Comparative Evaluation of Detection Assays for *Chlamydia trachomatis*

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Received 30 November 1992/Accepted 12 March 1993

The performances of a commercial nucleic acid hybridization test (Gen-Probe Pace 2 *Chlamydia trachomatis*) and two commercial enzyme immunoassays (EIAs) (Abbott Chlamydiazyme and Pharmacia *Chlamydia* EIA) were evaluated against cell culture for the detection of *C. trachomatis* infection, with cervical swabs obtained from 1,037 women visiting a public sexual health center. The positivity rate by cell culture was 4.7%. Sensitivity and specificity for each test were as follows: Gen-Probe, 95.8 and 98.3%; Chlamydiazyme, 80.4 and 99.3%; Pharmacia EIA, 80.8 and 99.1%. Analysis of discrepant results with probe confirmation assay (Gen-Probe) and direct immunofluorescence (Syva Microtrak) revealed 12 cases of *C. trachomatis* infection for which culture was negative, resulting in the definition of a true-positive case as opposed to a culture positive. The positivity rate by true-positive definition was 5.9%, and sensitivity and specificity for each test were as follows: Gen-Probe, 96.7 and 99.6%; Chlamydiazyme, 77.5 and 100%; Pharmacia EIA, 77.0 and 100%; cell culture, 80.0 and 100%. We conclude that the Gen-Probe Pace 2 *C. trachomatis* test is a sensitive and specific alternative to cell culture for the detection of *C. trachomatis*.

*Chlamydia trachomatis* is an important cause of sexually transmitted disease. The diagnosis of chlamydial infection has traditionally been by cell culture and, more recently, by antigen detection with either direct immunofluorescence or enzyme immunoassay (EIA). The development of nucleic acid hybridization assays promises to provide a rapid, sensitive, and specific alternative to the techniques currently in use. In this study, we compared cell culture with Abbott Chlamydiazyme EIA, Pharmacia *Chlamydia* EIA, and Gen-Probe Pace 2 *Chlamydia* nucleic acid hybridization assay.

**Enrollment of patients.** Women presenting to the Melbourne Sexually Transmitted Diseases Clinic between July 1989 and May 1990 were asked to participate in the study. Known prostitutes were excluded because of a previously recognized low prevalence of infection. Women who participated were interviewed with a standard questionnaire which sought data regarding their age, reason for testing, urogenital symptoms, antibiotic history, and partner’s health. Any additional pathology was noted.

**Sample collection.** To avoid disruption of the Clinic’s usual protocol, swabs were collected in the following order: (i) *Neisseria gonorrhoeae* culture, (ii) Papanicolaou stain, (iii) *C. trachomatis* culture, (iv) Gen-Probe Pace 2 *C. trachomatis* test, (v) Abbott Chlamydiazyme, and (vi) Pharmacia chlamydia EIA.

The collection sequence for the Gen-Probe, Abbott, and Pharmacia tests was altered each week to avoid any possible bias to specimen quality. All specimens were stored at 4°C and transported to the laboratory within 24 h of collection, where they were either tested immediately or kept at −70°C awaiting batch analysis, after which they were refrozen at −70°C in case further testing was required.

**Cell culture method.** All specimens were cultured in cycloheximide-treated HeLa 229 cells with conventional coverslip and vial technology. Each coverslip was stained after fixation in a two-step procedure with 20 μl of murine monoclonal antichlamydial antibody (specificity for major outer membrane protein) and 20 μl of adsorbed, affinity-purified, antimurine fluorescein isothiocyanate conjugate (Dako). Coverslips were mounted with DePeX (BDH Chemicals, Melbourne, Australia) and scanned at a magnification of ×100 with a Leitz Dialux microscope with inclusion morphology confirmed at ×400 or ×1,000. All of the culture-positive specimens were quantitated by inclusion counts per coverslip: 1+, 1 to 4 inclusions per coverslip; 2+, >4 inclusions per coverslip and <4 inclusions per ×400 field; and 3+, >4 inclusions per ×400 field.

**Gen-Probe Pace 2 assay.** On receipt, specimens for the Gen-Probe Pace 2 assay were immediately vortexed and the swabs were removed after as much fluid as possible had been expressed. The supernatants were stored at −70°C until tested in accordance with the manufacturer’s instructions.

**Pharmacia EIA.** Specimens for the Pharmacia EIA were stored at −70°C until tested in accordance with the manufacturer’s instructions. All positive specimens were retested with the confirmatory assay.

**Abbott Chlamydiazyme EIA.** Specimens for the Abbott Chlamydiazyme EIA were stored at 4°C and processed within 3 days of receipt in accordance with the manufacturer’s instructions. All positive specimens were retested with the confirmatory assay.

**DNA probe competition assay.** When required, a DNA probe competition assay was performed by Gen-Probe, San Diego, Calif. The Gen-Probe Pace 2 assay was repeated to show that the initial test results were reproducible, and a duplicate assay was performed in the presence of a 100-fold excess concentration of unlabelled chlamydia probe identical to the original labelled probe. If the reaction was specific, the excess unlabelled probe competed with the labelled probe.
probe, causing a significant reduction in the relative light unit count compared with the original result. To demonstrate that the positive assay was not the result of nonspecific binding, an additional assay using a 100-fold excess of heterologous probe (Mycoplasma pneumoniae) together with the standard labelled chlamydia probe was performed.

**Confirmatory assays (Pharmacia and Abbott).** Confirmatory assays were used to confirm the specificity of a positive Pharmacia or Abbott EIA result. The positive specimen was retested and simultaneously assayd in parallel with a blocking antibody to inhibit the antichlamydial antibody step. A significant (50% or more) decrease in the optical density of the result was considered to confirm the presence of chlamydial antigens in the specimen.

**Syva direct fluorescence antibody test.** A direct fluorescence antibody test (Syva Microtrak) was used to confirm EIA-positive results and was modified as follows: after EIA testing, the remaining specimen buffer was centrifuged at 14,000 rpm for 5 min in an Eppendorf centrifuge, after which all the supernatant was removed except for 100 µl which was used to resuspend the pellet. Duplicate 25-µl volumes of the resuspended pellet were applied to wells of an immunofluorescence slide, air dried, and fixed with methanol fixative before being stained according to the manufacturer’s protocol.

Cervical swabs from 1,037 women were assayed by the three test procedures. The sensitivities, specificities, and positive and negative predictive values of each method compared with those of cell culture are shown in Table 1. The positivity rate by cell culture was 4.7%.

Further analysis of cases in which culture was negative but other tests were positive showed 12 cases of *C. trachomatis* infection which culture failed to identify. These cases were confirmed by performing direct immunofluorescence (Syva Microtrak) on the centrifuged supernatants from the EIAs and by retesting the Gen-Probe specimens with the probe competition assay (Table 2). A definition of true positive and true negative was made by including these specimens with cell culture-positive specimens. True-positive specimens were defined as those which were culture positive and/or were positive by one or more EIA tests and confirmed by immunofluorescence and/or were positive by Gen-Probe and confirmed by probe competition assay. True-negative specimens were negative by cell culture, EIA, and Gen-Probe. The sensitivities, specificities, positive and negative predictive values, and positivity rate were then recalculated compared with true positives (Table 3).

A record of the swab collection order was maintained to detect any variation which could prejudice the test results. There were 43 false-negative swabs, and the analysis of collection order showed that 12 were third swabs, 14 were fourth, 11 were fifth, and 6 were sixth swabs.

The inadequacy of cell culture as a “gold standard” for *C. trachomatis* infection has been recognized for some time, and sensitivity estimates range between 80 and 90% (2, 11, 16, 17). EIAs and direct immunofluorescence stains for *C. trachomatis* have been extensively evaluated, with reported sensitivities ranging from less than 70 to more than 95% (1, 3, 4, 6, 8–10, 13, 14, 18, 19). Culture performed together with an antigen detection system has been shown to detect more positives than a single assay, and it has been suggested that a more satisfactory gold standard for *C. trachomatis* would be a combination of tests (8). The Gen-Probe Pace 2 nucleic acid hybridization assay for *C. trachomatis* is a relatively recent development and has been shown to be more sensitive and specific than the earlier Pace 1 and 125I-labelled probes (14–16).

This study shows that with cell culture as the gold standard, the Gen-Probe *C. trachomatis* Pace 2 assay is more sensitive than both the Abbott Chlamydiazyme and Pharmacia Chlamydia EIAs. Further analysis of cases in which cell culture was negative but other tests were positive showed that there were 12 cases of *C. trachomatis* infection which culture failed to identify. When these additional positives were added to the cell culture positives to give the total of true positives, the Gen-Probe assay proved to be more sensitive than tissue culture and the EIAs. These findings support those of Iwen et al. (5), who in a similar study demonstrated sensitivity, specificity, and positive and nega-
TABLE 3. Sensitivities, specificities, and positive and negative predictive values for the EIAs and nucleic acid probe test and cell culture compared with true positives (as defined) as the gold standard.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sens. (percent)</th>
<th>Spec. (percent)</th>
<th>PPV (percent)</th>
<th>NPV (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott Chlamydiazyme</td>
<td>77.5 (45/58)</td>
<td>100 (961/961)</td>
<td>100 (45/45)</td>
<td>98.6 (961/974)</td>
</tr>
<tr>
<td>Pharmacia Chlamydia EIA</td>
<td>77.0 (47/61)</td>
<td>100 (973/973)</td>
<td>100 (47/47)</td>
<td>98.6 (973/987)</td>
</tr>
<tr>
<td>Gen-Probe Pace 2 Chlamydia test</td>
<td>96.7 (58/60)</td>
<td>99.6 (948/952)</td>
<td>93.6 (58/62)</td>
<td>99.7 (948/950)</td>
</tr>
<tr>
<td>Cell culture</td>
<td>80.0 (49/61)</td>
<td>100 (971/971)</td>
<td>100 (49/49)</td>
<td>98.7 (971/983)</td>
</tr>
</tbody>
</table>

a The positivity rate was 5.9% by true-positive definition.

b Sens., sensitivity (percent); numbers in parentheses, ratios of number of positives detected to total number of positives.

c Spec., specificity (percent); numbers in parentheses, ratios of number of negatives detected to total number of negatives.

d PPV, positive predictive value (percent); numbers in parentheses, ratios of number of positives detected to total number of true positives.

e NPV, negative predictive value (percent); numbers in parentheses, ratios of number of negatives detected to total number of true negatives.

REFERENCES


We acknowledge the assistance of R. Pringle with computer data analysis.

We acknowledge also the generous support of the Gen-Probe and Pharmacia companies.