplification (TMA) and Dual Kinetic Assay (DKA).

Target Capture uses capture oligonucleotides and magnetic microparticles to capture target nucleic acid and separate potentially inhibiting substances from samples, thereby isolating the desired nucleic acid targets for amplification. **Transcription-Mediated Amplification (TMA)** uses specific ribosomal RNA target via DNA intermediates to potentially produce billions of copies of target nucleic acid. **The Dual Kinetic Assay (DKA)** uses single stranded chemiluminescent DNA probes that are complementary to a region of each target amplicon and are labeled with different acridinium ester molecules. The labeled probe combines with amplicon to form stable RNA:DNA hybrids. The labeled RNA:DNA hybrids are measured in a luminometer. In DKA, differences in the kinetic profiles of the labeled probes specific for *C. trachomatis* and *N. gonorrhoeae* allow for the differentiation of signal; kinetic profiles are derived from measurements of photon output during the detection read time. The chemiluminescent detection reaction for *C. trachomatis* signal has very rapid kinetics and has the “flasher” kinetic type. The chemiluminescent detection signal for *N. gonorrhoeae* is relatively slower and has the “glower” kinetic type. Assay results are determined by a cut-off based on the total Relative Light Unit (RLU) and the kinetic curve type.

Turn-Around Time[2]: 1-3 days

Advantages: The APTIMA Combo 2 is FDA cleared for urine specimens (sensitivity for urine specimens is equal to or slightly higher than for swab specimens). Direct tube sampling combined with penetrable cap technology simplifies specimen processing. Centrifugation of samples and swab expression is not required and all specimen types may be batched together. Because TMA is isothermal, no thermocycling equipment is needed. Since the amplification target is RNA and RNA is more labile than DNA the potential for cross contamination is reduced. The utilization of Target Capture should reduce or eliminate potential inhibitors. The Dual Kinetic Assay allows simultaneous detection of chlamydia and gonorrhea. Gen-Probe also has Analyte Specific Reagents (ASR) available for CT and GC testing that target a different region of the target rRNA to allow for confirmation testing in accordance with CDC recommendations[3]. The availability of single CT and GC ASR may reduce the price for confirmatory testing and/or screening for individual agents.

Disadvantages: The APTIMA Combo 2 is a combined test for CT and GC. It is generally not economical, practical or ethical to attempt to report only single results.

Proficiency Testing Programs: Available. Laboratories performing NAATs for chlamydia are required to enroll in CMS (HCFA) approved proficiency testing programs**.**

Specimen Adequacy[3] Proper specimen collection and transport is critical. Specific training on proper specimen collection and transport requirements is required for all clinicians collecting specimens. Training and periodic retraining should be a part of an ongoing quality assurance program. Laboratories are encouraged to periodically evaluate endocervical specimens for the presence of columnar and cuboidal epithelial cells, provide feedback, and as indicated, training to clinicians.

[1]Collection Sites and Specimen handling information is directly from product inserts.

[2]Turn-Around Time: is defined as the time the specimen is received in the testing laboratory to the time the specimen is reported out by the testing laboratory within the normal work week (excluding weekends and holidays).

[3]Screening Tests to Detect Chlamydia trachomatis and Neisseria gonorrhoeae Infections – 2002 MMWR October 18, 2002/Vol. 51/No. RR-15

STRAND DISPLACEMENT AMPLIFICATION (SDA) TEST

Reagent Manufacturer: Becton Dickinson (BD ProbeTec ET System)

FDA Cleared (Approved) Collection Sites[1]: The BDProbeTec ET System is designed to detect the presence of Chlamydia trachomatis in endocervical swabs, male urethral swabs and male and female urine specimens using the appropriate collection method. Specimens may be from symptomatic or asymptomatic females for the BDProbeTec ET CT and GC Assays, from symptomatic or asymptomatic males for the BDProbeTec ET CT Assay, and from symptomatic males for the BDProbeTec ET GC Assay.

Specimen Handling (Product Inserts)[1]: The **CULTURETTE** DIRECT collection swab and Mini-Tip **CULTURETTE** DIRECT swab must be stored and transported to the laboratory and/or test site at 2^0 to 27^0C within 4-6 days of collection. Storage up to 4 days has been validated with clinical specimens; storage up to 6 days has been demonstrated with seeded specimens. NOTE: if specimens cannot be transported directly to the testing laboratory under ambient temperatures (15^0 to 27^0C) and must be shipped, an insulated container with ice should be used with either overnight or 2-day delivery vendor. BD has FDA clearance for their "wet swab" transport system. In the "wet swab" transport system the swab dilution medium has been added to the transport tube. In addition, BD has received FDA clearance for their BD Viper specimen processing station.

If the test site adds the UPP[2], store and transport urine specimens to the test site at 2^0 to 8^0C within 4-6 days of collection. Do not freeze the specimen.

If the UPP is added at the collection site, store and transport urine specimens to the laboratory or test site at 2^0 to 8^0C within 4-6 days of collection or at 15^0 to 27^0C within 2 days of collection. Do not freeze the specimen.

Principle of Test: The SDA is an amplified DNA assay based on the simultaneous amplification and detection of target DNA using amplification primers and a fluorescent labeled detector probe. The SDA reagents are dried in two separate disposable microwell strips. The processed sample is added to the priming microwell that contains the amplification primers, fluorescent labeled detector probe, and other reagents necessary for amplification. After incubation, the reaction mixture is transferred to the amplification microwell, which contains two enzymes (a DNA polymerase and a restriction endonuclease) necessary for the SDA. The amplification microwells are sealed to prevent contamination and then incubated in a thermally controlled fluorescent reader that monitors each reaction for the generation of amplified products. The presence or absence of chlamydia is determined by comparing specimen results to a pre-determined cutoff value. A positive result has a reading greater than or equal to the cut-off value. A value less than the cut-off is a negative result.

Turn-Around Time[3]: 1-3 days

Advantages: The SDA is FDA cleared (approved) for urine specimens (sensitivity for urine specimens is slightly lower compared to swab specimens). The SDA offers an optional amplification control to monitor each specimen for the presence of an inhibitor that may produce a false negative result. The SDA has a high throughput, up to 564 patient specimens (with no amplification control, chlamydia only) per 8 hour shift. The new "wet swab" transport effectively reduces the specimen processing time and may reduce the cost of labor.

Disadvantages: The SDA has been reported to have some problems with cross contamination from the internal control. In addition several papers have indicated that the SDA GC test may cross react with other Neisseria strains. The new "wet swab" transport is more expensive than the previous swab transport system and may add to the diagnostic cost of the test.

Proficiency Testing Programs: Available. Laboratories performing NAATs for chlamydia are required to enroll in CMS (HCFA) approved proficiency testing programs.

Specimen Adequacy[4] Proper specimen collection and transport is critical. Specific training on proper specimen collection and transport requirements is required for all clinicians collecting specimens. Training and periodic retraining should be a part of an ongoing quality assurance program. Laboratories are encouraged to periodically evaluate endocervical specimens for the presence of columnar and cuboidal epithelial cells, provide feedback, and as indicated, training to clinicians.

[1]Collection Sites and Specimen handling information is directly from product inserts.
[2]UPP-Urine Processing Pouch
[3]Turn-Around Time: is defined as the time the specimen is received in the testing laboratory to the time the specimen is reported out by the testing laboratory within the normal work week (excluding weekends and holidays).
[4]Screening Tests to Detect Chlamydia trachomatis and Neisseria gonorrhoeae Infections – 2002 MMWR October 18, 2002/Vol. 51/No. RR-15

Appendix A

Analyte Specific Reagents

The Analyte Specific Reagents regulation rule was issued in the Federal Register November 21, 1997, Volume 62, Number 225. The rule essentially allowed certain individual reagents to be available for laboratories to use in their own in-house tests. The do not require manufacturer's to submit a 510(k) or PMA.

The APTIMA CT and GC Analyte Specific Reagent (ASR) products can be used for the detection of *Chlamydia trachomatis* or *Neisseria gonorrhoeae* rRNA. The manufacturer may make no performance, stability, or specimen claims for these products. Reagents are specific for an analyte(s) and are not sold as a "kit". These reagents are not FDA cleared and the customer must verify their performance. For additional information on test verification and validation see the Federal Register January 24, 2003, Volume 68, Number 16, Section 493.1253.

The manufacturer is not allowed to troubleshoot any possible performance related problems with or for the testing laboratory. The manufacturer may refer a laboratory that needs assistance to another laboratory running the assay. The manufacturer isn't allowed to provide any procedures to the testing laboratory. In addition, the manufacturer cannot solicit customers for these products. However, they can respond to unsolicited requests for information form laboratories or physicians.

The testing laboratory must include a disclaimer statement when reporting results as follows: "This test was developed and its performance characteristics determined by (Specific Laboratory Name). It has not been cleared or approved by the U.S. Food and Drug Administration."

In addition to a disclaimer statement, results should be reported consistent with the CDC Screening Guidelines[1], "When additional testing has been performed the laboratory should report the results of both the screening test and the additional tests, as well as the overall interpretation."

[1]Screening Tests to Detect Chlamydia trachomatis and Neisseria gonorrhoeae Infections – 2002 MMWR October 18, 2002/Vol. 51/No. RR-15

Appendix B
Training/Education Resources for Laboratorians

- National LabTraining Network

The National Laboratory Training Network (NLTN), dedicated to improving laboratory practice of public health significance through quality continuing education, is a training system sponsored by the Association of Public Health Laboratories (APHL) and Centers for Disease Control and Prevention (CDC).

- National Chlamydia laboratory committee

The National Chlamydia Laboratory Committee (NCLC) was created in 1995 by the Association of Public Health Laboratories (APHL) in conjunction with the Centers for Disease Control and Prevention (CDC) to provide a forum for the laboratory subcommittees of the ten Regional Infertility Prevention Project Advisory Groups to discuss issues and concerns regarding chlamydia screening tests and to develop a common position acceptable to the Regions. This workgroup does not establish national policy. However, it does play a major role in contributing to local, regional, and national strategies on laboratory issues related to chlamydia screening.

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