JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1986, p. 329–332
0095-1137/86/020329-04$02.00/0

Vol. 23, No. 2

# Evaluation of Chlamydiazyme for the Detection of Genital Infections Caused by *Chlamydia trachomatis*

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Received 13 September 1985/Accepted 9 November 1985

**Chlamydiazyme is a 4-h enzyme-linked immunoassay that detects an antigen of *Chlamydia trachomatis* directly in clinical specimens. This immunoassay was compared with cell culture for the diagnosis of chlamydial infections of the genital tract. The assay was evaluated at five clinics with a total of 1,277 cervical specimens of which 239 were culture positive. At three of these clinics where urethral samples were taken from males, 99 of 363 samples were culture positive. The sensitivity of the assay averaged 89.5% for detecting cervical infections and 78.8% for detecting male urethral infections. Specificity was 97.0% when samples from either males or females were tested. Some patients who were culture negative were infected with chlamydiae according to both Chlamydiazyme and a monoclonal antibody test that detected a chlamydial antigen distinct from the antigen detected by Chlamydiazyme. If the 15 females and 2 males who were positive by both immunoassays but culture negative were considered positive for chlamydial infection, the specificity of the assay was 98.4% in females and 97.7% in males. Chlamydiazyme is a simple and relatively rapid immunoassay that has sufficient sensitivity and specificity to supplant culture in the detection of genital chlamydial infections.**

The treatment and control of genital chlamydial infections has been hampered by the lack of a diagnostic test that is rapid and suitable for routine use in the clinical microbiology laboratory. The standard method for diagnosis is the propagation of chlamydiae in cultured mammalian cells and the subsequent staining of inclusions. Specificity is near 100% when experienced microscopists examine the cultures. Sensitivity, which is influenced by sampling procedures, cultural conditions, and staining methodology, probably varies between 70 and 90% (9).

Deficiencies in culture include loss of viability due to either toxic swabs (4) or storage of specimens before culture is performed. In addition, identification by culture requires 2 or 4 to 6 days if a second passage is used to increase sensitivity. The expense of culture and the need for experienced personnel further detract from this method as a routine diagnostic procedure.

Commerical assays, which serve as an alternative to culture, include a direct immunofluorescent antibody (IFA) test (13) and enzyme-linked immunoassays (EIAs) (3). In this paper, we describe the performance of Chlamydiazyme, which is a 4-h immunoassay that detects a chlamydial antigen directly in clinical samples. The performance of Chlamydiazyme during early stages of development was previously reported (1, 6). The data reported here were obtained at five clinical locations by using the Chlamydiazyme reagents that are now commerically available from Abbott Laboratories.

## MATERIALS AND METHODS

**Specimens.** Chlamydiazyme was evaluated at five clinical sites with samples from female patients. At three of these clinics, specimens from males were also tested. Written informed consent was obtained from each patient before samples were taken. The population studied and the incidence of *Chlamydia trachomatis* infection for each site are presented in Table 1. Separate swabs were used for the culture of chlamydiae and for antigen detection by immunoassay. The exocervix was cleared of mucus with a cotton swab before samples were taken. Specimens for the culture of chlamydiae from females were taken with a cotton (sites I, II, IV, V) or dacron (site III) swab which was rotated in the cervical os for 15 to 30 s. The male urethral specimen for culture was taken with a cotton swab, except at site III where a dacron swab was used. Swabs for Chlamydiazyme were supplied with the kits and were used to obtain specimens from the same anatomical sites sampled for culture.

**IFA and culture.** At sites III and IV, the Syva MicroTrak direct IFA test (13) was run concurrently with Chlamydiazyme and cell culture. For the direct IFA test, a separate swab was taken to prepare a smear on a glass slide (site IV), or the smear was made from the swab for culture before it was placed in sucrose-phosphate transport medium (2-SP) (4) (site III). Swabs used for culture were placed in 2-SP or Eagle minimum essential medium and tested within 8 h or frozen. No samples stored in minimum essential medium were frozen. One-tenth of the swab eluate was placed on each of duplicate McCoy cell monolayers. McCoy cell cultures for inoculation were grown on cover slips in glass vials in all laboratories, except at site IV where microtiter trays (15) were used. The inoculated cells were centrifuged at 2,000 to 2,500 × *g* at 35°C for 30 min. Cell culture medium was supplemented with cycloheximide. After 48 to 72 h of incubation at 35°C, one of the duplicate monolayers was stained with iodine (passage 1). At site I, an additional passage 1 monolayer was stained with IFA monoclonal antibody (11). At all sites, passage 2 was performed by scraping cells off the duplicate cover slip and inoculating monolayers with this suspension. These cultures were centrifuged, incubated, and stained with iodine as described for passage 1. Culture was considered positive if passage 1 or 2 monolayers contained one or more chlamydial inclusions.

**Chlamydiazyme procedure.** The swab for Chlamydiazyme was placed in a transport tube containing 0.1 ml of storage reagent and was tested within 72 h after sample collection. Specimen dilution buffer (1 ml) was added to elute chlamyd-

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TABLE 1. Population studied and prevalence of chlamydial infection at five sites where Chlamydiazyme was evaluated

| Site | Population studied | Sex of patients | Prevalence[a] of chlamydial infection (%) |
|---|---|---|---|
| I | Community health clinic | Female | 43/167 (25.7) |
| II | STD clinic[b] | Female | 23/92 (25.0) |
| | | Male | 30/134 (22.4) |
| III | STD clinic | Female | 27/160 (16.9) |
| | | Male | 42/171 (24.6) |
| IV | STD clinic | Female | 85/553 (15.4) |
| V | Symptomatic patients at obstetric-gynecology office and STD clinic | Female | 61/305 (20.0) |
| | STD clinic | Male | 27/58 (46.6) |

[a] Number of patients culture positive/number tested.
[b] STD clinic, Clinic for sexually transmitted diseases.

ial antigens. The swab was incubated at room temperature for 10 min and then agitated on a Multi-tube Vortexer (Scientific Manufacturing Industries) at a setting of 4 for three cycles of 15 s each. Finally, the swab was pressed against the side of the tube to remove excess fluid and was discarded. A 0.2-ml sample of the swab eluate and a 25-mm bead, which captured chlamydial antigens, were added to wells of a plastic tray. Each test run included a positive control and three negative controls. After 1 h of incubation at 37°C, the bead was washed four times with water by using a Pentawash (Abbott Laboratories). Rabbit antibody to *C. trachomatis* (200 μl) was added, incubation was continued for 1 h, and the beads were washed. Bound immunoglobulin was detected by the addition of 200 μl of horseradish peroxidase-labeled goat anti-rabbit immunoglobulin G which was incubated for 1 h. The beads were washed and transferred to optically clear tubes, after which 300 μl of a substrate solution containing *o*-phenylenediamine and $H_2O_2$ was added. The tubes were incubated for 30 min at room temperature, and the reaction was stopped with 1 ml of 1 N $H_2SO_4$. The optical density was measured at 492 nm on a spectrophotometer (Quantum; Abbott Laboratories) which had been blanked with a solution of enzyme substrate and $H_2SO_4$. Clinical specimens were considered positive if the optical density was ≥0.1 unit above the average of the three negative controls.

**Resolution of discordant results.** Fourteen 2-SP and 10 specimen dilution buffer samples from patients who were culture negative and Chlamydiazyme positive were available for further study. These samples had been stored at −20°C. After thawing, they were centrifuged at 1,050 × *g* for 30 min, and the supernatant fluid was decanted. The pellet was removed with a sterile cotton swab and smeared on a microscope slide. The slides were examined for chlamydial particles after staining by the Syva MicroTrak direct IFA technique.

## RESULTS

The sensitivity and specificity of Chlamydiazyme were calculated by comparing the EIA result with combined passage 1 and 2 cultures. At the one site where cultures were stained by IFA as well as iodine, three positive cultures were detected only by IFA. These three positive cultures were included with the iodine-positive results.

The comparisons of Chlamydiazyme and culture results obtained at each of the five sites are presented in Table 2. The average sensitivity was 89.5% for females and 78.8% for males. Specificity was 97.0% when samples from either males or females were tested. Based on an overall incidence of 18.7% for females, the predictive values of a positive and negative test were 87.3 and 97.6%, respectively. For the male population, whose incidence was 27.3%, the predictive values of a positive and negative test were 90.7 and 92.4%, respectively.

At two sites, Chlamydiazyme and the Syva direct monoclonal IFA test were evaluated concurrently by using culture as the standard method. The complete results of these studies will be reported by the individual investigators. These studies indicated that some samples from culture-negative patients were positive for chlamydial antigens according to the results of both Chlamydiazyme and the direct IFA test. Because of the agreement of the two antigen detection tests, we assume that culture failed to detect viable chlamydiae in these patients, who were probably infected. At site IV, 8 of 12 Chlamydiazyme-positive, culture-negative females were also positive by direct IFA. One female and one male patient at site III were Chlamydiazyme positive and direct IFA positive but culture negative.

The 2-SP fluid from 14 patients who were Chlamydiazyme positive but culture negative had been stored frozen by investigators and was examined by IFA for chlamydiae. Fluorescent particles consistent with chlamydial morphology were seen in two samples taken from females (one each from sites I and III). In addition, 6 of the 10 available specimen dilution buffer samples which were apparent false-positive specimens contained chlamydiae according to the IFA test. One of the six was a specimen from a patient who was already identified as infected on the basis of the direct IFA test. The five additional patients who were probably infected were four females at site V and one male at site II.

Thus, direct IFA on specimens at two sites and examination of 14 2-SP and 10 specimen dilution buffer samples by

TABLE 2. Sensitivity and specificity of Chlamydiazyme at five clinical laboratories

| Site | Sex | % Sensitivity (no. of samples EIA positive and culture positive/no. of samples culture positive) | % Specificity (no. of samples EIA negative and culture negative/no. of samples culture negative) | % Agreement with culture |
|---|---|---|---|---|
| I | Female | 79.1 (34/43) | 96.8 (120/124) | 92.2 (154/167) |
| II | Female | 73.9 (17/23) | 98.6 (68/69) | 92.4 (85/92) |
| | Male | 73.3 (22/30) | 98.1 (102/104) | 92.5 (124/134) |
| III | Female | 96.3 (26/27) | 95.5 (127/133) | 95.6 (153/160) |
| | Male | 83.3 (35/42) | 95.3 (123/129) | 92.4 (158/171) |
| IV | Female | 92.9 (79/85) | 97.4 (456/468) | 96.7 (535/553) |
| V | Female | 95.1 (58/61) | 96.7 (236/244) | 96.4 (294/305) |
| | Male | 77.8 (21/27) | 100 (31/31) | 89.7 (52/58) |
| All sites | Female | 89.5 (214/239) | 97.0 (1,007/1,038) | 95.6 (1,221/1,277) |
| | Male | 78.8 (78/99) | 97.0 (256/264) | 92.0 (334/363) |

TABLE 3. Sensitivity, specificity, and predictive values of Chlamydiazyme calculated with resolved data

| Sex | % Sensitivity (no. of samples EIA positive and infected[a]/total infected) | % Specificity (no. of samples EIA negative and not infected/total not infected) | Predictive Value (%) of positive test (no. of samples EIA positive and infected/no. of samples EIA positive) | Predictive value (%) of negative test (no. of samples EIA negative and not infected/no. of samples EIA negative) |
|---|---|---|---|---|
| Male | 79.2 (80/101) | 97.7 (256/262) | 93.0 (80/86) | 92.4 (256/277) |
| Female | 90.2 (229/254) | 98.4 (1,007/1,023) | 93.5 (229/245) | 97.6 (1,007/1,032) |

[a] Infected category includes samples from patients who were culture positive. Also included in this category are samples from patients who were infected according to examination by IFA of direct smears, 2-SP fluids, or specimen dilution buffer samples.

IFA indicated that 2 males and 15 females who were culture negative probably were infected with chlamydiae. If these 17 patients were infected, the resolved specificity of Chlamydiazyme was 97.7 and 98.4% for males and females, respectively (Table 3).

## DISCUSSION

The standard method for diagnosis of genital chlamydial infections is isolation with cultured mammalian cells. Several researchers (9, 15) have emphasized the need for a diagnostic test which, unlike culture, can be performed in most microbiology laboratories. Chlamydiazyme is an immunoassay that is easy to perform, and several hundred samples can be assayed in 4 h. The test results are obtained by the quantitative measurement of optical density, thereby eliminating the need for subjective microscopic evaluation of samples.

The sensitivity of the EIA in males ranged from 73.3 to 83.3%, whereas the sensitivity in females was between 73.9 and 96.3%. The variation in sensitivity was probably due in part to site-to-site differences in patient sampling techniques and in the efficacy of cell culture. Specificity was less variable and was consistently above 95% for both males and females. These sensitivity and specificity data were obtained by testing populations with high prevalences of chlamydial infections. The evaluation of Chlamydiazyme in low-risk populations requires further clinical trials.

False-positive results could have been due to the failure of culture to detect all chlamydial infections. Studies in which multiple cervical swabs were taken have shown that culture performed with a single swab does not detect all culture-positive women (7). In addition, a rise in antibody titer or seroconversions in some culture-negative males with nongonococcal urethritis indicated that culture is not 100% sensitive in detecting male urethral infections (5, 14). Schachter (9) estimated that the sensitivity of culture with a single swab is 70 to 80% for cervical infections, whereas the sensitivity for a male urethral culture is approximately 90%. False-positive results could also have been due to the detection of chlamydial antigens in specimens taken from patients harboring no viable elementary bodies. The use of separate swabs for direct antigen detection and culture was a necessity, because solutions designed to preserve viability, such as 2-SP, interfere with the performance of Chlamydiazyme. Variations due to sampling techniques, particularly in patients with low numbers of chlamydiae, could have adversely affected the correlation of EIA with culture.

Resolution of some false-positive results was achieved at two sites where direct examination of specimens by fluorescent monoclonal antibody was performed. These results, in addition to the observation of chlamydiae in 2-SP and specimen dilution buffer samples, increased the specificity from 97.0 to 98.4% in females. Specificity in males increased from 97.0 to 97.7%. The monoclonal antibody used to resolve these discordant results reacts with the major outer membrane protein (12, 13). The antiserum used in Chlamydiazyme has been purified and reacts only with the chlamydial lipopolysaccharide (unpublished data). Reactivity with chlamydial lipopolysaccharide but not the major outer membrane protein was demonstrated by electrophoresis of solubilized chlamydiae and subsequent immunoblotting by a published procedure (2). It is unlikely that antigens cross-reacting with both the antichlamydial major outer membrane protein and lipopolysaccharide antibodies would be present simultaneously in chlamydiae-negative specimens.

Early detection and treatment of chlamydial infections in women, many of whom are asymptomatic, would decrease the incidence of serious complications, which include endometritis, salpingitis, and perihepatitis (10). In addition, the test would be useful in the detection of chlamydial infections in pregnant women whose infants are at high risk for inclusion conjunctivitis and pneumonitis (8). In males, chlamydial urethritis, which is largely indistinguishable from gonococcal urethritis on the basis of symptoms, could be correctly diagnosed. Correct diagnosis would be particularly important in the management of males with concurrent gonococcal and chlamydial infections, of which many would be treated on the basis of a Gram stain with an antibiotic that is appropriate only for gonorrhea.

In summary, Chlamydiazyme fulfills a need for a routine diagnostic test for the identification of patients with genital chlamydial infections. The correct diagnosis and treatment of patients will be an important adjunct to public health programs designed to prevent infections caused by *C. trachomatis*.

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