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Blocking Antibody Assay for Confirmation of Urogenital Chlamydia Infection

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We evaluated the Syva MicroTrak Chlamydia Blocking Antibody Assay as a confirmatory assay for the detection of *Chlamydia trachomatis* in urogenital specimens. During a 5-month period, 109 positives were obtained (4.9%) by the MicroTrak II Enzyme Immunoassay as implemented with the Syva XL automated enzyme immunoassay instrument. Of 98 evaluable samples, 92 (93.9%) were confirmed as positive by the blocking assay, and 3 blocking-negative samples had organisms detected by direct fluorescent-antibody analysis (blocking sensitivity, 96.8%). We found that direct fluorescent-antibody analysis of samples with a specimen-to-cutoff absorbance ratio of ≤2.0 was a reasonable confirmation alternative and was more cost-effective than the blocking assay.

A number of enzyme immunoassays (EIA) are commercially available for the detection of *Chlamydia trachomatis* in urogenital specimens. The sensitivities, specificities, and predictive values of these tests vary and are highly dependent on the type of population being tested, i.e., asymptomatic versus symptomatic and low prevalence versus high prevalence, and on the quality of the specimens submitted for examination (3). The Centers for Disease Control and Prevention recently published guidelines on screening tests for chlamydial infection and recommended that positive EIA tests be confirmed by a supplemental test if a false-positive test is likely to have some adverse outcome (1). Supplemental tests include culture, direct immunofluorescence or a second nonculture test, and blocking antibody assays. The purpose of this study was to assess the performance of the Syva MicroTrak Blocking Antibody Assay when used in conjunction with the Syva MicroTrak II EIA and the Syva XL automated instrument.

Urogenital samples were obtained from adult patients seen at the Hospital of the University of Pennsylvania. Samples included 27.6% from the Emergency Department/Walk-In Clinic, 42.6% from Obstetrics and Gynecology (including labor and delivery), 16.7% from other outpatient areas (private physician offices), and 13.0% from inpatient services. Samples were collected in MicroTrak II EIA collection devices and processed according to the manufacturer's procedures. Samples were held at 4°C until testing.

Detection of chlamydial antigen in urogenital specimens was performed by the MicroTrak II *Chlamydia* EIA (Syva Company, San Jose, Calif.), in which specimens are processed and analyzed with the Syva XL automated enzyme immunoassay instrument according to the manufacturer's instructions. Briefly, antigen is detected with a polyclonal rabbit antibody directed against chlamydial lipopolysaccharide (LPS). Any sample that gave a specimen-to-cutoff absorbance ratio of >1.0 was considered positive, as outlined in the manufacturer's instructions. Confirmation of positive EIA results was performed by the Syva MicroTrak II *Chlamydia* EIA Blocking Assay by using the Syva XL instrument according to the manufacturer's instructions. The blocking reagent contains a monoclonal mouse anti-chlamydial LPS. The mouse anti-LPS antibody competes with the detection antibody reagent for chlamydial LPS binding and, in the presence of antigen, reduces the absorbance value. Each sample is run in the presence and absence of blocking reagent. A sample that exhibits ≥50% blocking, compared with that of the simultaneous control well, in the presence of blocking reagent is considered a confirmed positive. Direct fluorescent-antibody (DFA) staining was performed by the Syva MicroTrak *Chlamydia trachomatis* Direct Specimen Test according to the manufacturer's instructions.

The testing algorithm for the samples was as follows and generally followed the manufacturer's recommendations (Fig. 1). Clinical samples were initially screened by the Syva EIA assay. If positive, the sample was retested by the blocking assay. If negative, the sample was diluted 1:10 in sample buffer and retested. This dilution was performed to determine whether the initial test was negative because of an excess of antigen and is recommended by the manufacturer. Samples that exhibited negative results in the blocking assay were analyzed by DFA staining of the original samples for the presence of organisms.

Statistical analysis was performed with InStat v2.01 (GraphPad, San Diego, Calif.).

From November 1993 through March 1994, 2,203 patient samples were analyzed for the presence of chlamydial antigen. Four samples were urethral samples obtained from males, and 2,199 (99.8%) were cervical samples. A total of 109 (4.9%) samples were positive in the initial screening procedure, and all were from cervical samples (Fig. 1).

Of 109 initially positive samples, 98 samples were positive by repeat EIA testing. Ninety-two samples (93.9%) were confirmed as positive by the blocking antibody assay. Of the six unblocked samples, three were found to contain elementary bodies by direct immunofluorescence staining. Thus, the sensitivity of the blocking antibody assay was 96.8% (92 of 95).

Eleven samples (10.1%) were negative upon repeat EIA testing. These samples were diluted 1:10 and retested by EIA. One sample was positive, and this was confirmed by the blocking assay. DFA analysis of the other 10 specimens showed that 4 were positive. For the 98 samples that were positive by the repeat EIA, the false-positive rate (i.e., positive EIA and neg-

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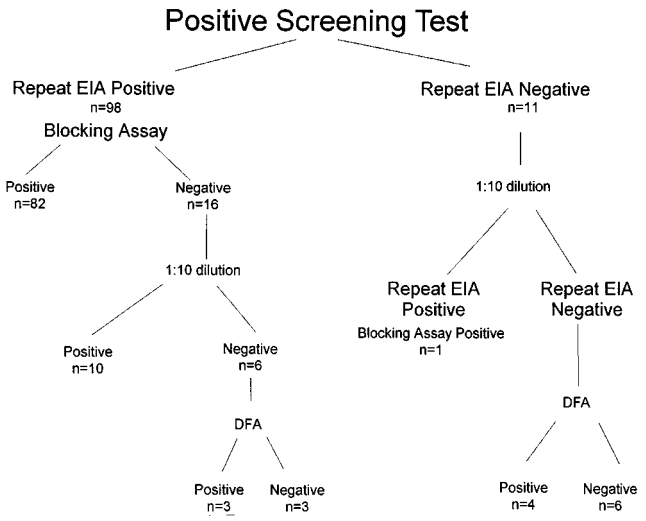

FIG. 1. Testing algorithm and summary of results for confirmation of chlamydial infection by the blocking antibody assay.

ative DFA) was 3.1% (3 of 98); however, 6 of 11 (54.5%) repeat EIA samples that were negative were considered falsely positive. If all the samples are considered, the false-positive rate for the screening procedure in our population was 8.3% (9 of 109). Thus, some type of confirmation strategy is needed for initially positive samples.

We analyzed the distribution of optical density (OD) and specimen-to-cutoff ratio (S/CO) values for both the first and second analyses of each sample. The high number of negative repeat EIA samples (10.1%) suggested that there was some variability in the EIA. Because of variations in EIA testing, the absolute OD values from one run to another might differ; however, the S/CO ratio should not be significantly different. Both the absolute OD value (0.919 versus 0.801) and the S/CO ratio (3.92 versus 2.80) were significantly lower in the second run compared with those from the initial testing ($P < 0.0001$ by the paired t test). The mean S/CO ratio for the negative repeat EIA samples was significantly lower than the S/CO ratio for the positive repeat EIA samples that were unblocked (1.52 versus 3.54) ($P < 0.0175$ by the unpaired t test).

We analyzed the number of days from initial testing to confirmatory testing to determine whether the time to confirmatory testing affected test performance. Confirmatory testing was performed on the same day or after 1 day on approximately two-thirds of the positive samples (67.9%). The percentages of samples tested after the first day were as follows: day 2, 0.9%; day 3, 5.5%; day 4, 20.2%; day 5, 2.8%; day 6, 0.9%; and day 7, 1.8%. For samples from the days on which there were enough samples to analyze (days 0, 1, and 4), there were significant decreases in S/CO ratios for specimens tested the same day and on day 1 after initial testing. However, specimens tested on day 4 did not show a significant decrease in the S/CO ratio. We further analyzed the negative cutoff values and S/CO ratios for the positive controls assayed during the course of the study. The mean negative cutoff value was 0.2782 (range, 0.2453 to 0.3445), with a coefficient of variation of 10.9%. The mean S/CO ratio for the positive assay controls was 4.81 (range, 3.4 to 6.6), with a coefficient of variation of 19.2%. The apparent decreases in OD and S/CO values upon repeat testing of samples on certain days suggest significant run-to-run variation. This phenomenon could account for the number of samples that were negative by the EIA assay upon repeat testing, especially those samples that were near the cutoff.

As an alternative to using the blocking assay to confirm all positive samples, we found that using DFA analysis to confirm initial specimens with S/CO ratios of ≤ 2.0 was a reasonable, cost-effective alternative. A total of 9 of 11 (81.8%) negative repeat EIA samples had S/CO ratios of ≤ 2.0, whereas 14 of 98 (14.3%) positive blocked samples had S/CO ratios of ≤ 2.0. This suggests that confirming samples with S/CO ratios of ≤ 2.0 would maximize the detection of chlamydiae in negative repeat EIA samples without having to confirm all of the other positive samples. In our study, 2 of the 109 samples tested (1.8%) would be falsely classified as positive.

The results of this study show that the blocking assay for the confirmation of chlamydial antigen in urogenital specimens performed reasonably well. The sensitivity of the blocking assay was good; however, a high proportion of samples could not be confirmed as positive upon repeat testing. There appeared to be significant run-to-run variation that resulted in lower OD and S/CO values upon confirmatory testing. The reasons for this are unclear, however, and deserve further investigation. Our results further support the recommendation made by the Centers for Disease Control that initially positive samples should be confirmed by a second test.

Using the blocking reagent to confirm all initial positives was found to add significant reagent expense to our assay. The blocking assay to confirm all initially positive samples had a reagent cost of $780 for the 109 samples. If DFA is used to confirm positives with S/CO ratios of ≤ 2.0, then the reagent cost is only $86, since only 21% of the initially positive samples would be confirmed by the DFA test. In addition, the DFA analysis of the sample can be performed in less time than the EIA takes, and thus the time to a reporting of the confirmed results is improved. Some of the disadvantages of DFA analysis, however, are that it requires relatively expensive equipment and expertise in interpreting the results. Other investigators have examined the benefits of selective confirmation of positive results (2, 4). Their conclusions are consistent with those of a recent report by Chan et al. (2) on the cost-effectiveness of DFA staining as a confirmatory assay for *C. trachomatis* infection in genital tract specimens.

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