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Comparison of the Clearview Chlamydia Test, Chlamydiazyme, and Cell Culture for Detection of *Chlamydia trachomatis* in Women with a Low Prevalence of Infection

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Two antigen detection systems, Clearview Chlamydia (Unipath Ltd., Bedford, United Kingdom) and Chlamydiazyme (Abbott Laboratories, North Chicago, Ill.), were compared with culture for the diagnosis of chlamydia infection in women attending gynecological clinics. *Chlamydia trachomatis* was isolated from 43 (4.5%) of the 965 women tested. In comparison with tissue culture, the Clearview Chlamydia and Chlamydiazyme tests had sensitivities of 79.0 and 74.4%, respectively, and both had a specificity of 99.6%. The results show that the Clearview Chlamydia test is comparable to Chlamydiazyme for the detection of *C. trachomatis* from endocervical specimens in a population with a low prevalence of infection.

Chlamydia trachomatis is a major cause of sexually transmitted disease in North America (3, 16). Previously, the diagnosis of this infection relied on isolation of the organism in cell cultures. However, culture techniques have been shown to have a sensitivity of less than 100% because of problems with the transport of specimens, the type of swab used, and the culture conditions themselves (2, 5, 6, 13). The introduction of commercial kits with species-specific monoclonal antibodies that detect chlamydial antigens in the outer membrane protein and genus-specific antibodies to cell wall lipopolysaccharide has permitted the development of techniques for more rapid testing of specimens. Both enzyme-linked immunoassay and direct fluorescent-antibody (DFA) methods are available (7, 8, 11, 14, 15). Several new rapid tests that are easier to perform and that do not require the purchase of expensive supplementary equipment have also been introduced (4). The Clearview Chlamydia (Unipath Ltd., Bedford, United Kingdom) test is a solid-phase sandwich immunoassay that uses a chromatographic principle and a color-labeled monoclonal antibody to a genus-specific lipopolysaccharide epitope to detect the presence of antigen. The purpose of this study was to compare the sensitivities and specificities of the Clearview Chlamydia and the Chlamydiazyme (Abbott Laboratories, North Chicago, Ill.) tests with those of standard tissue culture.

Four specimens were collected on endocervical swabs from each of 965 patients attending gynecological clinics at the Mount Sinai Hospital in Toronto. The order of collection of specimens by use of the four swabs from the test kits was rotated every 100 patients. The mean age of these patients was 30.5 years, with a range of 14 to 82 years. In collecting the specimens, the endocervix was first cleansed to remove excess mucus. The various test kit swabs were then inserted into the cervical canal individually and rotated to collect cellular material. The Clearview Chlamydia and Chlamydiazyme swabs were transported in the kits provided by the manufacturers. For the tissue culture, a plastic Dacron-tipped swab (Prolab Diagnostics, Toronto, Ontario, Canada) was used to collect the specimen. The swabs were placed in vials (14 by 15 mm) containing 1.5 ml of chlamydia transport medium and three glass beads. The transport medium was Eagle minimum essential medium (MEM) supplemented with 10% fetal bovine serum, glucose, gentamicin, vancomycin, and amphotericin B. If the cultures were not inoculated on the same day, they were stored at −70°C and processed within 48 h of receipt. The fourth swab, collected for culture of *Neisseria gonorrhoeae*, was placed into Amies transport medium with charcoal (NCS, Mississauga, Ontario, Canada) and processed within 2 h by a standard methodology (9).

A shell vial technique (7) with cycloheximide-treated McCoy cells (Connaught Laboratories, Toronto, Ontario, Canada) was used to isolate *C. trachomatis*. The specimens were vortexed for three cycles of 15 s each. Excess fluid was removed from each swab by pressing and rotating it against the side of the transport vial. Prior to inoculation, the growth medium (MEM, vitamins, L-glutamine, fetal bovine serum, vancomycin) was decanted from the shell vial, and 0.3 ml of the inoculum was added to two vials; this was done for the specimens from each patient. The vials were centrifuged at 3,000 × *g* for 1 h at 20°C. The inoculum was then aspirated, and 1 ml of overlay chlamydia medium (MEM, vitamins, amino acids, fetal bovine serum, vancomycin, cycloheximide) was added. Both vials were incubated at 36°C in 5% CO_2 for 48 h. After incubation, one vial was fixed with methyl alcohol and stained with fluorescein-conjugated monoclonal antibodies directed against the major outer membrane protein of *C. trachomatis*, using the MicroTrak Culture Confirmation Reagent (Syva Co., Palo Alto, Calif.) according to the instructions of the manufacturer. The stained coverslip was examined with a fluorescence microscope for the presence of intracytoplasmic inclusions. If any inclusions were present, the specimen was considered to be positive by culture. A blind subculture was performed by using the second vial if the original culture was negative. This was done by scraping the cells off the coverslip, vortexing, and inoculating the cells into a new monolayered shell vial, which was processed the same way as the samples from patients were.

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TABLE 1. Comparison of the Clearview Chlamydia and Chlamydiazyme tests with cell culture and cell culture plus antigen test for the detection of *C. trachomatis*

Test and result (no.)	No. of test results			
	Clearview Chlamydia		Chlamydiazyme	
	Positive (% sensitivity)	Negative (% specificity)	Positive (% sensitivity)	Negative (% specificity)
Cell culture				
Positive (43)	34 (79.0)	9	32 (74.4)	11
Negative (922)	4	918 (99.6)	4	918 (99.6)
Cell culture plus antigen test				
Positive (46)	37 (80.4)	9	35 (77.7)	11
Negative (919)	1	918 (99.9)	1	918 (99.9)

The Clearview Chlamydia test was performed by placing the swab into a flexible plastic extraction tube containing 0.6 ml of extraction buffer supplied in the kit. The tube was heated at 80°C for 10 min in a heater block to release the lipopolysaccharide antigen. The swab was then removed, ensuring that as much fluid as possible remained. This was achieved by gently squeezing the extraction tube against the swab. The sample was then left to cool for 5 min. However, the sample extract could be left at room temperature for 3 h before testing. The extraction tube was then capped with the attached dropper-filter, and five drops of the extract were then placed onto the absorbent pad in the sample window of the test strip. This strip was allowed to sit for 15 min and examined. If antigen is present in the sample, it combines with the latex-labeled antibody contained in the absorbent pad. This, in turn, is carried up the membrane strip by capillary action, where it contacts a band of immobilized monoclonal antichlamydia antibody. Here, the antigen is captured and forms a visible blue line that indicates that the sample is positive. Some of the latex is carried farther along the strip, where it is bound to a band of immobilized anti-mouse antibody, forming a visible blue line in the control window, indicating that the test has been completed. The majority of specimens were processed within 24 h of receipt, even though the manufacturers state that they can be stored refrigerated for at least 5 days before testing.

The Chlamydiazyme assay was performed according to the instructions of the manufacturer. All reactive Chlamydiazyme tests were confirmed by using a previously described blocking technique (12).

Because of the reported lack of sensitivity of tissue culture as a "gold standard," those specimens that were negative by culture but that were reactive by the Clearview Chlamydia and Chlamydiazyme tests were further studied by DFA, using the Syva MicroTrak Culture Confirmation reagent. The unused portion of the Chlamydiazyme specimen was centrifuged in a cytospin apparatus (Shandon Ltd., Cheshire, United Kingdom) at 2,000 × *g* for 15 min. The deposit was fixed and stained with the MicroTrak reagent, according to the instructions of the manufacturer. The slides were then examined for elementary bodies. Five or more elementary bodies were considered indicative of a positive specimen.

C. trachomatis was isolated from 4.5% (43 of 965) of the specimens by using tissue cultures. Of these, 40 specimens (93%) were positive in the initial culture and the remaining 3 specimens became positive only after the blind passage. Thirty-four (79.0%) and 32 (74.4%) of these 43 positive specimens were also positive by the Clearview Chlamydia and Chlamydiazyme tests, respectively (Table 1). There were a total of 13 specimens by the Clearview Chlamydia test and 15 specimens by the Chlamydiazyme test that gave discrepant results when they were compared with culture results. The positive predictive values for the Clearview Chlamydia and Chlamydiazyme tests were 89.5 and 88.9%, respectively. The negative predictive values were 99.0% for Clearview Chlamydia and 98.8% for Chlamydiazyme. The sampling order of the specimens was examined to determine whether it affected the results of the tests. No bias was observed, since positive results for each of the tests evaluated were equally distributed over the course of the study.

In three additional culture-negative specimens, the Clearview Chlamydia test, the Chlamydiazyme test, and DFA were all positive. If these specimens are considered to be true positives, the number of patients infected with *C. trachomatis* increased to 46, for a prevalence of 4.8%. By using these criteria, the sensitivity of tissue culture in the study would be 93.5% (43 of 46 patients), which is similar to the 92.3% sensitivity found by Mahony et al. (11) by a comparable methodology. By including these three additional culture-negative, antigen-positive patients identified by the Clearview Chlamydia and Chlamydiazyme tests, the sensitivities of the tests would be 80.4 and 77.7%, respectively; the specificity for both tests would be 99.9% (Table 1).

The one other published evaluation of the Clearview Chlamydia test was done in a patient population with a prevalence of *C. trachomatis* infection of 17.5%. Arumainayagam et al. (1) found that the sensitivity of the test was 93.5% and that the specificity was 99% compared with those of culture. It is possible that the increased sensitivity found in this study was due to the fact that these patients were symptomatic, and therefore, there may have been a larger number of infectious particles in the specimens (6, 10). Since the protocol of our study required the collection of four separate swabs, it is possible that this may have affected the sensitivities of both tests because fewer organisms may have been available on the latter swabs. With the exception of one patient, all those infected with chlamydia in the present study were asymptomatic. The symptomatic patient was referred to the clinic because of abdominal pain and vaginal bleeding. The prevalence of *N. gonorrhoeae* was 0.52% (5 of 965 specimens). Every patient from whom *N. gonorrhoeae* was isolated was also infected with *C. trachomatis*. These findings reflect the low prevalence of sexually transmitted disease present in the women attending gynecological clinics at our hospital. The results of this study are also consistent with the experience of other investigators (8, 11) who have

evaluated nonculture methods for the diagnosis of *C. trachomatis* in similar populations with a low prevalence of infection.

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