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Evaluation of Sanofi Diagnostics Pasteur Chlamydia Microplate EIA Shortened Assay and Comparison with Cell Culture and Syva Chlamydia MicroTrak II EIA in High- and Low-Risk Populations

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Seven hundred thirty-two female urogenital samples were collected for *Chlamydia trachomatis* testing by both the Sanofi Diagnostics Pasteur (Chaska, Minn.) Chlamydia Microplate EIA by the shortened protocol and the Syva (San Jose, Calif.) MicroTrak II EIA, and the results were compared with those obtained by cell culture. For the analysis of samples from female patients, the patients were divided into high- and low-risk categories. An additional 121 male urethral samples were collected and tested by the Sanofi Microplate EIA and cell culture; for the analysis of samples from male patients, the patients were divided into asymptomatic and symptomatic categories. All specimens positive by enzyme immunoassay (EIA) were confirmed by a blocking assay following the respective manufacturer's instructions. Specimens negative by EIA that fell within a gray zone 30% below the cutoff and negative cultures with one or more corresponding positive EIA results were tested further by cytocentrifugation and direct immunofluorescent assay. The overall sensitivity, specificity, positive predictive value, and negative predictive value for Syva versus culture were 94, 98.8, 85.5 and 99.6%, respectively. After resolution, the results were 94.5, 99.6, 94.5, and 99.6%, respectively. The parallel results for the Sanofi Microplate EIA versus culture were 94.0, 98.7, 83.9, and 99.6%, respectively, and after being resolved, the results were 94.9, 100, 100, and 99.6%, respectively. In the small male population tested, the resolved results of the Sanofi Microplate EIA versus culture demonstrated sensitivity, specificity, positive predictive value, and negative predictive value of 100, 100, 100, and 100%, respectively. The present study demonstrated that the Sanofi Microplate EIA shortened protocol is highly sensitive and specific in comparison with cell culture and the Syva MicroTrak II EIA.

Chlamydia trachomatis has been identified as the most common bacterial sexually transmitted disease in both men and women. *C. trachomatis* causes an estimated 4 million infections annually (15), with most infections being asymptomatic. Untreated chlamydial infections may lead to serious and costly complications such as salpingitis, infertility, and ectopic pregnancy (6). The estimated direct and indirect costs of chlamydial infections exceed $2.4 billion annually in the United States (16). Screening of women at moderate risk for chlamydial infection by direct antigen tests is proving to be beneficial for diagnosis as well as cost-effective (10). Cell culture is the most specific method for the detection of chlamydial infection and has been considered the "gold standard." However, the complex sample preparation and handling requirements and the need for special culture medium and expedient transportation are distinct disadvantages, especially in a laboratory handling large volumes of samples. With advances in technology such as PCR and immunological methods, cell culture is no longer regarded the true gold standard. Many recent studies have used a resolved standard or a blocking confirmatory test to improve the accuracy of the new assays (2, 14). In addition to cost and performance issues, laboratories handling large volumes of samples also require the capability to process large volumes of specimens in a shorter time. The study described here was undertaken to assess the new Sanofi Diagnostics Pasteur Chlamydia Microplate EIA by the shortened protocol with samples from patients in different risk categories and to compare the results with those obtained by cell culture and the established Syva MicroTrak II EIA.

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MATERIALS AND METHODS

Patient samples. Triplicate endocervical swab specimens were collected from female patients by family physicians and at the sexually transmitted disease clinic in Regina, Saskatchewan, Canada. Specimen collection was randomized for the Sanofi and Syva swabs after the culture swab was collected first. Male urethral specimens were collected and were used in a comparison between Sanofi EIA and cell culture only. Most of the male patients were from the Regina sexually transmitted disease clinic. For the male patients, the culture swab was collected first, and then the Sanofi swab was collected. All patient specimens were transported to the laboratory within 24 h of collection. Cell culture specimens were transported in 2-SP medium with an ice pack. Patient requisitions were checked for risk factors and symptoms for data analysis. Patients with any of the following risk factors were considered high risk: less than 25 years of age, more than two sexual partners in the last 6 months, change in sexual partners, mucopurulent cervicitis, or use of a nonbarrier form of contraception.

Culture. Specimens were shaken with glass beads before they were inoculated onto a confluent monolayer of McCoy cells previously grown for 24 h on coverslips in shell vials. After centrifugation at 2,500 × *g* at 35°C for 1 h, the supernatant was replaced with maintenance medium containing cycloheximide (1 μg/ml). After 72 h of incubation at 37°C, the medium was aspirated and the cells were fixed with methanol for 10 min prior to staining with a fluorescein isothiocyanate conjugate (Syva, San Jose, Calif.) comprising monoclonal antibodies to chlamydial reticulate and elementary bodies. The coverslip was mounted and examined for the presence of inclusion bodies in the cells by using a UV epifluorescence microscope (Olympus, Markham, Ontario, Canada). Positive and negative controls were included with each run.

Enzyme immunoassays (EIAs). Both the Syva MicroTrak II Chlamydia EIA and the shortened protocol of the Sanofi (Chaska, Minn.) Diagnostic Pasteur

Chlamydia Microplate EIA were performed according to the manufacturer's instructions.

Cytocentrifugation and direct fluorescent-antibody assay (DFA). All EIA-positive specimens and specimens that fell in the range of 30% below the cutoff, which were considered to be in the negative gray zone, were processed by the following procedures to confirm the result reported for the specimen.

(i) Testing of specimens by Syva EIA. Slides were prepared for DFA by centrifuging 200 μl of the sample at 1,500 rpm for 5 min in an Cytospin II centrifuge (Shandon, Pittsburgh, Pa.); this was followed by air drying and fixation of the sample in methanol. The slides were then stained with the Syva DFA stain according to the manufacturer's instructions.

(ii) Testing of specimens by Sanofi EIA and testing of culture retentate. Slides were prepared for DFA by centrifuging 400 μl of the sample at 10,000 × *g*. The pellets were resuspended in 20 μl of double-distilled water. Five microliters of the suspension was spotted onto a slide. After fixation with methanol, the slides were stained with a direct antigen stain, Sanofi Diagnostic Pasteur's Kallestad Direct Antigen, which stains for anti-major outer membrane protein and antilipopolysaccharide in the culture retentate.

All slides were examined with a UV epifluorescence microscope. A positive DFA test result was defined as five or more elementary bodies.

Blocking assays. In both the Syva and Sanofi blocking assays, specimens positive by the original Chlamydia EIA were retested in duplicate. One well received the standard assay reagents as usual, and the other well received the blocking reagent as well as the assay reagents. The absorbances of the two wells were used to calculate the percentage of blocking by the blocking reagent. If the blocking well had a 50% or greater reduction in absorbancy in comparison with that of the corresponding well without blocking reagent, the presence of *Chlamydia* antigen in the sample was considered confirmed.

Criteria for resolving discrepant results. If the EIA result did not match the culture result, the discrepancy was resolved as follows. If the culture was positive, the sample was considered positive for chlamydial infection, regardless of the EIA result. EIA samples whose results were in the negative gray zone (30% below the cutoff) were further tested by DFA and blocking assays to determine the presence of chlamydiae. If the culture result was negative and the EIA result was confirmed to be positive by the blocking assay, DFA testing of the sample used in the EIA or the culture retentate was done to further evaluate the sample. If any sample tested by DFA was positive with five or more elementary bodies, then the result for the sample was considered to be a confirmed positive result.

Statistical analysis. Female patients were divided into high- and low-risk populations according to the risk factors mentioned in the patient sample section. Male patients were divided into symptomatic and asymptomatic groups according to the information provided by the patients. Sensitivity, specificity, and positive and negative predictive values were calculated for direct comparison with those for culture and after resolution by DFA.

RESULTS

Seven hundred thirty-two triplicate specimens from female patients were obtained for the study. Four hundred ninety of these patients were considered low risk according to the information provided on the test requisitions, while 242 were categorized as being at high risk. Ninety-six paired specimens from asymptomatic men and 25 paired specimens from symptomatic men were obtained for a comparison between the Sanofi EIA and culture.

Table 1 provides the data for each patient category and the statistical analysis of each EIA in comparison with the resolved standard according to the criteria set forth above.

The shortened Sanofic EIA protocol was found to be highly sensitive and specific in comparison with cell culture and the Syva EIA. The shortened Sanofi EIA protocol gave good sensitivity after resolution for samples from the low-risk female population, in which the prevalence of *C. trachomatis* infection was 7%. The sensitivity increased as the prevalence rate went up, as noted in the high-risk population, which had an 11% prevalence rate. Resolution of discrepant specimens by DFA was completed on 8 culture-negative, Syva EIA-positive specimens and 11 culture-negative, Sanofi EIA-positive specimens. After resolution, 5 of 8 of the Syva EIA-positive specimens and all 11 of the Sanofi EIA-positive specimens were confirmed to be positive. This increased the positive predictive value for the Sanofi EIA from 82.8 to 100%.

TABLE 1. Sensitivity, specificity, and predicative values of each EIA in comparison with the resolved standard[a]

Sex and test	Group	No. of specimens	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Female						
Syva EIA	Overall	732	94.5	99.6	94.5	99.6
	High risk	242	100	99.1	92.6	100
	Low risk	490	90.0	99.8	96.4	99.4
Sanofi EIA	Overall	732	94.9	100	100	99.6
	High risk	242	96.2	100	100	99.5
	Low risk	490	93.9	100	100	99.6
Male, Sanofi EIA	Overall	121	100	100	100	100
	Asymptomatic	96	100	100	100	100
	Symptomatic	25	100	100	100	100

[a] PPV, positive predictive value; NPV, negative predictive value.

DISCUSSION

The recognition of *C. trachomatis* infection in women seeking routine gynecological care is problematic because the majority of infected women do not have symptoms or abnormal physical findings. This problem could also be applied to men; one study has shown that in a population with a prevalence of *C. trachomatis* infection of 6.5%, 16.6% of the infected males were asymptomatic (11).

For many years the cell culture technique has been regarded as the gold standard for screening for chlamydial infection. A major challenge with a comparison with culture is the relative low level of sensitivity of the cell culture technique (1, 4, 8, 9, 12, 14). Recent studies with other amplification techniques or studies that used multiple immunoassays suggested that the culture gold standard may have a sensitivity of only 55 to 65% (1, 4, 8, 9, 12). This is especially true for many clinical laboratories, in which transportation of the specimens to be processed may take more than 24 h, a factor that is known to reduce the sensitivity of the cell culture assay. An additional disadvantage of cell culture is that it is labor intensive, especially in the case of a laboratory that must handle a large volume of material.

Most of the commercial EIAs now provide a confirmation assay, such as a blocking reagent or a supplemental DFA stain, to increase the specificity of the assay. The sensitivity of the assay can also be increased by confirming the results for specimens whose results are in the defined gray zone (3, 7). For a laboratory that handles large volumes of material, the short assay time can increase the throughput of the laboratory and also makes same-day confirmation possible. In the present study we assessed the shortened Sanofi Chlamydia Microplate EIA protocol and compared the results obtained with those obtained by cell culture and the Syva EIA for samples from different patient groups.

One of the interesting findings with Sanofi's new shortened protocol is that the assay has excellent sensitivity and specificity for specimens from males. After resolution, the sensitivity and specificity for both symptomatic and asymptomatic males were 100%. These results are slightly better than those for specimens from both high- and low-risk female populations, which were similar to those of a study on the Syva EIA conducted by Moncada et al. (9). However, we did not test any specimens from males by the Syva assay in the present study; therefore, we cannot make the same comparison. On the basis of the present limited study, we found that 5.2% (5 of 96) of the

asymptomatic male patients attending the sexually transmitted disease clinic were infected with *C. trachomatis*. This is in agreement with other studies that have reported asymptomatic carriage rates ranging between 6 and 15% in sexually active adolescents (5, 13, 17). Because only a small male population was tested in the present study ($n = 121$), it will be necessary to have more samples from males to validate these results and to provide an accurate carriage rate for male patients attending the sexually transmitted disease clinic in Regina.

In conclusion, we have found that the new shortened Sanofi Chlamydia Microplate EIA protocol gives excellent sensitivity and specificity for samples from both male and female patients. The results are comparable to those of the Syva EIA and cell culture. The turnaround time for results by the new Sanofi EIA format is also shorter, enabling the laboratory to generate both initial and confirmed results within the same day. The new protocol takes approximately 2.5 h, which is 1.5 h less than the original assay. This shortened protocol allows the laboratory to do the blocking assay in the afternoon, thus providing a 1-day turnaround time, including the time to final confirmed results.

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