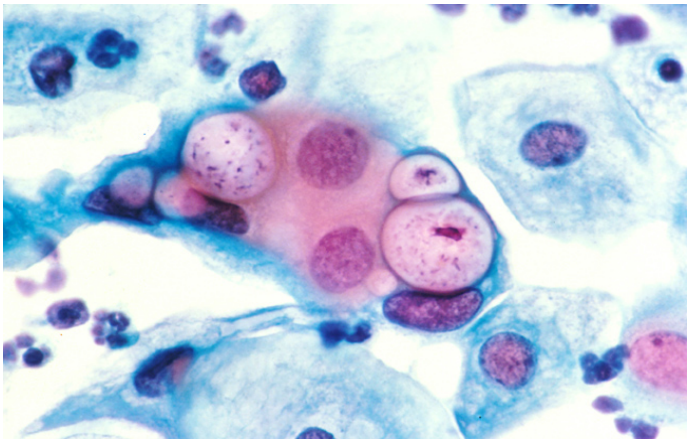
Laboratory Diagnostic Testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*



Expert Consultation Meeting Summary Report

January 13-15, 2009

Atlanta, GA

APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES

This report was produced in cooperation with the Centers for Disease Control and Prevention.

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In the last decade there have been major changes and improvements in STD testing technologies. While these changes have created great opportunities for more rapid and accurate STD diagnosis, they may also create confusion when laboratories attempt to incorporate new technologies into the existing structure of their laboratory. With this in mind, the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) convened an expert panel to evaluate available information and produce recommendations for inclusion in the Guidelines for the Laboratory Diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in the United States. An in-person meeting to formulate these recommendations was held on January 13-15, 2009 on the CDC Roybal campus. The panel included public health laboratorians, STD researchers, STD clinicians, STD Program Directors and other STD program staff. Representatives from the Food and Drug Administration (FDA) and Centers for Medicare & Medicaid Services (CMS) were also in attendance. The target audience for these recommendations includes laboratory directors, laboratory staff, microbiologists, clinicians, epidemiologists, and disease control personnel.

For several months prior to the in-person consultation, these workgroups developed key questions and researched the current literature to ensure that any recommendations made were relevant and evidence based. Published studies compiled in Tables of Evidence provided a framework for group discussion addressing several key questions.

The major recommendations of the consultation are summarized in the box below. The remainder of the following report summarizes the major discussions pertaining to chlamydia/gonorrhea diagnosis that were held over the course of the three day meeting and does not represent the final recommendations of the workgroup. The ensuing months will involve further expert discussion and literature review before the final development and publication of an STD Testing Guidelines document.

Summary of Major Conclusions:

- Nucleic acid amplification tests are recommended for detection of reproductive tract infections caused by *C. trachomatis* and *N. gonorrhoeae* infections in men and women with and without symptoms.
- Optimal specimen types for nucleic acid amplification tests are first catch urine from men and vaginal swabs from women.
- Nucleic acid amplification tests are recommended for the detection of rectal and oropharyngeal infections caused by *C. trachomatis* and *N. gonorrhoeae*. However, these specimen types have not been cleared by the FDA for use with NAATs and laboratories must establish performance specifications to satisfy CMS regulations for CLIA compliance prior to reporting results for patient management. (493.1253(b)(2))
- Routine repeat testing of nucleic acid amplification test positive screening specimens is not recommended.

Laboratory Diagnosis of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*

Topic: Performance Characteristics

Summary:

The sensitivity and specificity of the nucleic acid amplification tests (NAATs) are clearly the highest of any of the test platforms for the diagnosis of chlamydial and gonococcal infections. Since accurate diagnosis is the goal, there is no justification for the ongoing use of other technologies. Non-culture tests such as enzyme immunoassays (EIA) and DNA probe assays are inferior to NAATs with respect to performance. There are currently no point-of-care (POC) assays on the market that are suitable for routine use, although some may be of use in high risk populations where immediate treatment is the overriding concern due to poor follow up. The group felt that development of improved POC tests desirable.

There was recognition of the need to maintain the capability of both gonorrhea and chlamydia culture in at least some reference laboratories throughout the country. In particular, gonorrhea culture is the only method that can be used to monitor developing resistance to current treatment regimens, and viable isolates are needed.

Discussion:

- General agreement that NAATs have superior performance characteristics.
- All laboratories should be using NAATs since they are the best option for detecting *C. trachomatis* and *N. gonorrhoeae*.
- The guidelines should contain performance data from published reports. The possibility of publishing data from test manufacturer's package inserts was also considered.
- Committee members believe that chlamydia culture should be maintained in some laboratories to support research activities and/or aid in detection of LGV or rare infections caused by variant or mutated *C. trachomatis*.
- Maintenance of gonorrhea culture needs to be highlighted in the guidelines to support any activities related to the detection of antibiotic resistant gonorrhea isolates.
- Guidance for establishing performance specifications of a modified test system is essential if any recommendations are made regarding the use of tests and/or specimen types that are not cleared by the FDA.
- Serology is useful to support a diagnosis of inguinal LGV infection but not rectal LGV infections. NAAT is useful for the identification of LGV as well as non-LGV serovars of chlamydia, in both rectal and inguinal specimens. All commonly available NAATs will identify LGV as chlamydia but will not differentiate biovars. Although several laboratories have reported the use of laboratory developed tests for the differentiation of LGV strains, there is not enough evidence to support a recommendation for their use.
- Currently, there are only two non-culture tests cleared for the detection of chlamydial conjunctivitis and neither is widely available.

General Recommendations/ Conclusions:

1. Sensitivity and specificity ranges by test class will be based on published data, as well as on package inserts.

2. All culture and non-culture tests may generate false positive and false negative results. The laboratory staff must follow appropriate test procedures in a suitable environment to minimize test error.

3. Clinicians need to be aware of the limitations of any test in low prevalence populations that they serve.

4. National reference laboratory culture capability must be maintained for both *C. trachomatis* and *N. gonorrhoeae*. However, it is not recommended for routine testing for chlamydia.

5. Serologic testing is not recommended for non-LGV *C. trachomatis* diagnosis.

6. Serological tests are not recommended for rectal LGV diagnosis. They may be helpful in diagnosing inguinal infections.

7. Most currently FDA cleared NAATs will detect all of the LGV serovars (L1, L2, and L3) of chlamydia, as well as the biological LGV variant, but will not differentiate it from the trachoma biovar. The Roche PCR will not detect the new variant *C. trachomatis* (nvCT) that has been reported in Sweden. Laboratory procedures which have been developed for "in-house" NAATs for variants have been published, but the data is insufficient to make a recommendation for their clinical utility.

Additional Research Needs and Action Items:

- Data regarding the time period after treatment during which a NAAT for *C. trachomatis* and/or *N. gonorrhoeae* may be positive due to residual organism specific nucleic acid should be generated using all currently available NAATs.
- Studies should be conducted examining the performance of NAATs with ocular specimens.
- Further studies are needed to develop a NAAT that will differentiate the LGV from the trachoma biovar and to differentiate the trachoma biological variant (nvCT) from the wildtype trachoma biovar.
- Steps must be taken to aid in the maintenance of national reference laboratory culture capability for both *C. trachomatis* and *N. gonorrhoeae*.
- Set up and maintain an active website of current FDA cleared tests and cleared specimen types.

Topic: Screening Applications

Summary:
Urine is the preferred sample type for testing or screening men using NAATs. There is little need for urethral swab specimens and in some studies these samples are less sensitive than urine; urethral swab specimens and male urine were equivalent in specificity. For female screening, vaginal swab specimens are the preferred specimen type. Vaginal swab specimens are as sensitive as cervical swab specimens and there is no difference in specificity. Cervical samples are acceptable when pelvic examinations are done, but vaginal swab specimens are an appropriate sample type even when a full pelvic exam is being

performed. Cervical sample specimens are certainly acceptable for NAAT testing in those settings that combine Pap and sexually transmitted infection testing from the same sample, such as liquid cytology. There was some concern about some liquid cytology samples being more likely to result in inhibition of amplification or contamination in some assays, as well as, a concern that liquid cytology samples lead to testing of populations at low risk for infection. Female urine, while acceptable, may have reduced performance when compared to genital swab samples.

Due to target selection of the available assays, the sensitivity and specificity varies among NAATs when testing rectal and pharyngeal specimens for *C. trachomatis* and *N. gonorrhoeae*. However, these sample types are important for screening in specific populations. Creation of a repository of rectal and pharyngeal specimen types to facilitate the establishment of performance specifications was encouraged.

Discussion:

- The laboratory does not differentiate specimens based on whether they were collected from symptomatic or asymptomatic patients.
- The performance of NAATs for the detection of rectal infections caused by *C. trachomatis* and *N. gonorrhoeae* is superior to that of other tests.
- Culture for chlamydia and gonorrhea infections has limited utility for routine diagnosis.
- It would be useful to have a website listing available test parameters, including caveats as to features that may lessen performance.
- Pooling specimens has been shown to be an effective method to reduce costs without compromising test performance[1,2, 3,4].

General Recommendations/ Conclusions:

1. NAATs are recommended for detection of reproductive tract infections caused by *C. trachomatis* and *N. gonorrhoeae* infections in men and women with and without symptoms.

2. Urine is the preferred specimen type for testing males using NAATs[5,6,7].

3. Vaginal swabs are equal or superior to endocervical swabs or urine when processed with NAATs for the detection of *C. trachomatis* and *N. gonorrhoeae* in women. Vaginal swabs are therefore the preferred sample type for screening[8,9,10,11,12,13,14,15.]

4. NAATs have superior performance to culture for the detection of rectal and pharyngeal infections caused by *C. trachomatis* and *N. gonorrhoeae*. However, these specimen types have not been cleared by the FDA for use with NAATs and laboratories must establish performance specifications to satisfy CMS regulations for CLIA compliance prior to reporting results for patient management[16,17]. (493.1253(b)(2))

5. Pharyngeal specimens being assessed for *N. gonorrhoeae* should be tested using a NAAT that has been shown to be minimally affected by commensal *Neisseria spp*. Pharyngeal swab specimens have not been cleared by the FDA for use with NAATs and laboratories must establish performance specifications to satisfy CMS regulations for CLIA compliance prior to reporting results for patient management[16,18,19]. (493.1253(b)(2))

6. Routine repeat testing of NAAT positive screening specimens is not recommended[20].

7. Pooling specimens for testing with NAATs is an acceptable method to reduce costs without compromising performance[21,22.]

Topic: Laboratory Confirmation

Summary:

The meaning and interpretation of "confirmatory" varies. However, there was consensus that the currently available NAATs are sufficiently specific that repeat testing of positive samples is unlikely to yield meaningful, interpretable information. The possible exception to this is gonorrhea positives obtained using assays which have known clinical reactivity with non-gonococcal species. The remaining issue is how to deal with positive results obtained in low prevalence populations. The group agreed that this may be an issue that is addressed by NOT recommending testing of asymptomatic individuals in extremely low prevalence populations. A figure showing the relationship between positive predictive value and prevalence will be included in the Guidelines document to help clinicians decide whether their population is appropriate for screening or not.

Discussion:

- Confirmation of positive *C. trachomatis* and *N. gonorrhoeae* test results can be problematic as the failure to confirm the initial result may be wrong, and laboratories often have limited access to additional assays, other than those in routine use.
- Positive reactions with non-gonococcal *Neisseria* species have been reported with some NAATs, and in some anatomic sites.
- Repeat testing of NAAT positive specimens does not improve the positive predictive value of the test.
- The cost of repeat testing in screening programs to confirm positive C. trachomatis and N. gonorrhoeae test results may waste limited resources with no benefit.
- Laboratories report test results with appropriate interpretations to the clinician, who is responsible for understanding the test results to guide patient management.
- Laboratories should conduct routine environmental monitoring to minimize false positive test results due to contamination.
- Medico- Legal issues:
    - NAATs have an increased sensitivity and approach the specificity of culture. For this reason either one or both are appropriate for use in legal settings. However, due to its low sensitivity levels, culture would likely not be approved for legal evidence if it was evaluated today.
    - There are some questions surrounding whether or not the use of NAATs in children under 16 is considered an off-label test. A major difficulty lies in the inability to perform clinical trials in this population. A paper on this topic is anticipated for publication in *Pediatrics* the spring or summer of 2009 and will be evaluated upon publication. Recommendations will be included in the final guidelines[23].

General Recommendations/ Conclusions:

1. Routine repeat testing of NAAT positive specimens is not recommended for *C. trachomatis*[20,24,25,26].

2. Routine repeat testing of NAAT positive specimens is not recommended for *N. gonorrhoeae* unless there is a significant number of false-positive test results found in clinical specimens due to cross-reaction with non-gonococcal *Neisseria* species[27,28,29,30].

3. NAATs have been used in cases of adult rape or abuse and have shown to be superior to culture for the detection of chlamydia in such circumstances. Positive test results for gonorrhea from NAATs that have significant cross-reaction with non-gonococcal *Neisseria* species with clinical specimens should be retested with a different NAAT.

4. NAATs have been used in cases of pediatric sexual abuse and have shown to be superior to culture for the detection of chlamydia in such circumstances. Positive test results for gonorrhea from NAATs that have significant cross-reaction with non-gonococcal *Neisseria* species with clinical specimens should be retested with a different NAAT.

References

1. Gaydos, C.A., T.C. Quinn, D. Willis, A. Weissfeld, E.W. Hook, D.H. Martin, D.V. Ferrero, J. Schachter. Performance of the APTIMA Combo 2 assay for the multiplex detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in female urine and endocervical swab specimens. J. Clin. Microbiol 41: 304-309, 2003.
2. Van Der Pol, B., D.V. Ferrero, L. Buck-Barrington, E. Hook, C. Lenderman, T. Quinn, C.A. Gaydos, J. Lovchik, J. Schachter, J. Moncada, G. Hall, M.J. Tuochy, R.B. Jones. Multicenter evaluation of the BDProbeTec ET system for the detection of *Chalmydia trachomatis andNeisseria gonorrhoeae* in urine specimens, female endocervical swabs, and male urethral swabs. J. Clin. Microbiol. 39:1008-1016, 2001.
3. Martin, D.H., C. Cammarata, B. Van Der Pol, J.B. Jones, T.C. Quinn, C.A. Gaydos, K. Crotchfield, J. Schachter, J. Moncada, D. Jungkind, B. Turner, C. Peyton. Multicenter Evaluation of AMPLICOR and automated COBAS AMPLICOR CT/NG tests for *N. gonorrhoeae*. J. Clin. Microbiol. 38:3544-3549, 2000.
4. Van Der Pol, B., T.C. Quinn, C.A. Gaydos, K. Crotchfelt, J. Schachter, J. Moncada, D. Jungkind, D. H. Martin, B. Turner, C. Peyton and Robert B. Jones. Multi-Center Evaluation of the AMPLICOR and Automated COBAS AMPLICOR CT/NG Tests for the Detection of *Chlamydia trachomatis* J. Clin. Microbiol. 38:1105-1112, 2000.
5. Chernesky MA, Martin DH, Hook EW, Willis D, Jordan J, Wang S, Lane JR, Fuller D, Schachter J. Ability of new APTIMA CT and APTIMA *N. gonorrhoeae* assays to detect Chlamydia trachomatis and Neisseria gonorrhoeae in male urine and urethral swabs. J Clin Microbiol. 43: 127-31, 2005.
6. Gaydos, C.A., D.V. Ferrero, J. Papp. Laboratory aspects of screening males for *Chlamydia trachomatis* in the new millenium. Sex Transm. Dis. 35 suppl: S44-50, 2008.
7. Marrazzo J.M., J.M. Ellen, C. Kent, C. Gaydos, J. Chapin, E.F. Dunne , C.A.Rietmeijer. Acceptability of urine-based screening for *Chlamydia trachomatis* to asymptomatic young men and their providers. Sex.Transmit. Dis. 34:147-153, 2007.
8. Schachter J, Chernesky MA, Willis DE, Fine PM, Martin DH, Fuller D, Jordan JA, Janda W, Hook EW 3rd. Vaginal swabs are the specimens of choice when screening for Chlamydia trachomatis and Neisseria gonorrhoeae: results from a multicenter evaluation of the APTIMA assays for both infections. Sex Transm Dis. 32: 725-8, 2005.
9. Hobbs, M. M., van der Pol, B., Totten, P., Gaydos, C. A., Wald, A., Warren, T., et al. From the NIH: proceedings of a workshop on the importance of self-obtained vaginal specimens for detection of sexually transmitted infections. Sex Transm Dis, 35: 8-13, 2008
10. Kellogg, N. D., Baillargeon, J., Lukefahr, J. L., Lawless, K., & Menard, S. W. Comparison of nucleic acid amplification tests and culture techniques in the detection of Neisseria gonorrhoeae and Chlamydia trachomatis in victims of suspected child sexual abuse. J Pediatric & Adolescent Gynecology, 17: 331-339, 2004.
11. Knox, J., Tabrizi, S. N., Miller, P., Petoumenos, K., Law, M., Chen, S., et al. Evaluation of self-collected samples in contrast to practitioner-collected samples for detection of Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis by polymerase chain reaction among women living in remote areas. S Sex Transm Dis, 29: 647-654, 2002.
12. Schachter, J., McCormack, W. M., Chernesky, M. A., Martin, D. H., Van Der Pol, B., Rice, P. A., et al. Vaginal swabs are appropriate specimens for diagnosis of genital tract infection with Chlamydia trachomatis. J Clin Microbiol, 41: 3784-3789, 2003.

13. Shafer, M.-A., Moncada, J., Boyer, C. B., Betsinger, K., Flinn, S. D., & Schachter, J. Comparing first-void urine specimens, self-collected vaginal swabs, and endocervical specimens to detect Chlamydia trachomatis and Neisseria gonorrhoeae by a nucleic acid amplification test. J Clin Microbiol, 41: 4395-4399, 2003.

14. Chernesky MA, Hook EW 3rd, Martin DH, Lane J, Johnson R, Jordan JA, Fuller D, Willis DE, Fine PM, Janda WM, Schachter J. Women find it easy and prefer to collect their own vaginal swabs to diagnose *Chlamydia trachomatis* or *Neisseria gonorrhoeae* infections. Sex Transm Dis. 32:729-33, 2005.

15. Hsieh, Y-H., M.R. Howell, J.C. Gaydos, K.T. Mc Kee, Jr., T.C. Quinn, C.A. Gaydos. Preference among female army recruits for use of self-administered vaginal swabs or urine to screen for *Chlamydia trachomatis* genital infections. Sex. Transmit. Dis 30: 769-773, 2003.

16. Schachter J, Moncada J, Liska S, Shayevich C, Klausner JD. Nucleic acid amplification tests in the diagnosis of chlamydial and gonococcal infections of the oropharynx and rectum in men who have sex with men. Sex Transm Dis. 35: 637-42, 2008.

17. Kent CK, Chaw JK, Wong W, Liska S, Gibson S, Hubbard G, Klausner JD. Prevalence of rectal, urethral, and pharyngeal chlamydia and gonorrhea detected in 2 clinical settings among men who have sex with men: San Francisco, California, 2003. Clin Infect Dis. 41: 67-74, 2005.

18. Tabrizi,S.N,; Chen,S.; Cohenford,M.A.; Lentrichia,B.B.; Coffman,E.; Schultz,T.; Tapsall,J.W.; Garland,S.M. Evaluation of real time polymerase chain reaction assays confirmation of *Neisseria gonorrhoeae* in clinical samples tested positive in the Roche Cobas Amplicor assay. Sex Transm Infect. 80: 68-71, 2004.

19. Farrell,D.J. Evaluation of AMPLICOR *Neisseria gonorrhoeae* PCR using cppB nested PCR and 16S rRNA PCR. J Clin Microbiol. 37:386-390, 1999.

20. Schachter J, Chow JM, Howard H, Bolan G, Moncada J. Detection of Chlamydia trachomatis by nucleic acid amplification testing: our evaluation suggests that CDC-recommended approaches for confirmatory testing are ill-advised. J Clin Microbiol. 44:2512-7, 2006.

21. Kacena, K.A., S.B. Quinn, M.R. Howell, T.C. Quinn, G.E. Madico, C.A. Gaydos. Pooling urine samples for the screening of genital *Chlamydia trachomatis* infection in asymptomatic women using ligase chain reaction (LCR). J. Clin Microbiol. 36:481-485, 1998.

22. Kacena, K., S.B. Quinn, S.C. Hartman, T.C. Quinn, C.A. Gaydos. Pooling urine samples for the screening of *Neisseria gonorrhoeae* by ligase chain reaction (LCR): Accuracy and application. J Clin Microbiol 36:3624-3628, 1998.

23. Black, Carolyn M.; Driebe, Elizabeth M.; Howard, Laurie A.; Fajman, Nancy N.; Sawyer, Mary K.; Girardet, Rebecca G.; Sautter, Robert L.; Greenwald, Earl; Beck-Sague, Consuelo M.; Unger, Elizabeth R.; Igietseme, Joseph U.; Hammerschlag, Margaret R. Multicenter study of nucleic acid amplification tests for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in children being evaluated for sexual abuse. Pediatr Infect Dis J.. 28(7): July 2009.

24. Chernesky, M., Jang, D., Luinstra, K., Chong, S., Smieja, M., Cai, W., et al. High analytical sensitivity and low rates of inhibition may contribute to detection of Chlamydia trachomatis in significantly more women by the APTIMA Combo 2 assay. J Clin Microbiol. 44: 400-405, 2006.

25. Schachter, J., Hook, E. W., Martin, D. H., Willis, D., Fine, P., Fuller, D., et al. Confirming positive results of nucleic acid amplification tests (NAATs) for *Chlamydia trachomatis*: all NAATs are not created equal. J Clin Microbiol. 43: 1372-1373, 2005.

26. Scragg, S., Bingham, A., & Mallinson, H. Should *Chlamydia trachomatis* confirmation make you cross? Performance of collection kits tested across three nucleic acid amplification test platforms. Sex Transm Infect. 82: 295-297, 2006.

27. Moncada, J., Donegan, E., Schachter, J., Moncada, J., Donegan, E., & Schachter, J. Evaluation of CDC-recommended approaches for confirmatory testing of positivem *Neisseria gonorrhoeae* nucleic acid amplification test results. J. Clin. Microbiol. 46: 1614-1619, 2008.
38. Mangold, K. A., Regner, M., Tajuddin, M., Tajuddin, A. M., Jennings, L., Du, H., et al. *Neisseria* species identification assay for the confirmation of *Neisseria gonorrhoeae*-positive results of the COBAS Amplicor PCR.[erratum appears in J Clin Microbiol. 2007 Jul;45(7):2353]. J Clin Microbiol. 45: 1403-1409.
29. Peter-Getzlaff, S., Luethy, J., Springer, B., Peter-Getzlaff, S., Luethy, J., & Springer, B. Diagnostic value of molecular confirmation assays for *Neisseria gonorrhoeae*.[comment]. J Clin Microbiol. 45: 3856-3858, 2007.
30. Serisha, B., Sillis, M., Evans, J., Serisha, B., Sillis, M., & Evans, J. Confirmation of reactive gonorrhoea Gen Probe APTIMA Combo 2 assay using the alternative sequence mono-specific APTIMA gonococcus assay.[comment]. Int J STD AIDS, 19: 69, 2008.