

## Use of Gen-Probe Probe Competition Assay as a Supplement to Probes for Direct Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Urogenital Specimens

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**The potential for development of a cost-effective protocol for selective use of the Gen-Probe probe competition assay (PCA) in conjunction with PACE 2 for direct detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urogenital specimens that would not compromise patient care was investigated. To accomplish this, PCA data from testing performed over 12 months were retrospectively reviewed. Of 237 samples that were presumptively positive for *C. trachomatis* by initial probe assay and could be tested by PCA, positive PCA results were obtained for 100, 79, and 59%, respectively, of specimens that gave a signal of more than 1,500, 1,000 to 1,500, and less than 1,000 relative light units (RLU). For the 141 specimens that were presumptively positive for *N. gonorrhoeae* and could be tested by PCA, positive PCA results were obtained for 99, 80, and 42%, respectively, of samples with a signal of more than 1,500, 1,000 to 1,500, and less than 1,000 RLU. These data indicate that PCA should be a routine supplement to Gen-Probe PACE 2 for specimens with an initial signal by probe assay of less than 1,500 RLU and may not be necessary for samples yielding a signal of more than 1,500 RLU.**

Commercial nonisotopic DNA probe assays for direct detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urogenital swab specimens have been available for several years (Gen-Probe PACE 2 assays; Gen-Probe Inc., San Diego, Calif.). Data from evaluations of these assays have shown that, with few exceptions, the sensitivity of each is comparable to that of the respective conventional culture technique (cell culture for *C. trachomatis* and a selective solid medium for *N. gonorrhoeae*) (1, 3-10). To optimize specificity, a PACE 2 probe competition assay (PCA), which has been available since April 1994 for *C. trachomatis* and since June 1994 for *N. gonorrhoeae*, can be used as a supplement to the probe assays to determine whether a positive signal is due to the presence of the respective organism or to interfering material in the sample. Grossly bloody specimens (i.e., those with more than 80  $\mu$ l of whole blood in 1 ml of transport medium) may interfere with performance of this test, and lint, dust, or glove powder in the reaction tube may cause nonspecific chemiluminescence. Given the adverse consequences of reporting a false-positive result for *C. trachomatis*, experts at the Centers for Disease Control and Prevention recommended use of a competitive probe for verification of probe-positive samples (2); following the same guidelines for samples that are probe positive for *N. gonorrhoeae* is logical. However, PCA increases the turnaround time and cost of the probe assays; therefore, optimizing its use without compromising patient care is reasonable. To our knowledge, such an evaluation of PACE 2 PCA has not been reported. The purpose of this study was to determine if a cutoff value above which routine PCA was not necessary could be established. To accomplish this, PCA data from testing performed at our institution over a 12-month period were retrospectively reviewed.

Swab specimens (endocervical for females and urethral for males), collected by a health-care worker, were transported

within 24 h to the laboratory in the PACE specimen collection kit at room temperature. If the sample was grossly bloody, this was noted on the work sheet. Prior to testing, the transport tube was agitated on a vortex mixer, excess liquid was expressed from the swab, the swab was discarded, and the tube was again agitated. The initial PACE 2 probe assay was performed according to the manufacturer's directions, as described in detail previously (3-10). Appropriate controls (three negatives and one positive) were included in each run. A sample was considered presumptively positive if the following conditions were met: the specimen signal (measured in relative light units [RLU]) minus the mean of the signals of the three negative controls was  $\geq 350$  RLU for *C. trachomatis* and  $\geq 300$  RLU for *N. gonorrhoeae*.

PCA was performed according to the manufacturer's directions on all presumptively positive specimens for which a minimum of 200  $\mu$ l of material remained. For each patient sample, two tubes were prepared, one standard Gen-Probe tube and one PCA tube containing excess probe that was identical to the probe used in the initial assay except that it lacked the chemiluminescent label. Appropriate controls (three negatives and two positives [one standard and one PCA]) were included in each run. The steps included in the initial assay were then performed. Briefly, to each control tube and the two sample tubes 100  $\mu$ l of the chemiluminescently labeled probe reagent (*C. trachomatis* or *N. gonorrhoeae*, on the basis of the initial result) was added. The tubes were incubated for 1 h in a 60°C water bath, and 1 ml of separation suspension was added. After incubation at 60°C for 10 min, the tubes were placed on a magnetic separation unit for 5 min at room temperature. Supernatants were decanted, and the tubes were filled with wash solution and placed on the separation unit for 20 min. Supernatants again were decanted, and analysis was performed with a Leader instrument. If the reading in the standard Gen-Probe tube did not meet the requirement outlined above for a positive, the result was considered negative and the percent competition was not evaluated. For positive samples, a reduction of

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TABLE 1. Use of Gen-Probe PCA to eliminate false-positive reactions for *C. trachomatis* and *N. gonorrhoeae*

Organism	RLU reading for presumptively positive specimens	No. of specimens with RLU reading	No. (%) of specimens positive by PCA
<i>C. trachomatis</i>	<1,000	73	43 (59)
	1,000–1,500	24	19 (79)
	>1,500	140	140 (100)
Total		237	202 (85)
<i>N. gonorrhoeae</i>	<1,000	26	11 (42)
	1,000–1,500	10	8 (80)
	>1,500	105	104 (99)
Total		141	123 (87)

70% or more in the signal generated in the PCA reaction tube containing unlabeled probe compared with the signal generated in the standard tube with only labeled probe indicated that the specimen contained the respective agent (*C. trachomatis* or *N. gonorrhoeae*) and did not give a reaction due to interfering material in the sample. If the amount of material remaining after the initial assay was insufficient for PCA, a presumptively positive result was reported with the suggestion that another sample be evaluated for verification.

During the 12 months, 264 specimens were probe positive for *C. trachomatis* and 179 were positive for *N. gonorrhoeae*. Sufficient amounts of samples were available for PCA testing of 237 specimens considered presumptively positive for *C. trachomatis* and 141 specimens considered presumptively positive for *N. gonorrhoeae*. Results are summarized in Table 1. For *C. trachomatis*, positive PCA results were obtained for 85% of all specimens tested: 100% of those with an initial probe reading of more than 1,500 RLU, 79% of those with an initial signal of 1,000 to 1,500 RLU, and 59% of those with an initial reading of less than 1,000 RLU. For *N. gonorrhoeae*, positive PCA results were obtained for 87% of all samples tested: 99, 80, and 42%, respectively, of those with an initial reading of more than 1,500, 1,000 to 1,500, and less than 1,000 RLU. Of the specimens with which confirmation was not obtained, five (three initially positive for *C. trachomatis* and two that were positive for *N. gonorrhoeae*) were grossly bloody. The initial probe signals for four of these five samples were within 55 RLU of the cutoff for positivity; the fifth sample (presumptively positive for *N. gonorrhoeae*) had an initial reading of 1,041 RLU. The sample that gave an initial signal of more than 1,500 RLU with the *N. gonorrhoeae* probe (RLU reading of 5,870) but was negative after PCA was from a 20-year-old female whose chief complaint was dysmenorrhea. Her physical examination was normal, and she was not given empiric antimicrobial therapy. Technical error could not be excluded in this case.

On the basis of the above results, a protocol for routine performance of PCA was established, testing only those samples yielding an initial reading of 1,500 RLU or less. During the 2.5 months in which this has been our practice, PCA has been performed on 16 samples presumptively positive for *N. gonorrhoeae* and 53 samples presumptively positive for *C. trachoma-*

*tis*. Positive PCA results were obtained for 7 (44%) of those that were presumptively positive for *N. gonorrhoeae* (38% [5 of 13] and 67% [2 of 3], respectively, with initial RLU readings of less than 1,000 and 1,000 to 1,500) and for 31 (58%) of those that were presumptively positive for *C. trachomatis* (43% [16 of 37] and 94% [15 of 16], respectively, with initial RLU readings of less than 1,000 and 1,000 to 1,500).

In summary, our data strongly support the use of PCA as a supplement to Gen-Probe PACE 2 assays for direct detection of *C. trachomatis* and *N. gonorrhoeae*, especially if the initial probe signal is less than 1,500 RLU. Our data also indicate that it is reasonable to establish a cutoff value (in our case, 1,500 RLU) above which PCA would not routinely be performed. PCA, however, should be available for samples with probe signals above the cutoff value if the clinician has reason to suspect a false-positive result. Moreover, the cutoff value should be determined on the basis of data from the laboratory in which the assays are performed, and a policy of selective use of PCA should be instituted only with the support of clinicians who utilize the test results.

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