JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1989, p. 2811–2814
0095-1137/89/122811-04$02.00/0

Vol. 27, No. 12

TestPack Chlamydia, a New Rapid Assay for the Direct Detection of *Chlamydia trachomatis*

P. COLEMAN,* V. VARITEK, I. K. MUSHAHWAR, B. MARCHLEWICZ, J. SAFFORD, J. HANSEN, G. KURPIEWSKI, AND T. GRIER

Infectious Disease and Immunology Research, Abbott Laboratories, Abbott Park, Illinois 60064

Received 2 February 1989/Accepted 15 August 1989

TestPack Chlamydia (Abbott Laboratories) is a rapid enzyme immunoassay for the direct antigen detection of *Chlamydia trachomatis* in endocervical specimens. The assay is self-contained, requires no specialized equipment, and yields results in less than 30 min. The clinical performance of TestPack Chlamydia versus chlamydial cell culture was evaluated with a total of 1,694 paired endocervical specimens. Discordant samples were further investigated by immunofluorescent staining and by Chlamydiazyme immunoassay, with confirmatory procedures. The sensitivity of TestPack Chlamydia with less-than-48-h-old specimens was 76.5%, while culture sensitivity was 86.7%. TestPack Chlamydia specificity was determined to be 99.5%. These results indicate that TestPack Chlamydia is an accurate test for chlamydial infection, with a positive predictive value of 96.2%. This assay is suitable for low-volume chlamydial testing in physician offices, clinics, and smaller laboratories.

Infection with *Chlamydia trachomatis* is the most common sexually transmitted disease (STD) in the United States, with an estimated 3 to 5 million new cases annually (2, 17), and it is a disease which may lead to serious disease sequelae (5, 6, 15). *C. trachomatis* is an obligate intracellular pathogen that historically has been detected by cell culture techniques (14, 19). Chlamydial cell culture is a time-consuming and expensive procedure which requires highly trained personnel and is limited by specimen viability and cytological interpretation. These factors have curtailed the use of chlamydial cell culture as a routine detection method in all but the larger, well-equipped laboratories which analyze a large volume of specimens.

Culture limitations have led to the emergence of several assays which detect chlamydial antigen directly in patient samples and thereby circumvent specimen-viability concerns. The most common direct antigen detection assays are the enzyme immunoassay and the direct immunofluorescent-antibody (DFA) assay (1, 16). The limitation of the enzyme immunoassay is the requirement for a spectrophotometer and an assay time of 1.5 to 5 h, while DFA requires a fluorescence microscope and clinical experience in differentiating chlamydial staining from specimen artifacts. Because of these limitations, both enzyme immunoassay and DFA methods for chlamydial antigen detection have been used primarily by specialized, well-equipped, large-volume testing laboratories.

TestPack Chlamydia (Abbott Laboratories, Abbott Park, Ill.) is a new assay for the direct detection of chlamydial antigen in endocervical specimens. It is a rapid enzyme immunoassay (less than 30 min from sample to result) that is self-contained and does not require either specialized equipment or extensive training. The performance of this assay was evaluated with cultured specimens from five clinical locations. A series of ancillary antigen detection methods were used to resolve discordant samples. The data show that TestPack Chlamydia is a rapid, sensitive, and specific assay for the direct detection of chlamydial antigen from endocervical swab specimens. This assay is well suited for use by small-volume testing laboratories and at the office of the physician, where early diagnosis is most desirable for the immediate initiation of appropriate therapy.

MATERIALS AND METHODS

Specimens. Specimens were obtained from five geographically dispersed sites, and informed consent was obtained from patients prior to sampling. Patients undergoing antibiotic therapy were excluded from this study. The exocervix was cleared of mucus, and the endocervix was then sampled by inserting a swab and rotating for 10 to 30 s; samples were taken in a random order for culture and for immunoassay. The STD-EZE Sample Collection and Transport Kit (Abbott Laboratories) was used to collect and transport TestPack Chlamydia specimens. STD-EZE specimens were held at 4°C until shipment at ambient temperature to Abbott Laboratories.

Culture. Swab specimens to be cultured for chlamydiae were placed in sucrose–phosphate transport medium (2-SP) (3) and were cultured on cover slips as previously described (9). All culture samples were either held at 4°C for not more than 12 h before culture or snap frozen and then cultured. Specimen cultures were stained with antichlamydial fluorescent antibodies (MicroTrak Culture Confirmation kits; Syva Co., Palo Alto, Calif.). Culture was performed at the site, and inclusion-forming units (IFU) per cover slip were recorded.

DFA. DFA assay (MicroTrak Direct Specimen test; Syva Co.) was used to stain 2-SP samples. 2-SP (250 μl) was diluted 1:2 with phosphate-buffered saline (PBS), centrifuged at 10,000 rpm for 30 min. (Microfuge B; Beckman Instruments, Inc., Fullerton, Calif.), and the resulting pellet was suspended in a minimal volume of PBS and stained according to the instructions of the manufacturer.

Chlamydiazyme assay and blocking assay. An additional specimen was collected only at one site (site 5) for assay by Chlamydiazyme (Abbott Laboratories). Swabs extracted for TestPack Chlamydia assay and found to be positive were reextracted and assayed with Chlamydiazyme reagents. Chlamydiazyme results were resolved as specific or false-positive reactions by performing in parallel a confirmatory

* Corresponding author.

TABLE 1. Prevalence of chlamydial-cell-culture-positive specimens by assay validation site

Site	Investigated population	Prevalence [no. positive/total (%)]
1	STD clinic	17/123 (13.8)
2	STD clinic, OB-GYN[a] office	71/731 (9.7)
3	Student health clinic	21/177 (11.9)
4	STD clinic	57/424 (13.4)
5	STD clinic, prenatal clinic, and OB-GYN office	31/239 (13.0)
Total		197/1,694 (11.6)

[a] OB-GYN, Obstetric-gynecology.

blocking assay on the same sample. Briefly, blocking was accomplished by a bovine chlamydial-specific antibody which, when added to the Chlamydiazyme antibody, competes for chlamydial epitopes, while not recognizing cross-reactive epitopes. When Chlamydiazyme and the blocked Chlamydiazyme assay are run on the same swab extract, a 50%-or-greater decrease in absorbance of the blocked assay, compared with the Chlamydiazyme result, indicates the presence of chlamydial antigen. The confirmed presence of chlamydial antigen in patients not treated with antibiotics was taken as evidence of a chlamydial infection.

TestPack Chlamydia assay. A treated solid phase for the capture of specimen antigen is present as a vertical bar on a reaction disk which is perpendicular to a horizontal bar containing *C. trachomatis* antigen. An STD-EZE endocervical swab sample is extracted, vortexed, filtered with a sample clarification device, and then transferred to the reaction disk. The disk is washed and a rabbit anti-chlamydial antibody is added. This is followed 5 min later by a goat anti-rabbit β-galactosidase conjugate. After waiting an additional 5 min and after a second wash, a chromogenic substrate (chlorophenol red-β-D-galactopyranoside) is added to the reaction disk. If the specimen is negative, color develops only on the horizontal bar, producing a negative (−) sign. If the specimen contains *C. trachomatis* lipopolysaccharide antigen, color develops on both the horizontal and the vertical bars, producing a positive (+) sign. Specimens for TestPack Chlamydia assay were transported to Abbott Laboratories and blindly assayed within 24 h of receipt.

Statistical methods. Sensitivity, specificity, and predictive values for a positive and negative test were calculated by standard procedures (7).

RESULTS

A total of 1,750 paired endocervical swabs collected from five validation sites were separately cultured for chlamydiae and were assayed by TestPack Chlamydia. A total of 56 of the 1,750 samples (3.2%) were eliminated from the investigation because of cell culture toxicity. Of the remaining samples, 197 specimens were chlamydial cell culture positive, for an overall prevalence of 11.6% (197 of 1,694). Prevalence of infection for each assay validation site is shown in Table 1. A total of 112 of the chlamydial culture-positive specimens were also evaluated by the Chlamydiazyme confirmatory blocking assay, and 112 specimens (100%) demonstrated the confirmed presence of chlamydial antigen.

Specimens tested in TestPack Chlamydia (a total of 1,694 samples) were divided into two groups: those specimens assayed within 48 h of patient specimen collection (708 samples) and those specimens assayed at greater than 48 h from patient specimen collection (986 samples). The corresponding culture specimens for both sets of samples were independently prepared as described in Materials and Methods. Of the 708 specimens tested within 48 h of sampling, 85 were chlamydial culture positive (12.0%), while 623 specimens were negative (Table 2). TestPack Chlamydia sensitivity versus chlamydial cell culture was 72.9% (62 of 85), while specificity was 97.4% (607 of 623). Cell culture detected 23 specimens (15 with five or fewer IFU per cover slip) that were negative by TestPack Chlamydia. Conversely, 16 samples were positive by TestPack Chlamydia and negative by culture. Of these samples, 8 (61.5%) had both DFA-staining elementary bodies in 2-SP and chlamydial antigen in the paired swab by confirmed Chlamydiazyme results. Five specimens demonstrated chlamydial antigen in the reextracted swab only by confirmed Chlamydiazyme. Both specimens of one pair of swab specimens were inadvertently run in TestPack Chlamydia. The first swab specimen was positive by TestPack Chlamydia, and the reextraction of this swab was chlamydial antigen positive by confirmed Chlamydiazyme results, while the second "duplicate" swab was negative by both tests. A third swab for culture was also negative. This observation sheds some doubt upon the uniformity of replicate swab specimens.

TABLE 2. Sensitivity and specificity comparison of TestPack Chlamydia and chlamydial cell culture at five assay validation sites (TestPack Chlamydia evaluation of <48-h-old specimens)[a]

Site	% Sensitivity TPk (no. TPk positive/no. chlamydial culture positive)	% Specificity TPk (no. TPk negative/no. chlamydial culture negative)
1	71.4 (5/7)	98.3 (59/60)
2	25.0 (1/4)	100.0 (49/49)
3	85.7 (12/14)	96.5 (82/85)
4	80.0 (24/30)	97.9 (231/236)
5	66.7 (20/30)	96.4 (186/193)
Total	72.9 (62/85)	97.4 (607/623)

[a] Predictive value of positive = TestPack Chlamydia (TPk) positive and chlamydial culture positive/TPk positive = 62/78 = 79.5%. Predictive value of negative = TPk negative and chlamydial culture negative/TPk negative = 607/630 = 96.3%.

The resolved assay data versus confirmed chlamydial infection (cell culture positive and/or chlamydial antigen positive by confirmed Chlamydiazyme assay or DFA stain-

TABLE 3. Sensitivity and specificity comparison of TestPack Chlamydia and culture at five assay validation sites (TestPack Chlamydia evaluation of <48-h-old specimens)[a]

Site	% Sensitivity TPk (no. TPk positive/no. confirmed infection positive)	% Sensitivity culture (no. culture positive/no. confirmed infection positive)	% Specificity TPk (no. TPk negative/no. confirmed infection negative)
1	71.4 (5/7)	100.0 (7/7)	98.3 (59/60)
2	25.0 (1/4)	100.0 (4/4)	100.0 (49/49)
3	87.5 (14/16)	87.5 (14/16)	98.8 (82/83)
4	82.4 (28/34)	88.2 (30/34)	99.6 (231/232)
5	73.0 (27/37)	81.1 (30/37)	100.0 (186/186)
Total	76.5 (75/98)	86.7 (85/98)	99.5 (607/610)

[a] Predictive value of positive = TestPack Chlamydia (TPk) positive and confirmed infection positive/TPk positive = 75/78 = 96.2%. Predictive value of negative = TPk negative and confirmed infection negative/TPk negative = 607/629 = 96.5%.

TABLE 4. Sensitivity and specificity comparison of TestPack Chlamydia and chlamydial cell culture at five validation sites (TestPack Chlamydia evaluation of >2-day-old specimens)[a]

Site	% Sensitivity TPk (no. TPk positive/no. chlamydial culture positive)	% Specificity TPk (no. TPk negative/no. chlamydial culture negative)
1	40.0 (4/10)	95.7 (44/46)
2	77.6 (52/67)	98.2 (600/611)
3	57.1 (4/7)	100.0 (71/71)
4	85.2 (23/27)	97.7 (128/131)
5	100.0 (1/1)	100.0 (15/15)
Total	75.0 (84/112)	98.2 (858/874)

[a] Predictive value of positive = TestPack Chlamydia (TPk) positive and chlamydial culture positive/TPk positive = 84/100 = 84.0%. Predictive value of negative = TPk negative and chlamydial culture negative/TPk negative = 858/886 = 96.3%.

TABLE 5. Sensitivity and specificity comparison of TestPack Chlamydia and culture at five assay validation sites (TestPack Chlamydia evaluation of >2-day-old specimens)[a]

Site	% Sensitivity TPk (no. TPk positive/ no. confirmed infection positive)	% Sensitivity culture (no. culture positive/ no. confirmed infection positive)	% Specificity TPk (no. TPk negative/ no. confirmed infection negative)
1	45.5 (5/11)	90.9 (10/11)	97.8 (44/45)
2	80.5 (62/77)	87.0 (67/77)	99.8 (600/601)
3	57.1 (4/7)	100.0 (7/7)	100.0 (71/71)
4	86.2 (25/29)	93.1 (27/29)	99.2 (128/129)
5	100.0 (1/1)	100.0 (1/1)	100.0 (15/15)
Total	77.6 (97/125)	89.6 (112/125)	99.7 (858/861)

[a] Predictive value of positive = TestPack Chlamydia (TPk) positive and confirmed infection positive/TPk positive = 97/100 = 97.0%. Predictive value of negative = TPk negative and confirmed infection negative/TPk negative = 858/885 = 96.9%.

ing) are presented in Table 3. TestPack Chlamydia sensitivity was 76.5% (75 of 98), while cell culture sensitivity was 86.7% (85 of 98). TestPack Chlamydia demonstrated a resolved specificity of 99.5% (607 of 610) with less-than-48-h-old specimens. The predictive value of a positive test result was 96.2% (75 of 78), while the predictive value of a negative test result was 96.5% (607 of 629), indicating highly accurate test results.

TestPack Chlamydia reactivity with less-than-48-h-old male specimens has been limited by lack of samples (sensitivity versus culture = 6/7 = 85%, and specificity = 26/26 = 100%). More samples are required to determine the performance of TestPack Chlamydia with male specimens.

A total of 986 specimens were assayed by TestPack Chlamydia at a mean of 6 days after sample collection (range 3 to 28 days). The comparison of TestPack Chlamydia reactivity versus chlamydial cell culture detection is summarized in Table 4. As shown, 112 samples were chlamydial culture positive (11.3%), while 874 samples were negative. TestPack Chlamydia sensitivity was 75.0% (84 of 112), and specificity was 98.2% (858 of 874). A total of 28 samples were TestPack Chlamydia negative and cell culture positive (10 samples had five or fewer IFU per cover slip). A total of 16 samples were culture negative and TestPack Chlamydia positive, of which 13 specimens were demonstrated to be chlamydial antigen positive by DFA or Chlamydiazyme confirmatory assay. The resolved TestPack Chlamydia data for greater-than-2-day-old specimens are presented in Table 5. TestPack sensitivity versus confirmed infection was 77.6% (97 of 125), while culture sensitivity was 89.6% (112 of 125). Specificity was determined to be 99.7% (858 of 861). The resolved predictive value of a positive test result equaled 97.0% (97 of 100), and the predictive value of a negative test result equaled 96.9% (858 of 885). Although the data in Table 5 compare favorably with the less-than-2-day-old specimen data presented in Table 3, indicating that the chlamydial antigen is relatively stable, we presently recommend that samples for TestPack Chlamydia be assayed within 48 h of collection.

TestPack Chlamydia specimen extraction buffer ([SEB] 1.2 ml of reagent A plus 2 drops of reagent B) was tested for its effect on bacterial viability in the following manner. First, bacterial isolates from cervical swabs were grown at 37°C on BBL chocolate agar II plates (Becton Dickinson and Co., Cockeysville, Md.), harvested, and suspended to approximately 10^8 cells per ml in PBS. Three gram-positive and three gram-negative isolates, identified as two *Corynebacterium* species, *Streptococcus faecium*, *Acinetobacter lwoffii*, *Haemophilus parainfluenza*, and a group II J bacillus, respectively, were tested. Samples of these bacterial suspensions were incubated with PBS or SEB for 10 min and then replated on chocolate agar plates. At the highest inoculum tested, 10^6 cells per plate, the PBS-incubated bacterial controls grew luxuriantly, while the SEB-incubated bacterial samples demonstrated no detectable growth at 48 h. The six bacterial isolates were also negative in the TestPack Chlamydia assay at 10^7 cells per ml. Second, cervical swab specimens were either plated directly on chocolate agar or incubated in SEB for 10 min, vortex extracted, and then plated without filtration. While control swabs grew numerous colony types, of 25 SEB-treated swab specimens, only one specimen demonstrated any growth, with three isolated colonies of a gram-positive cocci that were grouped as *Staphlococcus* species. These data indicate that TestPack Chlamydia SEB is bactericidal for at least some cervical bacteria, thereby reducing the potential bacterial infectivity of TestPack Chlamydia-processed samples.

DISCUSSION

In this paper we describe the performance of TestPack Chlamydia, determined with clinical specimens. The assay was evaluated versus chlamydial cell culture, and most discordant positive specimens were further analyzed by DFA or Chlamydiazyme assay or both, with confirmatory procedures. Certain limitations and advantages of rapid chlamydial testing were discerned in this study. TestPack Chlamydia demonstrated a sensitivity 10 to 13% lower than that of culture when assayed with clinical specimens (see Tables 3 and 5). Of the 51 total TestPack Chlamydia-negative, culture-positive samples, almost half (25 of 51) contained 5 or fewer IFU per entire culture cover slip and these samples could be construed to contain low levels of antigen. Alternately, with so few staining particles on the culture cover slip, aberrant IFU readings could have occurred, since 7 of 15 of the fewer than five IFU culture samples contained no discernable chlamydial staining in 2-SP culture samples when retested. In either case, a standardized culture system with the potential to amplify a single viable organism from patient samples is generally accepted to be more sensitive than antigen detection methods. TestPack Chlamydia is limited, as are other detection methods, in that the quality of sample collection is solely dependent on the examining physician or practitioner. It is imperative that the columnar epithelium, which may contain

intracellular chlamydiae, be sampled and that the specimen be properly processed and vortex extracted. TestPack Chlamydia is recommended only for endocervical specimens at this time, since more data are required to assess assay performance with male specimens. In addition, TestPack Chlamydia was evaluated in a population with a total chlamydial prevalence of 11.6% (see Table 1) and further studies are necessary to determine assay performance in lower-prevalence populations.

The advantages of rapid antigen detection and lower sensitivity must be weighed against the advantages of culture when considering test applications. The advantages of TestPack Chlamydia are generation of results in less than 30 min, built-in reagent controls to determine whether reagents were added properly after specimen preparation, low demands on user expertise, and the availability of nationwide training and education programs to reduce user variability. TestPack Chlamydia is designed for the detection of chlamydial infection in low-volume diagnostic settings such as small laboratories and primary-care physician offices, where rapid chlamydial identification allows for the immediate treatment and effective follow up of patients. Rapid identification of infected patients has the potential of shortening the duration of clinical illness and reducing the period of infectivity (4), and this may also be applicable to chlamydial infections. It is beneficial to detect chlamydial infections in order to prevent the sequelae that lead to morbidity and increased medical costs (18). The indolent nature of chlamydial infection limits the effectiveness of empirically treating just symptomatic patients. Evidence has accumulated which suggests that testing of patients with multiple risk factors for chlamydial infection will detect a large proportion of those infected, even if asymptomatic (8, 10–13).

In summary, TestPack Chlamydia demonstrated positive and negative predictive values of 96.2 and 96.5%, respectively, versus confirmed chlamydial infection with less-than-48-h-old specimens. This high degree of correlation was achieved without specialized equipment and by visually reading positive and negative TestPack Chlamydia results. TestPack Chlamydia can be a useful diagnostic tool in providing quality health care to women at risk for chlamydial infection.

ACKNOWLEDGMENTS

The excellent technical assistance of Rebecca Deming, Ronald Falen, Hope Hagan, and Charles Van Sant is appreciated.

LITERATURE CITED

1. **Caldwell, H. D., and J. Schachter.** 1983. Immunoassay for detecting *Chlamydia trachomatis* major outer membrane protein. J. Clin. Microbiol. **18**:539–545.
2. **Centers for Disease Control.** 1985. *Chlamydia trachomatis* infections: policy guidelines for prevention and control. Morbid. Mortal. Weekly Rep. **34**(Suppl. 3S):53S–74S.
3. **Clyde, W. A., Jr., G. E. Kenny, and J. Schachter.** 1984. Cumitech 19, Laboratory diagnosis of chlamydial and mycoplasmal infections. Coordinating ed., W. L. Drew. American Society for Microbiology, Washington, D.C.
4. **Gerber, M.** 1986. Diagnosis of group A beta-hemolytic streptococcal pharyngitis, use of antigen detection tests. Diagn. Microbiol. Infect. Dis. **4**:5S–15S.
5. **Grayston, J. T., and S.-P. Wang.** 1975. New knowledge of chlamydiae and the diseases they cause. J. Infect. Dis. **132**: 87–105.
6. **Grayston, J. T., S.-P. Wang, L. J. Yeh, and C.-C. Kuo.** 1985. Importance of reinfection in the pathogenesis of trachoma. Rev. Infect. Dis. **7**:717–725.
7. **Griner, P., R. Mayewski, A. Musklin, and P. Greenland.** 1981. Selection and interpretation of diagnostic tests and procedures. Ann. Intern. Med. **94**:553–600.
8. **Handsfield, H., L. Jasman, P. Roberts, V. Hanson, R. Kothenbeutel, and W. Stamm.** 1986. Criteria for selective screening for *Chlamydia trachomatis* infection in women attending family planning clinics. J. Am. Med. Assoc. **255**:1730–1743.
9. **Howard, L., P. Coleman, B. England, and J. Herrmann.** 1986. Evaluation of Chlamydiazyme for the detection of genital infections caused by *Chlamydia trachomatis*. J. Clin. Microbiol. **23**:329–332.
10. **Kent, G., H. Harrison, S. Berman, and R. Keenlyside.** 1988. Screening for *Chlamydia trachomatis* infection in a sexually transmitted disease clinic: comparison of diagnostic tests with clinical and historical risk factors. Sex. Transm. Dis. **15**:51–57.
11. **Lindner, L., S. Gerrling, J. Nettum, S. Miller, and K. Altman.** 1988. Clinical characteristics of women with chlamydial cervicitis. J. Reprod. Med. **33**:684–690.
12. **Magder, L., H. Harrison, J. Ehret, T. Anderson, and F. Judson.** 1988. Factors related to genital *Chlamydia trachomatis* and its diagnosis by culture in a sexually transmitted disease clinic. Am. J. Epidemiol. **28**:298–308.
13. **Nogucki, M., T. Okamoto, N. Itoh, S. Hieda, H. Yabushita, and M. Ishihara.** 1988. *Chlamydia trachomatis* infection in female patients. Curr. Ther. Res. Clin. Exp. **44**:461–467.
14. **Reeve, P., J. Owen, and J. D. Oriel.** 1975. Laboratory procedures for the isolation of *Chlamydia trachomatis* from the human genital tract. J. Clin. Pathol. **28**:910–914.
15. **Schachter, J.** 1982. Sexually transmitted *Chlamydia trachomatis* infection. Postgrad. Med. **72**:60–69.
16. **Tam, M. R., W. R. Stamm, H. H. Handsfield, R. Stephens, C.-C. Kuo, K. K. Holmes, K. Ditzenberger, M. Krieger, and R. C. Nowinski.** 1984. Culture-independent diagnosis of *Chlamydia trachomatis* using monoclonal antibodies. N. Engl. J. Med. **310**:1146–1150.
17. **Thompson, S. E., and A. E. Washington.** 1983. Epidemiology of sexually transmitted *Chlamydia trachomatis* infections. Epidemiol. Rev. **5**:96–123.
18. **Trachtenberg, A., A. Washington, and S. Halldorson.** 1988. A cost-based decision analysis for chlamydia screening in California family planning clinics. Obstet. Gynecol. **71**:101–108.
19. **Wentworth, B. B., and E. R. Alexander.** 1974. Isolation of *Chlamydia trachomatis* by use of 5-iodo-2-deoxyuridine–treated cells. Appl. Microbiol. **27**:912–916.