Comparison of the Clearview Chlamydia, the PACE 2 Assay, and Culture for Detection of *Chlamydia trachomatis* from Cervical Specimens in a Low-Prevalence Population

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The Clearview Chlamydia assay (Wampole Laboratories, Cranbury, N.J.), the PACE 2 DNA probe assay (GenProbe, San Diego, Calif.), and culture were compared for their abilities to detect *Chlamydia trachomatis* from cervical specimens in a population with a low prevalence (3.9%) of chlamydial infections. A consensus reference method was used. The consensus reference method defined a positive specimen as one with a positive culture result or positive by both of the two nonculture methods. Of the 940 specimens tested, 37 were positive; 36 were positive by culture, 28 were positive by the PACE 2 assay, and 27 were positive by the Clearview assay, giving sensitivities of 97.3, 75.5, and 72.9%, respectively, and specificities of 100, 97.1, and 98.9%, respectively. There was a direct correlation between the number of inclusion-forming units detected by culture and the ability of the two nonculture methods to detect the positive specimens.

Chlamydia trachomatis is the leading cause of sexually transmitted diseases in the Western world and plays a major role in female infertility. This organism is most prevalent in sexually active young adults, who are often asymptomatic. Therefore, it is imperative to accurately identify patients infected with this organism in order to reduce the level of transmission and prevent serious sequelae.

Although culture is the "gold standard" among the available methods of detecting *C. trachomatis* from clinical specimens, it, too, can miss positive specimens (11). There have been several evaluations in which culture has been compared with other nonculture methods. Even with the same nonculture assay there are wide variations in the reported sensitivities and specificities of nonculture methods; these differences may depend on several parameters, including the specimen handling procedures and the tissue culture technique used as the reference method (1, 2, 4–7, 10, 12–14). Therefore, one approach that can be used to expand the reference method is to include two nonculture methods to discriminate the culture-negative, non-culture-positive specimens (11). In the study described here, we compared the Clearview Chlamydia assay (Wampole Laboratories, Cranbury, N.J.) and the PACE 2 DNA probe assay (GenProbe, San Diego, Calif.) with culture for their abilities to detect the presence of *C. trachomatis* in clinical specimens from a population with a low prevalence of infection. The reference method used was a consensus method in which a positive result was defined as a specimen that was positive by culture or positive by both nonculture methods.

Cervical specimens were obtained from patients seen in the Obstetrics and Gynecology Clinic at the University of California Irvine Medical Center. Both symptomatic as well as asymptomatic patients were included in the evaluation. After cleansing the cervix, three swabs were obtained, one for each of the three detection methods used in the study. For culture, a cotton swab (American Scientific Products,

McGaw Park, Ill.) that was previously tested for toxicity (7) was used to collect the sample, and after the sample was obtained, the swab was placed in 1.0 ml of 2-SPG transport medium which contained amphotericin B (25 μg/ml) and gentamicin (50 μg/ml). Dry collection swabs were provided with the Clearview Chlamydia assay (Wampole Laboratories). Swabs and transport media were provided with the PACE 2 DNA probe assay (GenProbe). The order of collection of the swabs was rotated every 50 patients. Upon collection, all specimens were held at 4°C until assayed. To be included in the evaluation, specimens for culture and the Clearview assay were tested within 24 h of collection and specimens for the PACE assay were tested within 4 days.

Swabs in 1.0 ml of 2-SPG transport medium were vortexed for 2 min, and 0.1 ml of specimen was inoculated onto duplicate 24- to 48-h-old McCoy cell monolayers contained in glass vials (15 by 45 mm). Cultures were centrifuged at $1,000 \times g$ at 30°C for 1 h, and then 1.0 ml of Eagle minimum essential medium containing fetal bovine serum (10%), gentamicin (50 μg/ml), and cycloheximide (1.0 μg/ml) was added. After incubation at 37°C for 48 h, the cells were fixed in methanol for 10 min and stained with a fluorescein-labeled monoclonal antibody (MAb) specific to the chlamydial lipopolysaccharide (LPS; Ortho Diagnostics, Raritan, N.J.). Coverslips were examined for chlamydial inclusions with a fluorescence microscope (Olympus) equipped with epiillumination with a 100-W mercury vapor light source. The number of inclusion-forming units (IFU) per coverslip was counted and recorded. Toxic cultures were passed to fresh monolayers. Cells were disrupted by adding sterile glass beads to the vial and vortexing for 2 min. After inoculating 0.1 ml into duplicate shell vials containing McCoy cells, processing was continued as described above for the primary culture. Contaminated cultures were treated with a vancomycin-streptomycin mixture (25 μg/ml each) and were recultured.

The Clearview and PACE 2 assays were performed according to the manufacturers' directions as described previously (3, 13). In the Clearview assay, a dry swab that was collected from the patient was heated for 10 min at 80°C in

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0.6 ml of an extraction buffer in order to extract LPS from chlamydial organisms that may have been present. Upon cooling for 5 min, the swabs were removed and the resulting specimen extract was tested on a Clearview card. Five drops of extracted specimen was added to the sample window, and the specimen was allowed to react for 15 min with the reagents impregnated in the card. During this time if chlamydial LPS was present the antigen bound to the latex-labeled murine MAbs to chlamydial LPS contained in the sample window. The antigen-antibody complex moved up the Clearview card until it reached the test window, where it bound to unlabeled MAb to LPS, and this resulted in a visible band in the test window. Any band in this window was considered a positive test result. The excess latex-labeled MAb migrated and reacted with rabbit anti-mouse sera that was located in the control window. All assays had a band in the control window indicating the proper migration of the labeled MAb; however, if the sample was found in the test the proper migration of the labeled MAb; window to be strongly positive, then the control signal tended to be weaker than that for the specimens that tested negative.

Before testing by the PACE 2 assay, cervical swabs collected in GenProbe transport medium were removed from the transport medium after vortexing. Both sample and acridinium ester-labeled probe (0.1 ml each) were added to a tube, mixed and incubated at 60°C for 1 h. The separation reagent (1 ml) was then added to the tubes, which were mixed and incubated at 60°C for 10 min in a magnetic separation unit. The supernatants were then discarded after 5 min, and the tubes were filled with a wash solution and allowed to incubate at room temperature for 20 min, after which time the supernatants were discarded. Tubes were placed in a luminometer (LEADER 50; GenProbe), reagents were added, and hybridization signals were read for the relative light units (RLUs) generated by the acridinium ester label. Three negative reference samples and one positive control sample were included in each run of the assay. The cutoff for a positive assay was the mean of the negative controls plus 350 RLUs.

With the PACE 2 assay there was enough material to repeat the test; therefore, all specimens positive by the PACE 2 assay as well as any sample with discrepant PACE 2 assay results were repeat tested. An attempt was also made to repeat the Clearview assay with the extracted material; however, the amount of sample remaining was insufficient and therefore the assay could not be repeated. Predictive values were calculated by the method of Ransohoff and Feinstein (9).

A total of 944 sets of cervical specimens collected met all the study criteria. Of the 944 sets, 4 were eliminated from the data analysis because of the repeated toxicity of one specimen for tissue culture and the failure of 3 specimens to migrate in the Clearview assay. The overall results for the remaining 940 specimens on initial testing are given in Table 1.

Thirty-seven sets of specimens were positive by the reference method. Of these, 24 (54%) were positive by all three methods, 7 were culture positive only, 1 was positive by both nonculture methods only but was missed by culture, and 2 and 3 were missed by the PACE 2 and Clearview assays, respectively. The one specimen missed by culture was from a patient who had been on nitrofurantoin (Macrodantin) within 2 weeks of specimen collection. Therefore, on initial testing, culture detected 97% (36 of 37) of the positive specimens, the PACE 2 assay detected 76% (28 of 37) of the

TABLE 1. Overall test results

	Test result		Total no. of specimens
Culture	Clearview	PACE 2	
+	+	+	24
+	−	+	3
+	+	−	2
+	−	−	7
−	+	+	1
−	+	−	10
−	−	+	26
−	−	−	867

positive specimens, and the Clearview assay detected 73% (27 of 37) of the positive specimens.

Of the 944 cultures included in the study, 49 (5%) required a blind passage because of contamination or toxicity. All of these were treated with additional antibiotics before passage. One specimen was repeatedly toxic, as described above. The distribution of the IFU of each of 34 of the 36 specimens that were positive by culture is given in Fig. 1. The other two specimens that were culture positive were quantitated from a blind passage because of the toxicity of the initial culture, and therefore, results for the two specimens are not shown in Fig. 1. Of the 24 cultures that were positive by all assays, 22 could be evaluated for the number of IFU in the initial culture, and of these 20 of 22 (91%) had >200 IFU per culture. The two cultures with IFU below this level had 128 and 12 IFU per culture. Seven specimens were culture positive but negative by both nonculture methods, and of these, five had less than 100 IFU and the other two had 102 and 760 IFU. Two specimens that were negative only by the PACE 2 DNA probe assay also had low numbers of IFU, 11 and 22. Three specimens were negative by the Clearview assay only, with IFU counts of 1,590, 190, and 190. Therefore, results for cultures with <200 IFU were statistically more significant with those for specimens that gave discrepant results (10 of 12 [83%]) in contrast to the specimens that were positive by the three methods (2 of 22 [9%]; $P = 0.001$).

Thirty-seven specimens were positive by the Clearview assay, of which 10 were positive only by the Clearview assay
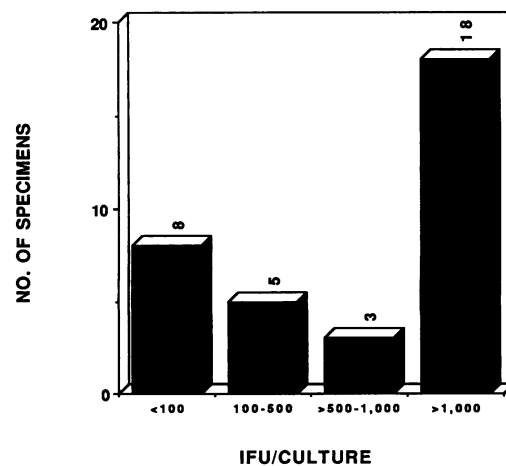

FIG. 1. Distribution of IFU per culture for 34 of the culture-positive specimens.
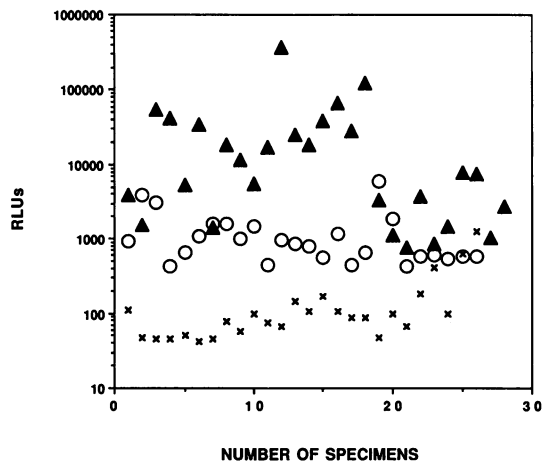
NUMBER OF SPECIMENS

FIG. 2. Distribution and comparison of the RLU obtained by the PACE 2 assay for the true-positive specimens (▲), the false-positive specimens on initial testing (○), and the repeat RLU for the initially false-positive specimens (×).

TABLE 2. Predictive values

Test	% Sensitivity	% Specificity	Positive predictive value (%)	Negative predictive value (%)
Culture	97.3	100	100	99.9
Clearview	72.9	98.9	73.0	98.9
PACE 2, initial	75.7	97.1	51.9	98.9
PACE 2, repeat	78.4	99.8	93.5	99.1

and thus were considered false-positive results. Of these 37, 15 (40.5%) had very weak reactions, which we referred to as a ± result. For data analysis we considered these borderline reactions to be positive. However, of these 15 weak reactions, 9 of 15 (60%) were negative by the reference method and the remaining 6 were positive by the reference method, with IFU counts ranging from 11 to 3,743.

Fifty-four specimens were initially positive by the PACE 2 assay. Of the 37 specimens that were truly positive by the reference method, the PACE 2 assay failed to detect 9 specimens (24.3%). When these specimens were repeat tested by the PACE 2 assay, one of the true-positive specimens initially missed was positive; the value by the initial test was 228 RLU and that by the repeat test was 877 RLU. The count for this specimen was 34 IFU. The other eight true-positive specimens remained negative. All eight of these specimens had counts of <1,000 IFU per culture.

Twenty-six specimens were repeat tested by the PACE 2 assay because initially they were positive only by the PACE 2 assay and therefore were considered to be false-positive results (Table 1). All but 2 of the 26 specimens were negative upon a repeat of the assay. The initial RLU readings for these 26 samples covered a wide range, from a low of 434 to a high of 5,890, with a geometric mean value of 983 and a mean of 1,314. Figure 2 shows the RLU for the initial and repeat PACE 2 assays for each of the 26 specimens. The two specimens that were positive by the PACE 2 assay on repeat testing but negative by the consensus reference method had low initial and repeat RLU, 587 and 625 RLU and 577 and 1,248 RLU, respectively. Although in general the true-positive specimens had a high RLU, there was some overlap in RLU between the true-positive and false-positive specimens (Fig. 2). The manufacturer of the PACE 2 DNA probe assay indicates that bloody specimens may interfere with test performance, but of the two samples that were false positive on repeat testing, only one contained visible blood. Of the remaining 24 samples that were initially positive, only 9 were bloody.

The predictive values for the assays compared with that for the consensus reference method are given in Table 2. The most sensitive and specific method for detecting the presence of C. trachomatis was culture. The initial PACE 2

assay gave a sensitivity similar to that of the Clearview assay. Although repeat performance of the PACE 2 assay showed an increase in the assay's sensitivity, the increase was minimal. However, repeat testing of the specimens with a positive RLU increased the positive predictive value of the PACE 2 assay significantly, from 52 to 94%. Therefore, the sensitivity of the nonculture methods was <80%, while that of culture was 97%. The specificity of the initial PACE 2 assay was lower than that of the Clearview assay.

In the present study, culture was the most sensitive and specific of the three methods tested for their ability to detect C. trachomatis in samples from a population with a low prevalence of chlamydial infections. We previously reported on the performance of an earlier version of the PACE assay in samples from a population with a prevalence of C. trachomatis infection of 10% (8). In the present comparison, we found that the PACE 2 assay had an improved sensitivity, 76% over the previous version, which gave a sensitivity of 60%, and also had a higher specificity than the previous version (95 versus 97%). The PACE 2 assay, however, had more false-positive results, resulting in a lower positive predictive value than the earlier version. In the present study, 26 specimens were repeat tested because results of the initial test did not agree with those of the consensus reference method. Of these, 24 were not positive on repeat testing. Therefore, in the present study we did parallel testing and thus had the index of suspicion about these 26 specimens and thus repeated the assays. However, in the normal diagnostic laboratory setting, in which only one assay is performed, these false-positive results most likely would not have been questioned. Therefore, we feel that a borderline zone for the RLU is needed, and if a result falls within the borderline zone, the assay should be repeated. In the case of this evaluation, this would have reversed the results for 24 patients. On the basis of our results with our patient population, we suggest that all specimens with positive RLU under 6,000 be repeated. This would have meant that 13 true-positive specimens would have been retested and that all 26 false-positive specimens would have been retested. Thus, a total of 39 specimens, or 4.2% of the total number of specimens tested, would have been repeat tested. In the study by Kluytmans et al. (3), they, too, found that samples that tested false positive by the PACE 2 assay were negative by both culture and polymerase chain reaction and had initial RLU that ranged from 448 to 5,106. Therefore, establishment of a borderline zone needs careful consideration.

In comparison with the PACE 2 assay, the Clearview assay had a lower sensitivity (73 versus 76%) but a higher specificity (99 versus 97%). In a previous evaluation of the Clearview assay, we obtained a higher sensitivity than that found in the present evaluation (95 versus 73%). In trying to explain these differences, a few possible explanations could be considered. In the earlier study (8), the prevalence of chlamydial infections in the population was twice that in the

population used in the present study. The number of IFU in the cultures could not provide a satisfactory explanation, since the level of recovery of IFU did not differ that greatly, with 32 and 24% of cultures yielding <100 IFU in the earlier (8) and the present studies, respectively. Although we attempted to use the same standardized system for culture, a subtle difference in the cell lines or media might have made the culture more sensitive in the present study. Differences in the lots of the Clearview assay product are also a possible explanation. A recent comparison (10) of the Clearview assay by three different laboratories in which there was a combined prevalence of chlamydial infections of 13% gave a sensitivity of 66% and a specificity of 98%, which are similar to the findings reported here. In a population with a prevalence of chlamydial infections of 4.5%, Skulnick et al. (12) reported that the Clearview assay had a sensitivity of 78% and a specificity of 99.6%. In the present evaluation, 10 specimens had false-positive results by the Clearview assay. Since there is often insufficient specimen for a repeat test, it may be prudent to obtain another specimen for testing by culture or another nonculture method to confirm the Clearview results for all specimens that test positive or that have a ± band intensity. Since 9 of the 10 false-positive specimens gave a result that was ± in intensity, it may be possible with the aid of a card reader to distinguish true-positive from false-positive specimens in the ± band intensity category. Another approach to this problem would be to consider as negative those specimens that have a low intensity band; however, this would lower the sensitivity of the assay even further.

It has been our experience, as well as that of other investigators, that specimens with low numbers of IFU are those that are commonly missed by the nonculture methods (4, 8). This is clearly a limitation of nonculture methods, resulting in a low sensitivity that needs to be understood by all who use a nonculture method as a sole means of detecting *C. trachomatis* in the laboratory.

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