Clinical Note

DNA probe assays for the detection of sexually transmitted diseases

BY CRAIG S. HILL

SEXUALLY TRANSMITTED diseases (STDs) continue to be one of the largest infectious disease problems in the U.S.A. Approximately 12–15 million persons are infected by one STD every year. *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) infections are the two most common STDs in the U.S.A. These diseases are the leading cause of pelvic inflammatory disease (PID) in females, which may lead to sterility and ectopic pregnancy if left untreated. Conjunctivitis and pneumonia can also occur in babies born to infected mothers. Although CT and NG are easily and inexpensively treated, they often go undiagnosed because they are commonly "silent," with no obvious symptoms. Coinfection with both diseases is quite common. It has been reported that up to 50% of patients infected with NG are also infected with CT.[1–4] It is critical to diagnose both diseases in order to properly treat them before complications occur.

Both NG and CT have traditionally been diagnosed with the use of bacterial culture for NG and cell culture for CT. NG culture is simple to perform, but has relatively long turnaround times. CT culture is considerably more complex and is only performed by a few specially trained laboratories in the U.S.A. Both techniques require that samples be delivered to the laboratory within a short period of time, and special storage conditions are required to keep the organisms viable until delivery to the laboratory.

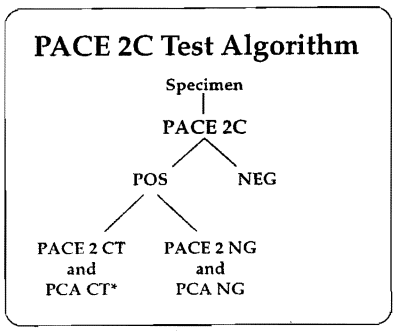
The limitations of culture have led to the development of many nonculture techniques over the last several years. Most of these are immunoassays for the detection of CT or NG antigens using urogenital swab samples. The majority of these assays are significantly less sensitive and specific in relation to culture. None of the immunoassays is capable of detecting both organisms in a single sample or single assay.

Many of the problems inherent to enzyme immunoassays (EIAs) have been overcome with the PACE 2 assay (Gen-Probe, Inc., San Diego, CA), which was first introduced in 1990. This assay uses acridinium ester-labeled DNA probes to detect CT or NG ribosomal RNA (rRNA) in urogenital samples. A single urogenital swab sample can be tested for both CT and NG. No special sample storage conditions are necessary, and samples are stable up to seven days at room temperature. A swab is taken from the male urethra or the female endocervical canal and placed in a test tube that is sent to the clinical laboratory for testing. Sample is added to each of two test tubes followed by addition of DNA probes specific for either CT or NG rRNA. The tubes are incubated for 1 hr to allow the DNA probes to hybridize to the target rRNA. After a brief wash, the tubes are read automatically in a luminometer, and the signal is output as relative light units (RLU). The entire procedure is very simple and takes less than 2.5 hr to perform. Results in laboratories throughout the world have shown very good performance in relation to culture. Sensitivity and specificity of the CT assay are typically about 93% and 99%, respectively.[5–9] The NG assay is usually about 95% sensitive and greater than 99% specific.[10–12] Positive results are verified by use of a probe competition assay, as has been recommended by the Centers for Disease Control and Prevention (CDC, Atlanta, GA).[13] The performance and ease of use of this assay, as well as the ability to detect both CT and NG from a single swab, have resulted in it becoming the most widely used nonculture CT and NG assay in the U.S.A.

A new version of the assay, PACE 2C, can detect both NG and CT not only from a single swab, but also in a single test tube. The assay procedure is very similar to the PACE 2 assay except that both probes are present in the single tube and both NG and CT can be detected simultaneously in the same tube. If either NG or CT is present in the tube, the assay will result in a positive signal. If a positive signal is obtained, then the remaining sample is tested in a PACE 2 assay using separate NG and CT probes to identify the organisms present (*Figure 1*). The primary advantage of this assay is that in the typical high-volume laboratory fewer assays need to be run, with a resultant saving in labor and costs to the laboratory. Performance of the assay has been as good as, and in some cases better than, culture. Clinical studies at several sites using 849 male and 1266 female patients demonstrated initial sensitivities of 89.9–97.1% in comparison with culture (*Table 1*). Specificity ranged from 97 to 98% except in symptomatic males, for whom specificity was 93.3%. When the probe-positive, culture-negative samples were rerun in a highly sensitive amplification assay, it was shown that CT and NG RNA were present in 37 of 55 (67%) of these samples. These data suggested that the majority of the apparent PACE 2C false positives were actually true positives missed by culture. The FDA did not allow use of these data in the package insert because the amplification assay used was a research-based assay.

The increased sensitivity of the PACE 2C assay in comparison to culture has been confirmed in other similar studies. Iwen et al. at the 1994 American Society for Microbiology (ASM) meeting reported the results of a study using the PACE 2C assay on endocervical samples from 394 patients admitted to an emergency room.[5] Prevalences in this population for CT and NG were 9.4% and 6.8%, respectively, with a coinfection rate of 37%. The sensitivity and specificity of the PACE 2C assay were 96.3% and 98.8% after discrepant resolution using DFA (direct fluorescence assay), repeat cell culture, and amplification assays. Culture sensitivity for CT and NG was lower than the PACE 2C assay at 89.2% and 88.9%, respectively.

These results show good performance for the PACE 2C assay compared to that observed with the standard culture. The assay is simple to run, and the transport system keeps specimens stable for up to one week. In addition, the assay can result in significant cost savings to laboratories with high sample volume. These advantages ensure the continued widespread use of the PACE 2 and PACE 2C assays in clinical laboratories for the diagnosis of CT and NG.



*Meets CDC recommendations for verification of positive CT results with a supplemental test.

Figure 1 *PACE 2C test algorithm. Specimens that test positive in the PACE 2C assay are tested in PACE 2 assays using separate NG and CT probes to identify the organisms present in the sample. Positive repeat assays may also be tested with the PCA (probe competition assay) to verify the presence of each organism.*

Table 1
Sensitivity/specificity after discrepant resolution

Female	
Symptomatic:	
High prevalence	92.8%, 97.3%
Low prevalence	94.2%, 97.8%
Asymptomatic:	
High prevalence	89.9%, 97.4%
Low prevalence	92.9%, 98.9%
Male	
Symptomatic	97.1%, 93.3%
Asymptomatic	93.3%, 95.8%

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