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A 1-Year Evaluation of Syva MicroTrak *Chlamydia* Enzyme Immunoassay with Selective Confirmation by Direct Fluorescent-Antibody Assay in a High-Volume Laboratory

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The Syva MicroTrak *Chlamydia* enzyme immunoassay (EIA; Syva Company, San Jose, Calif.) with cytospin and direct fluorescent-antibody assay (DFA) confirmation was evaluated on 43,630 urogenital specimens over a 1-year period in the Provincial Laboratory in Regina, Saskatchewan, Canada. This was a two-phase study intended to define a testing algorithm for *Chlamydia trachomatis* that would be both highly accurate and cost-effective in our high-volume (>3,000 tests per month) laboratory. The prevalence of *C. trachomatis* infection in our population is moderate (8 to 9%). In phase 1, we tested 6,022 male and female urogenital specimens by EIA. All specimens with optical densities above the cutoff value and those within 30% below the cutoff value were retested by DFA. This was 648 specimens (10.8% of the total). A total of 100% (211 of 211) of the specimens with optical densities equal to or greater than 1.00 absorbance unit (AU) above the cutoff value, 98.2% (175 of 178) of the specimens with optical densities of between 0.500 and 0.999 AU above the cutoff value, and 83% (167 of 201) of the specimens with optical densities within 0.499 AU above the cutoff value were confirmed to be positive. A total of 12% (7 of 58) of the specimens with optical densities within 30% below the cutoff value were positive by DFA. In phase 2, we tested 37,608 specimens (32,495 from females; 5,113 from males) by EIA. Only those specimens with optical densities of between 0.499 AU above and 30% below the cutoff value required confirmation on the basis of data from phase 1 of the study. This was 4.5% of all specimens tested. This decrease in the proportion of specimens requiring confirmation provides a significant cost savings to the laboratory. The testing algorithm gives us a 1-day turnaround time to the final confirmed test results. The MicroTrak EIA performed very well in both phases of the study, with a sensitivity, specificity, positive predictive value, and negative predictive value of 96.1, 99.1, 90.3, and 99.7%, respectively, in phase 2. We suggest that for laboratories that use EIA for *Chlamydia* testing, a study such as this one will identify an appropriate optical density range for confirmatory testing for samples from that particular population.

Chlamydia trachomatis infections are recognized as the most prevalent sexually transmitted disease, with an incidence of about 4×10^6 infections per year in the United States (5). Similar incidence rates have been reported for Canada and many other countries in the developed world.

Undetected infection puts the infected person at risk for serious long-term sequelae and sustains transmission within a community (1). Because symptoms are mild or absent in a majority of infected and infectious women and many men, diagnostic testing is the only way to detect infection. For this reason, screening of populations at risk for infection must be a part of *Chlamydia* control and prevention strategies.

The ideal testing method identifies the greatest number of true infections while keeping the false-positive rate very low, all at the least cost. In the case of *C. trachomatis*, these requirements point to nonculture methods. Culture, although very specific, lacks adequate sensitivity in clinical practice. It is also labor-intensive and is therefore impractical for high-volume screening.

Enzyme immunoassay (EIA) procedures provide labor savings that make high-volume screening practical and can give results in 3 to 4 h. Methods for confirming positive results, such as the direct fluorescent-antibody assay (DFA) or a blocking assay, can be used to increase specificity. The sensitivity of the testing algorithm can be enhanced by applying confirmatory procedures to specimens that lie within a negative gray zone (2).

The aim of our study was to define a testing algorithm that would provide the highest accuracy at the lowest cost in our high-volume (>3,000 tests per month) setting. We chose the Syva MicroTrak *Chlamydia* EIA (Syva Company, San Jose, Calif.) as our screening test because it showed excellent sensitivity and specificity in comparison with those for cell culture (3). Our confirmatory test was the Syva MicroTrak *Chlamydia trachomatis* Direct Specimen Test (DFA), used with the unused portion of the EIA specimen.

Each confirmatory test adds to the total cost of screening, so the most cost-effective testing algorithm consistent with acceptably high accuracy must identify those specimens whose EIA results have the highest probability of being false, and confirmatory testing must be confined to those specimens. In phase 1, we sought to define the optical density (OD) range within which confirmatory testing was required to produce accurate final results. Phase 2 was a 1-year-long trial of the performance and practicability of the resulting testing algorithm.

MATERIALS AND METHODS

Specimens were obtained by family physicians throughout Saskatchewan and at three sexually transmitted disease clinics. Endocervical swab specimens were collected from the females and urethral swab specimens were collected from the males by

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TABLE 1. Number of EBs per slide for specimens in each OD range (phase 1)

OD range[a]	No. of specimens	No. (%) of specimens with the following no. of EBs per specimen:					
		0	1	2	3	4	≥5[b]
Negative gray zone	58	28 (48)	7 (12)	2 (3)	5 (9)	9 (15)	7 (12)
0–0.499	201	8 (4)	4 (2)	5 (2.5)	10 (5)	7 (3.5)	167 (83)
0.500–0.999	178	0	1 (0.6)	0	1 (0.6)	1 (0.6)	175 (98)
1.000–1.999	168	0	0	0	0	0	168 (100)
≥2.000	43	0	0	0	0	0	43 (100)

[a] For EIA-positive specimens, the OD is expressed as AUs above the cutoff.
[b] Specimens were confirmed to be positive.

using the specimen collection kit provided by the EIA manufacturer. All specimens were transported to the laboratory within three days of collection.

Phase 1. A total of 6,022 male and female urogenital specimens were tested by the Syva MicroTrak *Chlamydia* EIA by following the directions in the package insert. All specimens with ODs above the cutoff value and those in the negative gray zone (within 30% below the cutoff value) were retested by DFA by the following protocol. A cytospin cup with a slide attached to it was inoculated with 200 μl of the unused portion of the EIA specimen and was centrifuged at 1,100 rpm in a Shadon Cytospin centrifuge (Shadon, Pa.). The slides were air dried, fixed with methanol for 5 min, and then stained with the MicroTrak Direct Specimen Reagent by following the manufacturer's instructions. The slides were read by using an Olympus epifluorescence microscope. A slide containing five or more elementary bodies (EBs) was considered positive.

The number of EBs per slide was recorded and was correlated with the OD of the specimen by EIA.

Phase 2. Between 1 January and 31 December 1991, a total of 37,608 specimens (32,495 from females; 5,113 from males) were tested by EIA. On the basis of the data obtained in phase 1, our testing algorithm included confirmation of all specimens with ODs of between 0.499 absorbance unit (AU) above the cutoff value and 30% below the cutoff value.

In June 1991, Syva informed us of a potential nonspecific reaction with the swabs used to collect male specimens and advised us to confirm all positive results on specimens collected with these swabs. In a small study to investigate this nonspecific reaction, we found that it was relatively rare and that, when a nonspecific reaction did occur, the specimen had an OD slightly above the cutoff value. However, we decided to extend the range of specimens on which we did confirmatory testing up to 1.99 AUs above the cutoff value for both male and female specimens in the last 6 months of the study to provide a comparison with the results for specimens tested in the first 6 months of the study.

RESULTS

The prevalence of chlamydial infection in our population was 9.2% in phase 1 and 7.9% in phase 2.

Phase 1. DFA confirmation was done on 648 specimens (10.8% of the total). EIA and DFA results agreed for 93% of the specimens (604 of 648): 553 specimens were positive by both assays and 51 specimens were negative by both assays. Assuming that all specimens with ODs of greater than 30% below the cutoff value were true negatives, the sensitivity and specificity of the EIA were 98.8% (553 of 560 specimens) and 99.3% (5,425 of 5,462 specimens), respectively; the positive and negative predictive values were 93.7 and 99.9%, respectively.

Table 1 shows the number of EBs per slide for specimens in five OD ranges, including the 30% negative gray zone. For EIA-positive specimens, the ODs are expressed as the number of AUs above the cutoff. Figure 1 shows the percentage of confirmed positive specimens in each range.

Table 1 and Fig. 1 show that 100% (211 of 211) of specimens with ODs equal to or greater than 1.00 AU above the cutoff

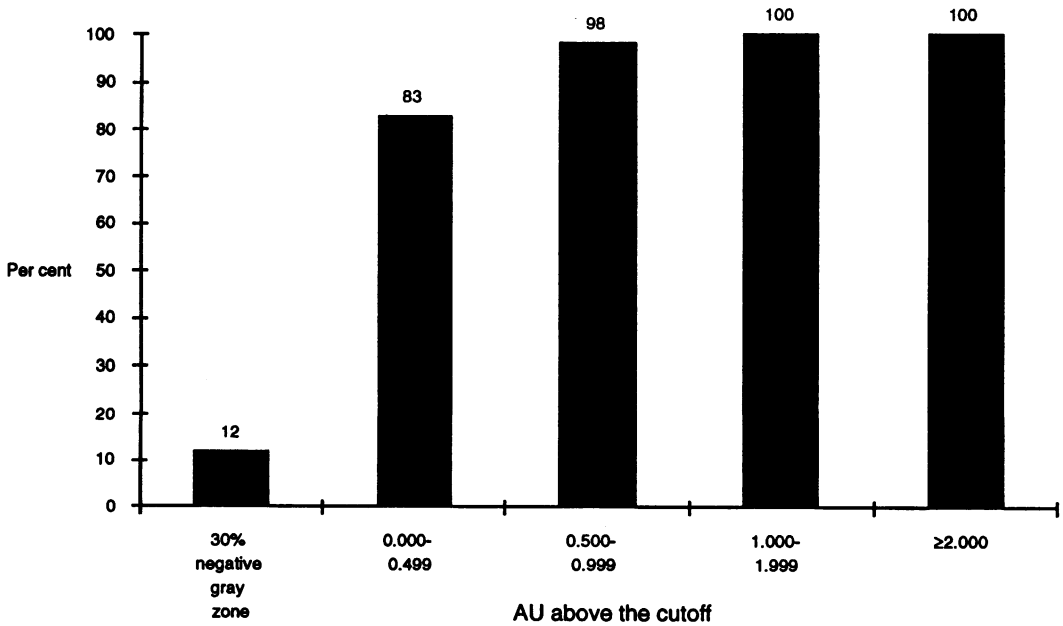

FIG. 1. Percentage of specimens in five OD ranges that were positive by the DFA confirmatory procedure in phase 1 of the study.

TABLE 2. Specimens requiring confirmatory testing in phase 2

Month	No. of specimens tested	No. (%) of specimens requiring confirmatory testing
January	3,092	163 (5.3)
February	3,059	129 (4.2)
March	3,041	138 (4.5)
April	3,308	150 (4.5)
May	3,384	136 (4.0)
June	2,939	119 (4.0)
July	3,230	188[a] (5.8)
August	3,128	217[a] (6.9)
September	3,214	221[a] (6.8)
October	3,371	274[a] (8.1)
November	2,977	291[a] (9.8)
December	2,865	188[a] (6.5)

[a] All specimens with ODs of 0 to 1.999 AUs above the cutoff value underwent confirmatory testing following receipt of the manufacturer's warning about nonspecific results for specimens collected with the swab used to collect specimens from males.

contained 5 or more EBs per slide and were therefore confirmed to be positive.

Of the specimens with ODs of between 0.500 and 0.999 AU above the cutoff value, 98.2% (175 of 178) were confirmed to be positive. The three exceptions had one, three, and four EBs per slide, respectively.

Of the specimens with ODs of between 0 and 0.499 AU above the cutoff value, 83% (167 of 201) contained five or more EBs per slide.

These data were used to set the criteria for confirmatory testing in phase 2.

Phase 2. Table 2 shows the number and percentage of specimens that required DFA confirmation each month. Note that the proportion of specimens requiring confirmatory testing was higher in the last 6 months of the study because the criterion for confirmatory testing changed to include all positive specimens with ODs of up to 1.99 AUs above the cutoff value.

Table 3 shows the performance of the EIA prior to confirmatory testing on male, female, and total specimens during phase 2.

DISCUSSION

We believe that this is the first large, 1-year-long study of an EIA for *C. trachomatis* with DFA confirmation performed on specimens from both males and females. In a recent publication, the Centers for Disease Control and Prevention (1) has recommended that confirmatory testing of specimens with positive results could be selective in populations in which the prevalence of *C. trachomatis* infection is greater than 5%. Since the prevalence in our population is well above 5%, our method meets the criterion of the Centers for Disease Control and Prevention.

Phase 1. The data from phase 1 demonstrated that specimens with ODs equal to or greater than 0.500 AU above the cutoff value can be safely interpreted as positive without confirmatory testing.

Confirmatory testing of specimens in the 30% negative gray zone is justified: 12% (7 of 58) of these specimens had five or more EBs per slide.

Phase 2. Phase 2 demonstrated the practicability of the present testing algorithm in a high-volume setting over a long period. DFA confirmation was required for only 4 to 5% of specimens tested during the first half of the year while we were following the original phase 2 protocol. By contrast, in phase 1 (when all positive specimens were required to undergo confirmatory testing), 10.8% of the specimens required retesting. In the second half of phase 2 (when all positive specimens with ODs of up to 1.999 AU above the cutoff value underwent confirmatory testing), 7.3% of specimens required retesting. The low proportion of confirmatory tests required by the original phase 2 algorithm provides a significant cost savings to the laboratory.

The MicroTrak EIA performed very well in both phases of the study. The sensitivity for both specimen types each month was greater than 90% (with one exception of 89.5%) and was usually much higher. Sensitivity was at or near 100% for male specimens for 6 of the 12 months. Specificity was similarly high; for female specimens, it never dropped below 99% during the entire 12 months.

During the months of September to December there was an increase in the rate of false-positive results for male specimens, consistent with the warning from the manufacturer. This problem was corrected at the beginning of 1992.

We found that we could perform the EIA in the morning and the confirmatory procedure in the afternoon. The cytospin apparatus can concentrate the cells onto one tiny spot on the slide, which dries within about 1 min. The staining and reading of the slide can be performed almost immediately. Scanning of this tiny spot also reduces the time required for the assay. This procedure gives us a 1-day turnaround time to a final confirmed result. We chose five EBs per slide as our criterion for the confirmation of a positive result. Some authors (4) have suggested that only two EBs are required for confirmation.

The objectives of the study were met: we identified a testing algorithm that provides a cost savings without compromising the predictive value of the assay. We suggest that, for laboratories that use EIA for *Chlamydia* testing, a study such as this

TABLE 3. Results of testing by the MicroTrak II *Chlamydia* EIA prior to confirmatory testing in phase 2[a]

Specimen	No. of specimens testing:				Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
	True positive	False positive	False negative	True negative				
Total	2,965	320	101	34,222	96.7	99.1	90.3	99.7
Female	2,211	179	85	30,020	96.3	99.4	92.5	99.7
Male	754	141[b]	16	4,202	97.9	96.8	84.2	99.6

[a] A total of 37,608 specimens were tested.

[b] These false-positive results occurred for 104 (73.8%) of these specimens in the second half of the year, after the manufacturer warned of the possibility of nonspecific results for specimens collected with the swab used to collect specimens from males in certain lots of their specimen collection kit. The problem was corrected in early 1992.

one will identify an appropriate OD range for confirmatory testing for samples from that particular population.

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