JOURNAL OF CLINICAL MICROBIOLOGY, July 1993, p. 1892–1895
0095-1137/93/071892-04$02.00/0

Vol. 31, No. 7

Confirmation of Positive Results for Chlamydial Antigen by the Chlamydiazyme Assay: Value of Repeated Testing and a Blocking Antibody Assay

MARGARET A. OLSEN[1]* AND ANTHONY R. SAMBOL[2]

Departments of Medical Microbiology and Pathology, Creighton University School of Medicine, Omaha, Nebraska 68178,[1] and Pathology Department, Saint Joseph Hospital, Omaha, Nebraska 68131[2]

Received 7 January 1993/Accepted 19 April 1993

We studied the specificity of the Abbott Chlamydiazyme test for detection of *Chlamydia trachomatis* antigen by means of a specific blocking antibody test. A total of 457 previously positive specimens were tested; 22 did not block in the blocking antibody test, 39 did not repeat as positive, and 396 were confirmed as positive. The distribution of A_{492} values obtained with specimens which did not repeat as positive was nonrandom and was concentrated between the cutoff values and 0.400. The positive predictive value of the Chlamydiazyme assay after initial testing was 86.7% (396 of 457), but the positive predictive value increased to 94.7% (396 of 418) if specimens which were not repeatedly positive were considered negative. We recommend routinely repeating the Chlamydiazyme assay for all specimens which give A_{492} values between the cutoff and 0.400 to eliminate many false-positive results. Use of the blocking antibody reagent can then be reserved for confirming only specimens which are repeatedly positive.

Accurate methods for diagnosis of sexually transmitted diseases are essential for appropriate patient care and prevention of transmission of the diseases. It is also very important to use methods which are highly specific, to avoid the devastating consequences of reporting a false-positive result to a patient. This is true for all sexually transmitted diseases, including *Chlamydia trachomatis*, which is estimated to cause 4 million infections annually in the United States (2).

Many types of assays are available for diagnosis of *C. trachomatis* infections, including culture, detection of antigen by enzyme immunoassay (EIA) or direct immunofluorescence, and, more recently, a DNA probe assay (6, 7). Culture is the most specific test available, but it is expensive and time and labor consuming. EIAs are widely used for detection of chlamydial antigen, because of their ease of performance and the ability to automate the procedure. The Abbott Chlamydiazyme assay is an EIA which employs polyclonal antisera to detect chlamydial antigen in patient specimens. Numerous evaluations of Chlamydiazyme have been published, with reported sensitivities ranging from 44 to 100% and specificities of 90 to 99% (7). The majority of reports have concluded that Chlamydiazyme has a specificity of greater than 95%.

Several options exist to verify EIA results positive for chlamydial antigen. Positive results can be confirmed by simultaneous culture of a duplicate specimen, repeating all positive results to confirm reproducibility, performance of direct immunofluorescence on sediment from the EIA tube, or performance of a blocking antibody test. A blocking antibody assay is available for confirmation of positive results with the Chlamydiazyme test. The blocking antibody test involves performance of the EIA in the presence of murine monoclonal anti-*C. trachomatis* antibody. If *C. trachomatis* is present in the specimen, the monoclonal antibody will compete with the polyclonal rabbit antiserum in the Chlamydiazyme kit for binding to chlamydial antigen and the signal produced after addition of conjugate and substrate will be decreased relative to a duplicate sample in which blocking antibody was not added. A positive blocking antibody test confirms that the antigen detected in the Chlamydiazyme assay was *C. trachomatis*.

We evaluated the use of the blocking antibody test on positive specimens collected during a 10-month period to determine the false-positivity rate of the EIA in our population. We also analyzed the repeatability of the Chlamydiazyme assay, and from the results of this evaluation, we propose a simple protocol for confirmation of positive results.

MATERIALS AND METHODS

Specimens. Specimens were collected from the endocervix, urethra, and conjunctiva by using the appropriate collection kits. The specimens were transported to the laboratory at ambient temperature and were stored at 4°C for no more than 5 days before testing.

Specimens which gave positive results in the Chlamydiazyme assay were stored frozen at −70°C in specimen dilution buffer until the blocking antibody assay was performed.

Chlamydiazyme assay. The Chlamydiazyme assay (Abbott Laboratories, Abbott Park, Ill.) was performed exactly as recommended by the manufacturer. The cutoff values were determined by adding 0.100 to the average of the absorbance values of the negative controls.

Blocking antibody test. The blocking antibody reagent (Abbott Laboratories) consists of a murine monoclonal antibody to chlamydial lipopolysaccharide. The blocking assay was performed in accordance with the manufacturer's guidelines as follows. A predetermined volume of rabbit anti-*C. trachomatis* antiserum (in the Chlamydiazyme kit) was pipetted into a clean tube. A 25-fold smaller volume of the blocking antibody (murine monoclonal) was then added to the tube. The Chlamydiazyme assay was begun by adding 200 μl of each specimen to be tested to duplicate wells in a

* Corresponding author.

TABLE 1. Results of blocking antibody assay

Type of specimen	No. of initial positives	No. of repeat negatives (%)[a]	No. of false positives by blocking (%)[b]	Total no. of false positives (%)[c]
Male urethral	34	1 (2.9)	1/33 (3.0)	2 (5.9)
Endocervical	422	38 (9.0)	21/384 (5.5)	59 (14.0)
Total	456	39 (8.6)	22/418 (5.3)	61 (13.3)

[a] Specimens not repeatedly positive.
[b] Specimens repeatedly positive but did not block in the blocking antibody assay.
[c] Total number of specimens not repeatedly positive and repeatedly positive specimens that did not block in the blocking antibody assay.

tray. Treated beads were added to each well, and the trays were incubated for 1 h at 37°C. The beads were then washed, and 200 μl of the unmodified rabbit anti-*C. trachomatis* antibody was added to one of the duplicate specimen wells, one of the duplicate positive control wells, and the negative control well. A total of 200 μl of blocked antibody (blocking antibody plus rabbit anti-*C. trachomatis*) was added to the other duplicate specimen well and the second of the duplicate positive control wells. The trays were incubated for 1 h at 37°C, and then the beads were washed. The remainder of the assay was performed exactly as the standard Chlamydiazyme assay.

After completion of the test, the A_{492} values of the positive and negative controls and specimens were determined spectrophotometrically. The absorbance value of the negative control was subtracted from the values of the positive controls and specimens (both blocked and unblocked) to obtain the net absorbance values.

Interpretation of results. A positive result in the blocking assay was indicated by a decrease in absorbance of 50% or more in the blocked sample compared with that of the control (unblocked sample). To be considered positive in the blocking assay, the control (unblocked sample) must have had a net absorbance of ≥0.100. Specimens in which the control sample did not have a net absorbance value of at least 0.100 were considered nonrepeaters.

If the absorbance of the unblocked specimen was >2.000, the blocking antibody test was repeated after diluting the specimen 1:10 in specimen dilution buffer.

RESULTS

During the study period, a total of 478 specimens were positive out of 4,514 specimens tested by the Chlamydiazyme assay. The prevalence rate of 10.6% was comparable to our average prevalence during that time period (unpublished results). Of the original 478 specimens which were positive in the Chlamydiazyme assay, 457 were stored frozen at −70°C and were retested with the blocking antibody assay.

The results of the blocking antibody assay are shown in Table 1. Of the 34 urethral specimens (all from males), 32 (94%) were confirmed as positive by the blocking antibody assay. A total of 422 endocervical specimens were tested with the blocking antibody assay, and only 86% (363 of 422) were confirmed positive. The control well (without blocking antibody) from 9% (38 of 422) of the previously positive endocervical specimens did not give a positive result on repeat assay, while 5.5% (21 of 384) of the repeatedly positive endocervical specimens did not block with the murine monoclonal antibody. One conjunctival specimen tested positive initially, and it was confirmed as positive in the blocking antibody test. Thus, of the 457 specimens originally interpreted as positive in the Chlamydiazyme assay, only 396 were confirmed as positive by repeat testing and the blocking antibody assay. The total number of false-positive and nonrepeating specimens was 61, which results in a false-positivity rate [(number of false-positive + nonrepeating results)/total number of initially positive results] of 13.3%. The false-positivity rate when urethral specimens were tested was 5.9% (2 of 34), whereas the false-positivity rate for endocervical specimens was 14.0% (59 of 422). The specificity of the Chlamydiazyme assay before repeating and blocking was 98.5% (4,036 of 4,097), and the positive predictive value was 86.7% (396 of 457).

The stratification of specimens by initial absorbance values in the Chlamydiazyme assay is shown in Table 2. The number of specimens which did not give a positive result in the blocking antibody assay (false-positive results) or did not repeat as positive are compared according to their absorbance values in the initial Chlamydiazyme assay. As seen in Table 2, 74% of the total false-positive and nonrepeating specimens had original absorbance values of ≤0.400, and 92% had absorbance values of ≤0.900. The initial absorbance values of nonrepeating specimens are heavily

TABLE 2. Absorbance values of specimens in the initial Chlamydiazyme assay and final results after the blocking antibody assay

Absorbance	No. in range (%)	No. of false positives	No. of nonrepeaters	% False positives + nonrepeaters in range[a]	% Total false positives + nonrepeaters in range[b]
Cutoff–0.200	53 (11.6)	6	22	52.8	45.9
0.201–0.300	38 (8.3)	2	9	28.9	18.0
0.301–0.400	31 (6.8)	2	4	19.4	9.8
0.401–0.500	21 (4.6)	0	0	0	0
0.501–0.600	21 (4.6)	2	2	19.0	6.6
0.601–0.700	16 (3.5)	2	0	12.5	3.3
0.701–0.800	18 (3.9)	3	0	16.7	4.9
0.801–0.900	14 (3.1)	1	1	14.3	3.3
0.901–1.000	8 (1.8)	0	0	0	0
1.001–1.500	37 (8.1)	0	0	0	0
1.501–2.000	22 (4.8)	1	1	9.1	3.3
>2.000	178 (38.9)	3	0	1.7	4.9

[a] Percentage of specimens with the indicated initial absorbance values which were judged false positive by the blocking antibody assay or did not give a positive result on repeat assay.
[b] Percentage of total false-positive plus nonrepeatedly positive specimens which had initial absorbance values in the indicated range.

weighted towards the lower ranges (<0.400) of absorbance values, and this tendency is highly significant ($\chi^2 = 80.8$, $P < 0.00005$). The proportion of specimens which gave a false-positive result in the blocking antibody assay is also weighted towards the lower ranges of absorbance values, but the trend does not reach statistical significance ($\chi^2 = 18.4$, $P = 0.07$).

As shown in Table 2, the percentage of specimens which gave a false-positive or nonrepeatable result was highest when the absorbance value in the initial Chlamydiazyme assay fell between the cutoff and 0.200. Almost 50% of the total false-positive or nonrepeatedly positive results had initial absorbance values in that lowest range. Thirty-seven percent (45 of 122) of specimens with absorbance values between the cutoff for a positive result and 0.400 were not repeatedly positive or were not confirmed as positive by the blocking antibody assay. The percentage of false-positive or not repeatedly positive specimens fell to 11% (11 of 98) when specimens with initial absorbance values between 0.400 and 1.000 were considered together and was only 2% (5 of 237) for specimens with high (>1.000) initial absorbance values.

DISCUSSION

Previous work has shown that the specificity of Chlamydiazyme for detection of *C. trachomatis* antigen is greater than 95%, but the positive predictive value varies, depending on the prevalence of infection in the population tested (1, 4, 5, 7, 11, 13–16). We studied the specificity of this EIA in a population with a prevalence of 10.6% and evaluated the use of a blocking antibody test to determine the need for confirmation of positive results.

Overall, we found that the positive predictive value of Chlamydiazyme in our population with a 10.6% prevalence of *C. trachomatis* infection was 86.7%. Ninety-four percent of male urethral specimens were confirmed as positive, but only 86% of endocervical specimens were confirmed as positive after repeated testing and performance of the blocking antibody test. Sixty-one of 457 total specimens were not confirmed as positive, and 39 (64%) of these were not repeatedly positive. Ninety percent of the specimens which were not repeatedly positive had absorbance values in the initial Chlamydiazyme assay of <0.400. Seventy-four percent of the total false-positive or nonrepeating specimens were in the low-positive ranges (cutoff to 0.400) on initial testing, and 92% had initial absorbance values of <1.000. Thus, specimens with a low absorbance value on initial testing have a higher probability of not being confirmed as positive than specimens with high absorbance values. These results correlate very well with that of Hipp et al. (4), who found that 73% of false-positive specimens, defined by negative cultures on duplicate specimens, had absorbance values in the Chlamydiazyme assay of <0.400.

In our study, specimens which initially were positive in the Chlamydiazyme assay were stored frozen at −70°C for subsequent testing by the blocking antibody assay. Some degradation of antigen may occur during a freeze-thaw cycle, which might result in negative results for chlamydial antigen on retesting. This probably did not contribute significantly to our results, however, because our repeat negative rate of 8.5% (39 of 457) is similar to that reported by others. Kellogg et al. (9) found that 198 of 210 (5.7%) genital specimens were repeatedly positive after storage of specimens at 4°C for no more than 48 h. Hallender et al. (3) found that 6.2% of 666 originally positive specimens were positive after retesting with the Chlamydiazyme assay. Thus, the repeat negative rate of 8.5% obtained in our study is slightly higher than the rates reported in these two previous studies, but the differences are not statistically significant (χ^2 test, $P = 0.23$). Our rate of 13.3% false-positive Chlamydiazyme results is very similar to the 12% false-positivity rate reported by Kellogg et al. (10). Thus, freezing of diluted specimens prior to confirmation of the Chlamydiazyme test does not appear to have much impact on the false-positivity rate determined by repeating the EIA or the blocking antibody assay.

Other investigators have used a variety of methods to confirm positive results with the Chlamydiazyme assay. Kellogg et al. (9) recommended testing of duplicate specimens to ensure reproducibility, and Schwebke et al. (15) found that a direct fluorescent-antibody test was helpful for confirmation of positive results with the Chlamydiazyme assay. Several authors have evaluated the blocking antibody test in conjunction with the Chlamydiazyme assay and have found the blocking test to be very useful in eliminating false-positive results (3, 12, 13, 17). Moncada et al. (13), in a multicenter evaluation, found that the specificity of Chlamydiazyme after performance of the blocking antibody assay was 99.9% compared with isolation of *C. trachomatis* and direct immunofluorescence. Kellogg and colleagues (8) tested Chlamydiazyme and the blocking antibody reagent with clinical isolates of bacteria and yeasts and found that the blocking antibody assay eliminated false-positive results due to large concentrations of some strains of gram-negative bacteria. This group also found that the percentage of false-positive Chlamydiazyme results, as defined by the blocking antibody assay, was increased in improperly collected specimens compared with specimens which contained endocervical and/or metaplastic cells (10). The authors found that as the number of endocervical and/or metaplastic cells increased in a specimen, the probability that a positive Chlamydiazyme result would be confirmed as a true positive also increased. Thus, they recommended use of the blocking antibody assay, particularly for specimens which were not collected properly.

Hallender et al. (3) compared the blocking antibody test with direct immunofluorescence on centrifuged material from the EIA tubes for confirmation of positive Chlamydiazyme results. They found that the results of the two tests correlated well, and there was good agreement between the number of elementary bodies and the absorbance value in the blocking antibody assay. Thus, it appears that either method can be used successfully for confirmation of results with the Chlamydiazyme assay.

Hallender and colleagues (3) also reported in the same study that the vast majority of specimens with false-positive results determined by the blocking antibody assay were collected from the cervix (105 of 109), while only four were urethral specimens. This agrees with our finding that 14% of initially positive endocervical specimens were not confirmed as positive after repeating the EIA and the blocking antibody test, whereas only 6% of initially positive urethral specimens had false-positive results. Kellogg et al. (10), as discussed above, found that endocervical specimen quality affected the incidence of false-positive results with the Chlamydiazyme assay. Because it is more difficult to collect endocervical specimens free of contaminating vaginal secretions than it is to collect urethral specimens, this may result in the increased percentage of false-positive results with endocervical compared with urethral specimens.

On the basis of our results, we recommend repeating the Chlamydiazyme assay for all specimens with absorbance

values between the cutoff and 0.400. We chose an absorbance value of 0.400 as the criterion for repeating the Chlamydiazyme assay because the number of nonrepeatedly positive results was significantly increased in specimens with these low initial absorbance values. With this simple protocol, approximately 25% of the specimens in our setting with initially positive results (122 of 457) would be retested (3% of total specimens), and the positive predictive value of Chlamydiazyme in our population would have increased from 86.7% to 94.3%. The blocking antibody assay can then be performed, if desired, only on those specimens which are repeatedly positive. Our results showed that the initial absorbance values of false-positive specimens defined by the blocking antibody test tended to cluster in the low ranges, but the trend was not statistically significant. Thus, it appears that the blocking antibody assay should be used for all repeatedly positive specimens and not only those with low absorbance values. This result is in contrast to that of Van Dyck et al. (17), who compared Chlamydiazyme and the blocking antibody assay with cell culture and found that the initial absorbance values of true-positive specimens were significantly higher than the initial absorbance values of specimens which were not confirmed as positive. Possibly the difference in results is due to the breakdown of our results according to repeatability and negative blocking antibody tests, whereas in the study of Van Dyck et al., all false-positive Chlamydiazyme results were considered together. Our results are supported by those of Hallander et al. (3), who found that the predictive value of a positive result with Chlamydiazyme increased with increasing absorbance values, but false-positive results (defined by negative blocking antibody assays) still occurred in 10% of the specimens with absorbance values greater than 0.500. We obtained an identical result with our population, in which 10.2% (15 of 157) of specimens which had initial absorbance values of >0.400 were falsely positive.

The decision to confirm all repeatedly positive specimens by the blocking antibody assay may depend on the prevalence of chlamydial infection in the patient population tested. A laboratory testing specimens from a population with a very high prevalence of infection (sexually transmitted disease clinic) may not choose to confirm positive results, because the positive predictive value of a positive result in a high-prevalence population would be quite high. When testing specimens from a low-prevalence population, the blocking antibody assay is very useful for discriminating false-positive from true-positive results. In this setting, the blocking antibody test is a useful adjunct to the Chlamydiazyme assay and will ensure that accurate results are reported to the physician and, ultimately, to the patient. The blocking antibody assay is a simple variation of the Chlamydiazyme test, and the protocol of confirming all repeatedly positive specimens can easily be incorporated into routine laboratory practice.

ACKNOWLEDGMENT

We thank Abbott Laboratories for their generous gift of the blocking antibody reagents.

REFERENCES

1. **Baselski, V. S., G. McNeeley, G. Ryan, and M. Robison.** 1987. A comparison of non-culture methods for detection of *C. trachomatis* infections in pregnant women. Obstet. Gynecol. **70:**47–52.
2. **Centers for Disease Control.** 1985. *Chlamydia trachomatis* infections. Policy guidelines for prevention and control. Morbid. Mortal. Weekly Rep. **34:**53S–74S.
3. **Hallander, H., P. Jonsson, and B. Gastrin.** 1992. Value of confirmation of Chlamydiazyme enzyme immunoassay results in the detection of *Chlamydia trachomatis*. Eur. J. Clin. Microbiol. Infect. Dis. **11:**550–552.
4. **Hipp, S. S., Y. Han, and D. Murray.** 1987. Assessment of enzyme immunoassay and immunofluorescence tests for detection of *Chlamydia trachomatis*. J. Clin. Microbiol. **25:**1938–1943.
5. **Howard, L. V., P. F. Coleman, B. J. England, and J. E. Herrmann.** 1986. Evaluation of Chlamydiazyme for the detection of genital infections caused by *Chlamydia trachomatis*. J. Clin. Microbiol. **23:**329–332.
6. **Iwen, P. C., M. S. Tina, M. H. Blair, and G. L. Woods.** 1990. Comparison of the Gen-Probe PACE 2 system, direct fluorescent-antibody, and cell culture for detecting *Chlamydia trachomatis* in cervical specimens. Clin. Microbiol. Clin. Chem. **95:**578–582.
7. **Kellogg, J. A.** 1989. Clinical and laboratory considerations of culture vs. antigen assays for detection of *Chlamydia trachomatis* from genital specimens. Arch. Pathol. Lab. Med. **113:**453–460.
8. **Kellogg, J. A., J. W. Seiple, and M. E. Hick.** 1992. Cross-reaction of clinical isolates of bacteria and yeasts with the Chlamydiazyme test for chlamydial antigen, before and after use of a blocking reagent. Am. J. Clin. Pathol. **97:**309–312.
9. **Kellogg, J. A., J. W. Seiple, and J. S. Levisky.** 1989. Efficacy of duplicate genital specimens and repeated testing for confirming positive results for Chlamydiazyme detection of *Chlamydia trachomatis* antigen. J. Clin. Microbiol. **27:**1218–1221.
10. **Kellogg, J. A., J. W. Seiple, C. L. Murray, and J. S. Levisky.** 1990. Effect of endocervical specimen quality on detection of *Chlamydia trachomatis* and on the incidence of false-positive results with the Chlamydiazyme method. J. Clin. Microbiol. **28:**1108–1113.
11. **Lefebvre, J., H. Laperriere, H. Rousseau, and R. Masse.** 1988. Comparison of three techniques for detection of *Chlamydia trachomatis* in endocervical specimens from asymptomatic women. J. Clin. Microbiol. **26:**726–731.
12. **Mardh, P.-A., A. N. Elbagir, and K. Stenberg.** 1990. Use of blocking reagent to confirm enzyme immunoassay results in chlamydial conjunctivitis. Eur. J. Clin. Microbiol. Infect. Dis. **9:**292–293.
13. **Moncada, J., J. Schachter, G. Bolan, J. Engelman, L. Howard, I. Mushahwar, G. Ridgway, G. Mumtaz, W. Stamm, and A. Clark.** 1990. Confirmatory assay increases specificity of the Chlamydiazyme test for *Chlamydia trachomatis* infection of the cervix. J. Clin. Microbiol. **28:**1770–1773.
14. **Ryan, R. W., I. Kwasnik, O. Steingrimsson, J. Gudmundssen, H. Thorarinssen, and R. C. Tilton.** 1986. Rapid detection of *Chlamydia trachomatis* by an enzyme immunoassay method. Diagn. Microbiol. Infect. Dis. **5:**225–234.
15. **Schwebke, J. R., W. E. Stamm, and H. H. Handsfield.** 1990. Use of sequential enzyme immunoassay and direct fluorescent antibody tests for detection of *Chlamydia trachomatis* infections in women. J. Clin. Microbiol. **28:**2473–2476.
16. **Smith, J. W., R. E. Rogers, B. P. Klatz, J. F. Brickler, P. L. Lineback, B. Van Der Pol, and R. B. Jones.** 1987. Diagnosis of chlamydial infection in women attending antenatal and gynecologic clinics. J. Clin. Microbiol. **25:**868–872.
17. **Van Dyck, E., N. Samb, A. Dieng Sarr, L. Van de Velden, J. Moran, S. Mboup, I. Ndoye, J. L. Lamboray, A. Meheus, and P. Piot.** 1992. Accuracy of two enzyme immunoassays and cell culture in the detection of *Chlamydia trachomatis* in low and high risk populations in Senegal. Eur. J. Clin. Microbiol. Infect. Dis. **11:**527–534.