Diagnosis of Chlamydial Infection in Women Attending Antenatal and Gynecologic Clinics

JAMES W. SMITH,^{1,2*} ROBERT E. ROGERS,^{2,3} BARRY P. KATZ,^{2,4} JOSEPH F. BRICKLER,^{1,2}
PATRICIA L. LINEBACK,^{1,2} BARBARA VAN DER POL,^{2,4} AND ROBERT B. JONES^{2,4}

Departments of Pathology,¹ Obstetrics and Gynecology,³ and Medicine,⁴ Wishard Memorial Hospital, Regenstrief Institute for Health Care, Indianapolis, Indiana 46202, and Indiana University School of Medicine, Indianapolis, Indiana 46223^{2}*

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Two antigen detection systems (MicroTrak [MT], Syva Co., Palo Alto, Calif.; and Chlamydiazyme [CZ], Abbott Laboratories, North Chicago, Ill.) were compared with semiquantitative culture for diagnosis of chlamydial infection in 1,059 patients. Cultures were done on microtiter plates and blind passaged once. Culture-negative but CZ- or MT-positive specimens were recultured. True positives were positive by either initial or repeat cultures. Of 827 nonpregnant and 231 pregnant patients, 9.1 and 12.1%, respectively, had positive cultures. Overall sensitivity of the initial culture was 48.5% without passage and 86.4% with passage. The sensitivity of CZ was 67%. The sensitivity of MT in our laboratory was 50%; however, further review of these specimens by Syva employees gave a combined sensitivity of 71.6%. MT and CZ were more sensitive for pregnant patients (MT, 84.6%; CZ, 85.7%) than for nonpregnant patients (MT, 65.5%; CZ, 60.0%). All the tests had specificities above 95%. Of the specimens that were positive after initial culture without subculture, MT-negative specimens had a mean of 3.7 inclusions in culture, and MT-positive specimens had a mean of 24.8 ($P = 0.002$); CZ-negative specimens had a mean of 4.3 inclusions, and CZ-positive specimens had a mean of 20.0 ($P = 0.026$). In addition, cultures of specimens from pregnant patients had more inclusions than did those from gynecology patients, but this was not statistically significant ($P = 0.096$). No method is ideal; however, MT and CZ were less sensitive than was this culture system for detecting chlamydial infection in patients in gynecology clinics and were of comparable sensitivity for pregnant patients.

Chlamydial infections in women are often only minimally symptomatic, or even asymptomatic. However, in pregnant women, the organism can be passed from mother to child at birth, resulting in neonatal conjunctivitis or pneumonia (1). In nonpregnant women, chronic salpingitis resulting from chlamydia often damages the fallopian tubes, with consequent ectopic pregnancy or infertility (12). In addition, asymptomatic women appear to be the major reservoir of infection in a community (6).

Until recently, diagnosis has been based on symptomatic presentation or a history of contact with such a person. Now, however, improved culture methods and the development of antigen detection tests offer the possibility of screening for chlamydial infections and treating them early to prevent their untoward consequences. Cultures for chlamydia, however, are not performed by most laboratories, and the relative usefulness of culture and antigen detection methods has not been as well studied in women not identified as being at high risk for infection. Most previous studies comparing methods have been of relatively high-risk patients attending sexually transmitted disease (STD), family-planning, or abortion clinics (2, 4, 5, 8, 11). The present study compares the usefulness of three diagnostic methods for women attending county hospital obstetrics and gynecology clinics. A microtiter culture system with blind passage is compared with antigen detection by direct immunofluorescence microscopy (MicroTrak [MT]; Syva Co., Palo Alto, Calif.) and by enzyme-linked immunosorbent assay

(Chlamydiazyme [CZ]; Abbott Laboratories, North Chicago, Ill.).

MATERIALS AND METHODS

Patient population. Patients evaluated in this study consisted of all the consenting pregnant females seen for the first time in the obstetrics clinic and all the consenting nonpregnant females under 35 years of age seen for the first time in the gynecology clinic of Wishard Memorial Hospital and its associated Regenstrief Institute outpatient clinics. These city-county health-care facilities serve Indianapolis and Marion County, Ind. Patients suspected of having STD were referred to STD clinics, and patients desiring abortion were referred to abortion clinics; therefore, they were not included in the study. Patients with acute salpingitis were not included, and thus all patients studied were either asymptomatic or had mild nonspecific symptoms. Specimens were obtained by the various physicians who staffed the clinics.

Specimen collection. To randomize the order of collection, the collection materials for each patient were placed in a large bag with the order of collection for the individual specimens numbered such that the specimens would be collected in random sequence, and each test would be first, second, or third an equal number of times. After removal of excess cervical mucus, the specimens were collected from the endocervix by using Dacron swabs. Syva collection kits were used for MT, and Abbott collection kits for females were used for CZ. The swabs for MT and CZ were handled according to the instructions of the manufacturers. Endocervical swabs for culture were placed in a small vial containing 1.5 ml of transport medium and stored at 4°C (<24 h) until

* Corresponding author.

transport to the culture facility, where they were used to inoculate tissue culture cells or were subjected to further storage at -70°C (<72 h) until tissue culture inoculation. The swab for the MT test was rolled over a slide well (diameter, 8 mm) immediately after collection, with the swab held parallel to the surface and with all portions of the swab surface used to apply material to the well. The swab for CZ was immediately placed into the tube containing specimen storage reagent.

Upon receipt in the laboratory, the CZ specimen tubes were placed in the refrigerator, and the test was performed within 5 days. For better control of fixation, MT slides were not fixed at the time of collection but were fixed upon receipt in the laboratory, according to the instructions of the manufacturer, by squeezing all of the acetone from an ampoule (provided by the manufacturer) on the surface of the slide and allowing it to evaporate completely. The slide was then placed in the refrigerator (2 to 8°C) until it was stained (within 3 days).

Slides for MT were stained according to the instructions of the manufacturer. MT reagent, fluorescein-conjugated monoclonal antibody against *Chlamydia trachomatis* outer membrane plus an Evans blue counterstain, was placed over the slide well for 15 to 30 min. The slides were then rinsed in distilled water and allowed to air dry. Mounting fluid (Syva) and a cover slip were added, and the slides were examined within 24 h by using an incident fluorescence microscope (American Optical Corp., Buffalo, N.Y.) with a mercury light source. Smears were scanned at $\times 500$ magnification with an oil immersion objective (magnification, $\times 50$), with positives being confirmed at $\times 1,000$ magnification. The presence of columnar and cuboidal cells mixed with polymorphonuclear leukocytes indicated that the specimen was adequate. Acellular smears and smears containing only squamous cells were considered inadequate. The entire well of each negative slide was examined. An MT was considered positive if it had 10 or more organisms per slide. Staining and examination of MT were performed the day after collection, except that Friday specimens were done on Monday.

The CZ procedure was performed according to the recommendations of the manufacturer. Briefly, 1 ml of specimen dilution buffer was added to each tube and incubated at room temperature for at least 15 min. The tube with the swab was vortexed for three cycles of 15 s each at a medium-to-high setting, and excess fluid was removed by pressing and rotating the swab against the side of the tube. In each batch of CZ, one positive and three negative controls were included. Vortexed specimens or controls were added to appropriate wells of the reaction tray, and one polystyrene bead was added to each well. After incubation at 37°C for 60 min, liquid was aspirated; each bead was washed; and then polyclonal rabbit antibody against *C. trachomatis* was added to each reaction well. After incubation at 37°C for 60 min, each bead was washed again; then, peroxidase-labeled anti-rabbit immunoglobulin G conjugate was added to each well, and the beads were incubated at 37°C for 60 min and washed. Beads were immediately transferred to tubes, and *o*-phenylenediamine was added to each specimen or control tube and to two blank substrate tubes. After incubation for 30 min at room temperature, 1 N sulfuric acid was added to each tube and mixed. Tubes were read on the Quantum II (Abbott) at A_{492} . The test was considered positive when the optical density reading was 0.100 greater than the mean optical density of the three negative controls.

Chlamydia culture procedure has been described in detail elsewhere (7). Briefly, before inoculation, each vial contain-

ing a swab specimen in transport medium was mixed on a vortex mixer at full speed for 3 to 5 s. Then, a 0.1-ml sample was inoculated into each of three wells of a tissue culture plate (96 wells; Costar, Cambridge, Mass.) containing a McCoy cell monolayer; 0.2 ml of minimal essential medium containing vitamins, glutamic acid, amphotericin B, and cycloheximide was added to each inoculated well; and the plate was centrifuged at $1,750 \times g$ for 1 h at 30°C . The remainder of the specimen was stored at -70°C for possible subsequent reculture.

After centrifugation, the plates were incubated at 35°C for 72 h. One well of monolayer cells from each specimen was then examined for inclusion bodies by immunofluorescence staining by using a monoclonal antibody.

For blind subculture, the medium was aspirated from the two remaining unstained monolayers, 0.1 ml of transport medium was added to the first well, and the cells were scraped loose with a blunt Pasteur pipette. This suspension was transferred to the second well, and again the cells were scraped. The pooled material from the two wells was used to inoculate a fresh monolayer of McCoy cells in a single well, 0.2 ml of medium was added, and the plate was centrifuged as described above. This plate was incubated for an additional 72 h and then stained and examined for inclusion bodies.

Each of the methods was performed independently, without knowledge on the part of the technician of the results of the other methods. After initial analysis of the results, specimens which were not positive in all three of the tests, but which were positive by at least one of the test systems, were then reevaluated by all three test systems. For culture, a portion of the initial specimen stored at -70°C was thawed and cultured as described above. For CZ, the remaining material frozen at -70°C was retested with the procedure described above. For MT, after examination in our laboratory, all slides were sent to Syva. MT of specimens for which there was a lack of agreement among the methods were examined by Syva employees as blind unknowns, along with an equal number of specimens negative by all three methods in our laboratories. In some instances, when Syva employees thought on initial examination that the stain was not satisfactory, the slides were refixed, restained, and then reexamined at Syva.

Specimens were considered to be positive if they were positive by initial culture or repeat culture (true positive). Specimens were considered to be false-positives when they were positive by MT or CZ, but negative by initial and repeat cultures.

RESULTS

Of 1,059 patients studied with all three methods, 231 were pregnant and 827 were not pregnant. For one patient, pregnancy status was not recorded. Overall there were 103 (9.7%) true positives, with 75 (9.1%) positive from nonpregnant patients and 28 (12.1%) positive from pregnant patients. Table 1 compares MT, CZ, primary culture, culture with blind passage, and true positive culture, as defined in Materials and Methods. Only MT specimens which were considered satisfactory by our laboratory were included in the evaluation of MT. For culture and CZ, the quality of specimens could not be determined, and all were included in the analyses. Primary culture without blind passage was positive in only 50 (48.5%) of 103 true positives, whereas initial culture with blind passage was positive in 89 (86.4%) of 103 (Table 1). Of 61 specimens initially culture negative

TABLE 1. Sensitivity, specificity, PPV, and NPV of various methods for diagnosing chlamydial infection^a

Method	Nonpregnant patients				Pregnant patients				Total			
	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Primary culture vs TP ^b	44.0			94.7	60.7			94.7	48.5			94.7
Primary culture with subculture vs TP	88.0			98.8	82.1			98.8	86.4			98.6
MT vs primary culture	84.6	94.4	45.8	99.7	86.7	89.9	50.0	98.3	85.4	93.4	47.3	98.9
MT vs primary culture with subculture	66.0	96.2	64.6	96.4	81.0	92.7	65.4	96.6	70.6	96.4	64.9	95.9
MT ^c vs TP	65.5	97.2	75.0	95.7	84.6	96.6	84.6	96.6	71.6	97.1	78.4	95.9
CZ vs primary culture	80.0	94.0	36.8	99.1	88.2	91.6	50.0	98.5	86.0	93.5	39.4	99.3
CZ vs primary culture with subculture	54.5	94.7	47.4	96.0	82.6	93.3	57.6	98.0	61.8	94.4	50.5	96.4
CZ vs TP	60.0	95.9	59.2	96.0	85.7	95.6	72.7	98.0	67.0	95.8	63.3	96.4

^a Sens, Sensitivity; Spec, specificity; TP, true positive.^b Positive in initial or repeat culture (see the text).^c Results are for 638 satisfactory specimens only and are the combined results of our laboratory and Syva laboratories.

but CZ or MT positive in our laboratory, 14 (23.0%) were positive after repeat culture (Table 2). All 14 were CZ positive, and 8 were MT positive in our laboratory. An additional three were MT positive by Syva employees.

MT specimens were satisfactory in only 638 (60.2%) of 1,059 specimens, 493 from nonpregnant, 144 from pregnant patients, and 1 from the patient whose pregnancy status was not recorded. Of these, 82 (12.8%) were true positives, 56 from nonpregnant and 26 from pregnant patients. In contrast, of 421 specimens unsatisfactory for MT, only 21 (5.0%) were true positives ($P < 0.001$). The results in Table 1 are for 74 specimens positive by MT in our laboratory or by Syva employees. For MT, our laboratory found sensitivities of 37.5% (nonpregnant), 76.9% (pregnant), and 50.0% (overall) compared with 65.5, 84.6, and 71.6% for the combination of laboratories. We read only 2 specimens as positive which Syva employees read as negative (both were culture positive), but they read 27 specimens as positive which our laboratory read as negative. Of these, 19 were culture positive, and 23 were CZ positive. They interpreted one specimen as positive which we found negative by MT, CZ, and culture. The MT appeared more sensitive for pregnant patients (84.6%) than for nonpregnant patients (65.5%) (Table 1). A positive predictive value (PPV) represents the likelihood that a patient with a positive test has disease, and a negative predictive value (NPV) represents the likelihood that a patient with a negative test is free of disease. Predictive values therefore are affected by the frequency of disease

in the population studied. For MT, the overall PPV was 78.4% and the NPV was 95.9% (Table 1).

Overall, CZ showed a sensitivity of 67.0% and a specificity of 95.8%; however, as with MT, sensitivity was greater for pregnant (85.7%) than for nonpregnant (60.0%) patients. Overall, the PPV was 63.3% and the NPV was 96.4%. CZ was repeated for all CZ-positive, initial-culture-negative, or CZ-negative, culture-positive specimens. The optical density of CZ-positive specimens was uniformly lower for the repeat test than for the initial one. Of 109 positive CZ specimens, 11 were negative when the test was repeated. Initially, all but one of these were only weakly positive (i.e., just above the threshold for positive), and only one had a positive culture when it was repeated. None of the 34 culture-positive, CZ-negative specimens were positive when the test was repeated. In our laboratory, only one specimen was CZ negative and MT positive, and it was culture negative. Syva employees noted four additional CZ-negative, MT-positive specimens, all of which were culture positive both initially and when recultured. Of 21 specimens which were CZ positive, MT negative, and culture negative in our laboratory, 10 were MT positive when reviewed at Syva.

To assess whether the intensity of infection correlated with the sensitivity of MT and CZ, we compared geometric mean numbers of inclusions detected in the primary culture with the results of MT and CZ by using the Student *t* test on the logarithm of the numbers of inclusions (Table 3). Specimens positive by MT or CZ had large numbers of inclusions noted in the primary culture, whereas MT- or CZ-negative specimens had fewer inclusions. This is also shown by the greater sensitivity of MT and CZ when compared with that

TABLE 2. Comparison of initial and repeat culture results for CZ- and MT-positive specimens

Positive test	No. of initial cultures		No. of initial or repeat cultures		No. changed
	+	-	+	Both -	
MT ^a	48	26	58	16 ^b	10
CZ	55	54	69	40	14
Culture	89		103		14 ^c

^a Combined results for 638 adequate specimens only.^b Fifteen MT-positive specimens were also CZ positive, and one was CZ negative.^c All the specimens were CZ positive and 11 were MT positive, but 30 culture-positive specimens were still MT and CZ negative.

TABLE 3. Correlation of CZ and MT results with inclusion counts in the primary culture

Test	No. of results	Inclusion count (geometric mean) ^a	Significance (P)
MT			
positive	26	24.8	
negative	15	3.7	0.002
CZ			
positive	43	20.0	
negative	7	4.3	0.026

^a Number of inclusions in the primary culture (without blind passage).

of the primary culture, rather than that of the culture with blind subculture or true positive culture (Table 1). It is assumed that culture-positive specimens requiring blind passage or repeat culture for detection of chlamydia contain fewer organisms than those positive in primary cultures. Specimens from pregnant patients with positive primary cultures had greater geometric mean inclusion counts in culture (31.0) than did those from nonpregnant patients (8.8), although this was not statistically significant ($P = 0.096$). Both MT and CZ showed greater sensitivity for pregnant than for nonpregnant patients (Table 1). The order of specimen collection did not significantly affect the results of any method (data not shown).

DISCUSSION

This study indicates that no currently available system is ideal for diagnosis of chlamydial infections. Culture without blind passage missed over one-half of the infections, and even with blind passage missed 14.6%, and this assumes that all the patients who were negative by MT, CZ, and culture were free of chlamydia (an unlikely assumption). Other more detailed studies of the effect of blind passage for diagnosis of chlamydial infections are consistent with these observations (6, 7). By comparisons with observed prevalences in populations at risk for chlamydial infections, we believe that the culture technique used in this study is as sensitive as that performed by most investigators and more sensitive than many. The use of shell vials for culture combined with immunofluorescence identification of inclusions with monoclonal antibodies may be somewhat more sensitive (10). However, vial culture for chlamydia is more cumbersome and expensive than is culture in microtiter plates and, therefore, is less applicable to the processing of large numbers of specimens than is microtiter plate culture (13). It is important to recognize that the sensitivity of culture as usually performed is less than 100%, and any improvement therein will only further reduce the sensitivity of direct antigen tests which are already less sensitive than culture is as it is usually performed, but increased sensitivity of culture may result in improved specificity for the direct test.

In comparing various methods for diagnosis of chlamydial infections, several factors are important. One is the population being evaluated. The numbers of organisms present are likely to be greater in symptomatic patients being evaluated for STD than in largely asymptomatic patients presenting for routine antepartum or gynecologic care (8). The numbers of recoverable organisms clearly influence the sensitivities of the tests (Table 3). Consequently, a test may appear fairly sensitive for STD clinic patients, but less so for other patients with a lower prevalence of infection and fewer organisms at an infected site. In addition, if a relatively insensitive culture method, such as culture without blind passage, is used as the "gold standard," a direct antigen test may appear more sensitive than when a more sensitive culture method is used. Both a direct antigen test and an insensitive culture method will detect organisms when there are many present, while only the more sensitive culture test may be positive when few are present. It should be noted that, although we have considerable experience in culturing *C. trachomatis* and every effort was made to maximize recovery from culture, our experience with these direct antigen tests is minimal. Thus, the results obtained with MT and CZ represent the type of results which can be expected when these procedures are first used at an institution with a competent microbiology laboratory, with specimens col-

lected by the types of personnel ordinarily involved in patient care. There is no doubt that greater attention to technique and experience in specimen collection (and, in the case of MT, specimen evaluation) would result in improved sensitivity. However, results of both antigen detection tests as now formulated are still going to fall short of results obtainable with good culture methods, at least for nonpregnant patients. Even those infections detected with good culture methods represent considerably less than 100% of the infected patients, as shown by the 14.6% of specimens which were culture positive only after reculture.

The importance of skilled personnel is emphasized by the greater ability of Syva employees than that of our personnel to detect infections with MT. Although our laboratory personnel are experienced in fluorescence microscopy, Syva employees were able to diagnose cases which we did not detect. Some of these could be diagnosed only after Syva employees refixed and restained the slides, suggesting that instructions for fixation from Syva may not give optimal fixation for some specimens and that longer fixation before staining might help.

The MT has a distinct advantage over the other tests (CZ and culture) in that it allows the quality of the specimen to be evaluated. We found the quality unsatisfactory in 40% of these specimens, which is greater than the 12% noted by Forbes et al. (3) and may reflect differences in the skills of the personnel collecting the specimen or applying it to the slide. Adequacy of specimens could not be determined for either culture or CZ. The importance of assessing specimen adequacy is indicated by the greater recovery of chlamydia by culture of specimens from patients with adequate MT specimens than from patients with unsatisfactory MT specimens. The sensitivity of MT observed in the present study is considerably less than in most studies from STD clinics (2, 3, 8, 9), although comparable to some studies of similar patient groups (2, 3, 8). However, if the MT-inadequate specimens are included in the overall analysis, the sensitivity of MT drops to <50% of the total specimens, as compared with that of the true positives. Thus, although a positive MT is probably most often indicative of infection, a negative or inadequate one does not rule it out.

CZ showed better reproducibility than did culture or MT and has the advantages that it is more rapid than culture and less labor intensive than MT and requires less skill for laboratory interpretation than both MT and culture. It was more sensitive than MT when the latter was evaluated in our laboratory and had sensitivity equivalent to that of MT when evaluations by Syva employees were included. CZ was less sensitive than culture with blind passage for nonpregnant women (60.0 versus 88.0%), although of comparable sensitivity for pregnant women (85.7 versus 82.1%).

The observed specificity of CZ was slightly less than that of MT. However, although 15 of 16 culture-negative MT-positive specimens were also CZ positive and were probably true positives not detected by culture, 10 of 25 CZ-positive, culture-negative specimens in which there was an adequate specimen for MT were negative by MT as well. These were likely CZ false-positives. Evaluation of CZ in a patient population extensively cultured for chlamydia, e.g., multiple cultures from multiple sites (6), might help resolve the issue of specificity. In the present study, the more thorough the culture used for comparison was, the greater the specificity of the direct tests was (Table 1).

The PPVs of the tests in the various populations are clinically relevant. For CZ, 36.7% of patients with positive tests were not confirmed by culture, whereas for MT, 21.6%

were not confirmed by culture. This is potentially an important consideration, since the social consequences of informing someone in a non-STD clinic setting that they have a sexually transmissible disease can, in many circumstances, far outweigh the medical consequences. Such considerations need to be borne in mind by the clinician in using any nonculture method. In addition, either test may be of value for screening selected populations, especially when culture is not available or is cost prohibitive. However, a negative test, even a negative culture, should not be interpreted as excluding infection, and retesting at appropriate time intervals may be of value in certain cases.

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