

## **Laboratory Diagnosis: *Chlamydia trachomatis*/ *Neisseria gonorrhoeae* Infections**

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Numerous culture and non-culture techniques are available to test for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in genital specimens. Culture was historically considered the “gold standard” for testing both pathogens and is still the recommended method for medical-legal cases where false-positive results are not acceptable. Culture methods however, in comparison to molecular tests, have been shown to generally be less than 90% sensitive for both species. One reason for the reduced sensitivity of culture is decreased viability of organisms when transporting specimens to or from off-site facilities (see Iwen, et al., Arch.

Pathol. Lab. Med. 1996; 120: 1019-1022). To overcome the issue of viability, a number of non-culture test methods have been evaluated, including tests to detect antigen or genetic material. Two such molecular tests are the Gen-Probe PACE 2 assays (Gen-Probe, San Diego, CA), one for *C. trachomatis* (PACE 2CT System) and one for *N. gonorrhoeae* (PACE 2NG System). These tests use nucleic acid hybridization techniques for the detection of ribosomal RNA (rRNA) and they are widely utilized in public health laboratories throughout the United States because of reliable performance characteristics and ease of use. Recently, Gen-Probe made available another nucleic acid probe test that simultaneously detects both *C. trachomatis* and *N. gonorrhoeae* from a single patient sample (PACE 2C System). Since reported co-infection rates of 15% to 40% are not uncommon, a probe test to detect both organisms from each specimen is a desirable alternative for testing. The NPHL uses this Gen-Probe PACE 2C System to screen genital specimens simultaneously for both *C. trachomatis*

and *N. gonorrhoeae*. The PACE 2C System uses chemiluminescent labeled, single stranded DNA probes that are complementary to the rRNA of both target organisms. After the rRNA is released from the target organisms, the labeled probes combine with this rRNA to form stable DNA:RNA hybrids. The labeled DNA:RNA hybrids are separated from the nonhybridized probes and the DNA:RNA hybrids, if present, emit a light reaction which is detected using a luminometer. The results of testing are reported quantitatively in relative light units (RLUs). A positive screen indicates the presence of *C. trachomatis*, *N. gonorrhoeae*, or both subsequently requiring additional testing for verification and to identify specific species.

### **Specimen Collection**

Endocervical and male urethral specimens are collected using the appropriate Gen-Probe PACE Specimen Collection Kit (Gen-Probe Urethral, Cat. No. 3275 and Gen-Probe Cervical, Cat. No. 3300). These two body sites are the only sites currently approved for testing with the combined assay. The proper swab should be used and placed back into the collection tube containing transport media after collection. The tubes are transported to the laboratory and held at room temperature until assayed. Samples not processed within 7 days are stored frozen at -20 to -70 °C.

### **Specimen Processing**

Upon receipt in the laboratory, swabs are processed according to manufacturer’s recommendations. The processing involves steps in preparation, hybridization, separation, and detection. Both negative and positive controls are included with each sample run. Upon reading each processed sample with a luminometer, a chemiluminescence reaction is detected which is converted into a RLU reading.

### **Interpretation**

A positive screen result is a RLU reading of greater than 300 plus the mean of the Negative Reference (approximately 350 RLUs total) which

indicates the possibility of *C. trachomatis* and/or *N. gonorrhoeae* rRNA present in the specimen. A negative screen result is considered when a RLU reading of less than 300 plus the mean of the Negative References is identified. All specimens with RLU readings of 300 or more are subjected to a second level of testing. Specimens with RLU readings of 300 to 1000 are considered “grey zone” and tested with Gen-Probe’s Probe Competition Assays (PCAs) for both *C. trachomatis* (CT -PCA) and *N. gonorrhoeae* (NG-PCA). The PCAs are supplemental DNA probe tests that use the technique of competitive nucleic acid hybridization. They are used to detect a nonspecific signal in specimens that test high-negative or low-positive in the PACE 2 Systems. For specimens with RLU readings of greater than 1000, clinical studies have shown them to be true-positive and not requiring supplemental PCA testing for verification. These true-positive specimens in our laboratory are followed up tested with individual *C. trachomatis* (PACE 2CT) and *N. gonorrhoeae* (PACE 2NG) assays to identify specific species. In rare instances, where follow-up testing with the individual assays have RLU readings between 300 and 1000, the result is retested using the PCA method for verification. If not enough sample is available for retesting, the result is reported as “inconclusive” and another sample requested. When identified as true positive by the confirmatory method, the specific etiological agent is reported.

#### **Performance Evaluation**

The PACE 2C assay has a reported sensitivity of 89.9% to 98.9% for endocervical and 93.3% to 97.1 % for male urethral specimens, with specificities greater than 95% for both sites. An evaluation by our laboratory comparing the PACE 2C assay with culture using a nucleic acid amplification test for discrepancy analysis, showed the sensitivity of culture for *N. gonorrhoeae* and *C. trachomatis* to be 88.9% and 89.2%, respectively, with a PACE 2C assay sensitivity of 96.3% and specificity of

98.8% (Iwen, et al., J. Clin. Microbiol. 1995; 33:2587-2591). It is important to note there are some limitations to consider when using this test methodology. Specimen adequacy cannot be assessed microscopically, so it is imperative that proper specimen collection techniques be used. Additionally, grossly bloody specimens (greater than 80 µL whole blood in 1 ml transport medium) may interfere with the performance of the probe test, thus requiring PCA testing. Finally, as is true with all laboratory tests, when positive and negative results contradict other clinical or patient information, verification tests, such as culture, should be considered.

#### **Conclusion**

Nucleic acid amplification tests are available for testing both pathogens. The high sensitivity and specificity of these tests, along with the ability to test urine, shows promise. However, studies comparing amplification tests with PACE 2 and PCA assays are limited and have had conflicting results. This, along with the higher cost for amplification testing, has limited their usefulness for large scale screening. NPHL currently process about 3000 genital samples per month. Cultures for both chlamydia and gonorrhea are offered for medical-legal cases and to test specimens such as conjunctiva, rectum, and from infants, nasopharynx and throat. The PACE 2C System is not FDA-approved for these sites, although the PACE 2CT for *C. trachomatis* has been approved.

### Screen for Chlamydia/Gonorrhea by DNA Probe

Availability:	Monday - Friday
Turnaround Time:	24 hours
Specimen:	Swab of endocervix or male urethra Swab of conjunctiva (for chlamydia only)
Container:	Gen-Probe transport container Send at room temperature
Causes for rejection:	Other sites and specimens submitted on wooden swabs are unacceptable
Reference range:	Negative for <i>C.trachomatis</i> and <i>N.gonorrhoeae</i>
Additional information:	Culture for both chlamydia and gonorrhea are available. Contact the Nebraska Public Health Laboratory for proper collection and transport information.