JOURNAL OF CLINICAL MICROBIOLOGY, July 1991, p. 1551–1553
0095-1137/91/071551-03$02.00/0

Vol. 29, No. 7

Evaluation of the Rapid CLEARVIEW Chlamydia Test for Direct Detection of Chlamydiae from Cervical Specimens

NICOLAS J. STRATTON,[1] LISA HIRSCH,[2] FRED HARRIS,[1] LUIS M. DE LA MAZA,[1] AND ELLENA M. PETERSON[1]*

Departments of Pathology[1] and Obstetrics and Gynecology,[2] University of California Irvine Medical Center, 101 City Drive, Orange, California 92668

Received 9 January 1991/Accepted 9 April 1991

The CLEARVIEW Chlamydia test (Unipath, Mountain View, Calif.), a 30-min immunoassay, was compared to a standard tissue culture technique for the direct detection of chlamydiae from 677 cervical specimens obtained from 667 patients. For data analysis, 15 specimens were eliminated because of toxicity in the culture and 14 were eliminated because of failure of the extracted specimen to migrate in the CLEARVIEW test, one of the latter group being culture positive. Of the remaining 648 specimens, 40 were culture positive, of which 38 were detected by the CLEARVIEW test, and 12 specimens were positive only by the CLEARVIEW test. Therefore, the CLEARVIEW in comparison with culture was easier to perform, more rapid, and in this low prevalence (6.2%) population had a 95.0% sensitivity, 98.0% specificity, and 76.0% positive and 99.7% negative predictive values.

Over the past 10 years several products have been introduced for the direct detection of *Chlamydia trachomatis* which make the laboratory testing for this important sexually transmitted pathogen more practical than the standard tissue culture method. Although culture is still regarded as the gold standard for the laboratory detection of *C. trachomatis*, few laboratories are able to perform it to its maximum sensitivity, whether this be due to specimen collection, transportation, or holding or culture techniques (1, 9). However, the first generation of direct assays, which in general take a few hours to complete and/or require specialized equipment, have been reported in some studies to have sensitivities that range from 60 to 85% and specificities that range from 94 to 99%; therefore, both sensitivity and specificity remain a problem with these assays (3, 6, 8, 10). In the past few years, the second generation of assays has been introduced, which do not require costly specialized equipment and take even a shorter time to complete. Although there are not as many reports in the literature on the performance of these assays, the data available so far seem promising (2, 4, 5). The CLEARVIEW Chlamydia, which is a 30-min single-reagent immunoassay, has been recently introduced for testing cervical specimens. In one report, the CLEARVIEW test had a sensitivity of 93.5% and a specificity of 99% when tested in a population with a prevalence of 17.5% of chlamydial infection (2). In this report, we compared the CLEARVIEW system with a standard tissue culture technique in a population with a low prevalence (6.2%) of chlamydial infection.

Cervical specimens were obtained from patients attending the Obstetrics and Gynecology Clinic at the University of California Irvine Medical Center. Upon cleansing the cervix, two swabs were obtained from each patient. The order of collection of the swabs was rotated every 25 patients. Specimens for culture were obtained with a cytobrush, or in the case of a pregnant female, in which collection using a cytobrush was not advisable, a cotton swab (American Scientific Products, McGaw Park, Ill.) was used. This swab or cytobrush was placed in 1 ml of 2-SPG (0.2 M sucrose, 0.02 M sodium phosphate [pH 7.2], 5 mM glutamic acid) (11) containing gentamicin (50 μg/ml) and amphotericin B (25 μg/ml). The swabs for testing by the CLEARVIEW assay were provided with the testing kits and upon collection were held dry. All specimens were refrigerated within 1 h of collection.

Cultures were performed as previously described (10). Specimens were processed and tested within 24 h of collection, with the majority within 6 h of collection. Briefly, swabs or cytobrushes were vortexed for 2 min in 1 ml of 2-SPG transport media. Two glass shell vials (15 by 45 mm) containing McCoy cell monolayers which had been seeded 24 h before culture on 12-mm coverslips were each inoculated with 0.1 ml of specimen. Vials were centrifuged for 1 h at 35°C at 1,500 × *g*, after which 1 ml of Eagle minimal essential medium containing fetal calf serum (10%), gentamicin (50 μg/ml), and cycloheximide (1 μg/ml) was added. Cultures were incubated for 48 h before fixation with methanol and staining with a monoclonal antibody (MAb) to the lipopolysaccaharide (LPS) of chlamydiae (Cultureset; Ortho Diagnostics, Inc., Raritan, N.J.). The number of inclusion-forming units (IFU) per coverslip was determined. With 402 cultures, a blind pass was performed from the extra vial inoculated. For the blind pass, glass beads were added directly to the vial, which was then vigorously vortexed for 2 min, and 0.1 ml of this was used to inoculate each of two vials, which were centrifuged, incubated, and stained as described for the primary cultures.

Specimens collected and transported on dry swabs provided with the CLEARVIEW Chlamydia assay were tested at the same time the cultures were inoculated. Swabs were placed in 0.6 ml of extraction buffer and heated at 80°C for 12 min in a heating block provided by the manufacturer. Upon cooling for 5 min, the swabs were removed, and the resulting specimen extract was tested on a CLEARVIEW card. To the sample window (Fig. 1), 5 drops of extracted specimen were added and allowed to react for 15 min with the reagents impregnated in the card. During this time, if chlamydial LPS antigen was present in the patient sample, the antigen would bind to the colored-latex-labeled murine MAb to chlamydial LPS contained in the sample window. The liquid sample

* Corresponding author.

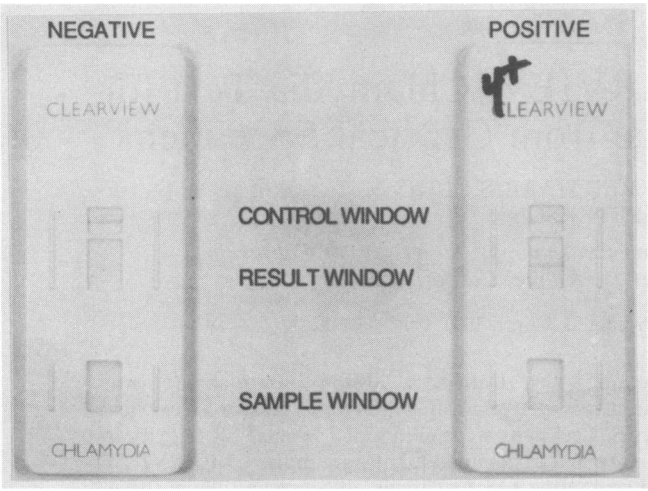

FIG. 1. A negative (left) and positive (right) specimen by the CLEARVIEW Chlamydia assay are shown. Extracted specimens are placed on the filter in the sample window, which contains a colored-latex-labeled murine MAb to chlamydial LPS. Upon rehydration with the extracted sample, chlamydial LPS, if present in the patient specimen, complexes with the labeled MAb, and this complex migrates up the card. The result window contains immobilized unlabeled murine MAb to chlamydial LPS, which serves to capture any colored antigen–latex-labeled-antibody complex that may be present. Therefore, if a chlamydial LPS-labeled-MAb complex has been formed in the sample window, a colored band forms in the result window. In all assays, a band is formed in the control window because of the migration of the excess colored-latex-labeled murine MAb to chlamydial LPS, which is captured by the immobilized rabbit anti-mouse antibody in the control window.

would migrate up the CLEARVIEW card filter until it reached the result window where, if the LPS antigen-antibody complex was present, it would bind to unlabeled anti-LPS MAb that was immobilized in the result window. This would result in a visible band in the result window, since the colored-latex-labeled chlamydial antigen-LPS antibody complex would have been captured by the unlabeled anti-LPS antibody present in the result window. Any band in this window was considered a positive result. The excess unbound colored-latex-labeled murine anti-LPS MAb would migrate to the control window and react with rabbit anti-mouse serum that was immobilized in the control window. Therefore, each assay had a built-in control that the latex-labeled MAb had migrated in the assay system. All assays had a positive band in the control window; however, in some cases, if the sample was strongly positive in the result window, then the control signal tended to be weaker than that seen with the negative specimens because of the smaller amount of free murine anti-LPS antibody available to migrate to the control window. An example of a positive and a negative specimen for chlamydiae assayed on a CLEARVIEW card can be seen in Fig. 1.

A total of 677 cervical specimens were obtained from 667 patients. The patients ranged in age from 13 to 62 years, with an average age of 26.9 years. Blind passes were performed on 402 of the specimens cultured, and of these, there was 1 culture-positive specimen detected on the blind pass. Of the total specimens tested, there were 15 specimens that were toxic to McCoy cell monolayers when they were initially cultured. These specimens were eliminated from the data analysis, since they were frozen at the time of the initial culture, and therefore repeat cultures were performed from frozen not fresh cultures, thus raising the possibility of a loss of viability in the specimen due to freezing. There were 14 specimens which failed to migrate in the CLEARVIEW system, and therefore they too were eliminated from the final analysis. One of these latter specimens was culture positive.

TABLE 1. Correlation of the CLEARVIEW color intensity with the number of IFU per coverslip

CLEARVIEW color intensity (1+ to 4+)	No. of specimens with the given no. of IFU per coverslip			
	Negative	<100	100–1,000	>1,000
1+	10	10	5	0
2+	1	0	3	2
3+	1	0	3	1
4+	0	2	0	12

Of the remaining 648 specimens from 641 patients, there were 40 (6.2%) that were culture positive. The distribution of IFU per coverslip in these positive specimens was 13 with <100 IFU, 12 with from 100 to 1,000 IFU, and 15 with >1,000 IFU. There were 9 (5.0%) positive specimens of the total 178 specimens collected by cytobrush, and of the 463 swabs used for culture, 29 (6.3%) were culture positive. The remaining specimens for culture were received with the cytobrush or swab already removed from the 2-SPG medium, and therefore how the specimen had been actually obtained was unknown. Therefore, in this study, there was no statistical difference ($P > 0.05$) between the culture-positive specimens as to whether they were obtained by a cytobrush or a swab. This is similar to the findings of Lees et al. (7), in which, comparing 2,024 cervical specimens collected by both cytobrush and swab, they failed to find a difference in culture results between the two collection devices.

The CLEARVIEW system detected 38 (95.0%) of the culture-positive specimens. The two culture-positive specimens not detected by the CLEARVIEW test had 4 and 325 IFU per coverslip. The correlation between the intensity of the color reaction by the CLEARVIEW Chlamydia and the IFU by culture for those 38 specimens that were positive by both methods can be seen in Table 1. There was a direct correlation between color intensity by the CLEARVIEW test and the number of IFU by culture. There were 12 specimens that were detected only by the CLEARVIEW test. None of these patients had been receiving antibiotics prior to having the specimens obtained. Of the specimens that were positive only by the CLEARVIEW test, the majority (83%) had an intensity of 1+, as can be seen in Table 1.

In this evaluation, we compared a rapid immunoassay, the CLEARVIEW Chlamydia, for the direct detection of chlamydiae with a standard tissue culture method. The tissue culture method is one which we have previously compared with other rapid-detection methods (10). In this study, we found the CLEARVIEW test to have a sensitivity of 95.0%, a specificity of 98.0%, and positive and negative predictive values of 76.0 and 99.7%, respectively. The sensitivity found with the CLEARVIEW test was higher than that we previously reported for the Chlamydiazyme (85%), Microtrak (75%), and PACE DNA probe (60%) assays (10). There is one other report of the CLEARVIEW assay, in a population with a higher prevalence (17.5%) for chlamydial infection than the 6.2% for chlamydial infection prevalence of the population in

this report (2). The authors of this previous study found that of the 376 endocervical samples examined with both culture and the CLEARVIEW Chlamydia, the CLEARVIEW test had a sensitivity of 93.5% and a specificity of 99%.

There were several features of the CLEARVIEW assay which made it very easy to perform. First, the specimen is taken and transported at room temperature on a dry swab, thereby eliminating transport fluid and refrigeration problems. These two features make this test practical for most outpatient facilities. Second, there is only one reagent to dispense, the total hands-on time of the assay is under one min, and the total time to completion of the assay is 0.5 h. Third, the only instrumentation required is a heating block for the 80°C extraction, which is provided by the manufacturer.

The reading of the CLEARVIEW result was subjective, and this may have contributed to some of the false-positive CLEARVIEW results. Of the 12 false-positive results, 10 were of a low-intensity (1+) color band. Also in the 1+ range were 15 of the 40 culture-positive samples. Perhaps if a card reader were available, then there could be differentiation within this 1+ range between the true- and the false-positive results. An instrument to measure the color intensity of the reaction would also eliminate any technical variation in interpretation among laboratory workers.

In summary, although not as sensitive and specific as a standard tissue culture assay, the CLEARVIEW system had a comparable or even higher sensitivity and specificity than some of the more laborious and technically demanding direct chlamydial assays. This assay has some definite advantages over many of the other direct assays currently available in that it requires no specialized equipment, transport media, or refrigeration, and it is rapid and technically easy to perform.

REFERENCES

1. **Aarnaes, S. A., E. M. Peterson, and L. M. de la Maza.** 1984. The effect of media and temperature on the storage of *Chlamydia trachomatis*. Am. J. Clin. Pathol. **81**:237–239.
2. **Arumainayagam, J. T., R. S. Matthews, S. Uthayakumar, and J. C. Clay.** 1990. Evaluation of a novel solid-phase immunoassay, Clearview Chlamydia, for the rapid detection of *Chlamydia trachomatis*. J. Clin. Microbiol. **28**:2813–2814.
3. **Chernesky, M. A., J. B. Mahony, S. Castriciano, M. Mores, I. O. Stewart, S. J. Landis, W. Siedelman, E. J. Sargeant, and C. Leman.** 1986. Detection of *Chlamydia trachomatis* antigens by enzyme immunoassay and immunofluorescence in genital specimens from symptomatic and asymptomatic men and women. J. Infect. Dis. **154**:141–148.
4. **Coleman, P., V. Varitek, I. K. Mushahwar, B. Marchlewicz, J. Safford, J. Hansen, G. Kurpiewski, and T. Grier.** 1989. TestPack Chlamydia, a new rapid assay for the direct detection of *Chlamydia trachomatis*. J. Clin. Microbiol. **27**:2811–2814.
5. **Hammerschlag, M. R., M. Gelling, P. Roblin, and M. Worku.** 1990. Comparison of the Kodak Surecell Chlamydia Test Kit with culture for the diagnosis of chlamydial conjunctivitis in infants. J. Clin. Microbiol. **28**:1441–1442.
6. **Hipp, S. S., Y. Haun, and D. Murphy.** 1987. Assessment of enzyme immunoassay and immunofluorescence tests for detection of *Chlamydia trachomatis*. J. Clin. Microbiol. **25**:1938–1943.
7. **Lees, M. I., D. M. Newnan, H. Plackette, P. W. Traynor, J. R. Forsyth, and S. M. Garland.** 1990. A comparison of cytobrush and cotton swab sampling for the detection of *Chlamydia trachomatis* by cell culture. Genitourin. Med. **66**:267–269.
8. **Lipkin, E. S., J. V. Moncada, M. Shafer, T. E. Wilson, and J. Schachter.** 1986. Comparison of monoclonal antibody staining and culture in diagnosing cervical chlamydial infection. J. Clin. Microbiol. **23**:114–117.
9. **Mahoney, J., and M. Chernesky.** 1985. Effect of swab type and storage temperature on the isolation of *Chlamydia trachomatis* from clinical specimens. J. Clin. Microbiol. **22**:865–867.
10. **Peterson, E. M., R. Oda, R. Alexander, J. R. Greenwood, and L. M. de la Maza.** 1989. Molecular techniques for the detection of *Chlamydia trachomatis*. J. Clin. Microbiol. **27**:2359–2363.
11. **Wang, S.-P., and J. T. Grayston.** 1964. Egg infectivity assay of trachoma virus. Proc. Soc. Exp. Biol. Med. **115**:587–591.