Detection of *Chlamydia trachomatis* in Genital Specimens by the Chlamydiazyme Test

MARY F. JONES, THOMAS F. SMITH, ARVID J. HOUGLUM, AND JOHN E. HERRMANN

Section of Clinical Microbiology, Mayo Clinic, and Olmsted County Health Department, Rochester, Minnesota 55901, and Abbott Laboratories, North Chicago, Illinois 60064

Received 6 April 1984/Accepted 25 May 1984

Cotton swabs were used to collect two specimens each from 416 patients (206 males, 210 females) attending a sexually transmitted disease clinic. The first swab was transported in Specimen Storage Reagent and extracted in Specimen Dilution Buffer for enzyme immunoassay by Chlamydiazyme (Abbott Laboratories); the second swab was extracted into 2SP and inoculated into McCoy cell cultures. In the first phase of the study (215 patients: 111 males, 114 females) enzyme immunoassay results were positive (optical density ≥ 0.1) in 30 of 35 instances in which *Chlamydia trachomatis* was isolated (sensitivity, 86%). Of 18 false-positive enzyme immunoassay results, 15 (83%) were cervical swabs (specificity, 90%). In a phase II study, using a modified Chlamydiazyme kit, 201 patients were tested (95 males, 106 females). Of 41 chlamydial isolates, 8 were not detected by the Chlamydiazyme test (sensitivity, 81%). Only three positive Chlamydiazyme test results could not be confirmed by culture (specificity, 98%). Overall, Chlamydiazyme assay provided a rapid (4 h), sensitive, and specific assay for the detection of chlamydial antigens.

*Chlamydia trachomatis* may be the most common agent of sexually transmitted disease and causes a clinical spectrum of disease paralleling that of *Neisseria gonorrhoeae* (1, 4). In recent years, substantial progress has been made in streamlining the methodology required for the inoculation, incubation, and examination of cell cultures required for the cultivation of these organisms in the laboratory (8). Presently, detection of chlamydial inclusions in cell cultures requires staining techniques which are generally performed 40 to 48 h postinfection. Immunological techniques for detecting chlamydial infection have been, for the most part, directed toward assays for chlamydial antibodies (11, 12). Immunofluorescence techniques have been used to identify chlamydial inclusions in ocular and genital scrapings (3, 6). More recently, fluorescent monoclonal antibody to *C. trachomatis* has been used to identify *C. trachomatis* elementary bodies in genital smears (9). Detection of *C. trachomatis* antigen by radioimmunoassay or enzyme immunoassay has been described (2, 10), but these procedures have not been applied to detection of antigen in clinical specimens.

We evaluated an enzyme immunoassay kit procedure requiring only 4 h testing time (Chlamydiazyme: Abbott Laboratories, North Chicago, Ill.) for the direct detection of chlamydial antigens in extracts of swab specimens. The specimens were obtained from 416 individuals attending a sexually transmitted disease clinic, and the enzyme immunoassay results were compared with conventional cell culture results.

**MATERIALS AND METHODS**

**Study population.** Individuals attending the Olmsted County Sexually Transmitted Disease Clinic for signs or symptoms of urethritis or cervicitis but who had not received antimicrobial therapy within the past 4 weeks were selected. Those patients whose specimens yielded a positive Chlamydiazyme but negative culture result were recalled for repeat testing.

**Specimens.** Two swabs were selected from each patient. For males, a cotton swab (American Scientific Products, McGaw Park, Ill.) was inserted 3 to 5 cm into the urethra, placed into the collection tube, and extracted into 0.1 ml of Chlamydiazyme Specimen Storage Reagent. A second urethral swab was obtained as described above, except that it was extracted immediately into 1 ml of 2SP transport medium. The entire swab was then discarded.

For women, two sequential endocervical specimens were obtained with a cotton swab; the first was inserted into the Specimen Storage Reagent-containing tube and the shaft of the swab was broken and discarded. The second swab was extracted in 2SP and the entire swab was discarded. All specimens were stored at 4°C and transported to the laboratory within 12 h of collection.

**Immunoassay of Chlamydiazyme assay.** Before the enzyme immunoassay was performed, 1 ml of Chlamydiazyme Specimen Dilution Buffer was added to each tube containing a swab specimen. The tubes were incubated at room temperature for 10 min and then placed in a Multivortex Unit (Scientific Manufacturing Industries, Emeryville, Calif.) for three 15-s cycles. The liquid was then expressed from the swab by pressing and rotating the tip of the swab against the side of the tube. The swab was discarded.

Samples (200 μl) of the Specimen Dilution Buffer specimen extract and control specimens (known negative and positive specimens supplied in kit) were placed in reaction tray wells of plastic plates. One treated polystyrene bead was added to each well, and the plate was incubated in a water bath (35 to 39°C) for 45 min. Each bead was washed and 200 μl of antibody to *C. trachomatis* (rabbit) was added to each well. After incubation (35 to 39°C) for 45 min, each bead was washed and reacted with 200 μl of horseradish peroxidase-conjugated antibody to immunoglobulin G (rabbit). Subsequent to incubation (35 to 39°C for 45 min) and washing, 300 μl of peroxidase substrate (o-phenyl-
TABLE 1. Detection of C. trachomatis by the Chlamydiazyme test (phase I) and culture in McCoy cells

<table>
<thead>
<tr>
<th>Chlamydiazyme</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>30</td>
<td>18</td>
<td>48</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>162</td>
<td>167</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>180</td>
<td>215</td>
</tr>
</tbody>
</table>

TABLE 2. Detection of C. trachomatis by the Chlamydiazyme test (phase II) and by culture in McCoy cells

<table>
<thead>
<tr>
<th>Chlamydiazyme</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>33</td>
<td>3</td>
<td>36</td>
</tr>
<tr>
<td>Negative</td>
<td>8</td>
<td>157</td>
<td>165</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>160</td>
<td>201</td>
</tr>
</tbody>
</table>

enediamine - 2HCl + H2O2) was added to each well. The reaction was stopped with 1 ml of 1 N sulfuric acid after incubation at room temperature for 30 min. Absorbance of the specimens was determined with a QUANTUM II (Abbott Laboratories) spectrophotometer at 492 nm. In the phase I study, a result was considered to be positive with an optical density (OD) reading of ≥0.1. For phase II, a specimen was considered positive if the OD exceeded that of the mean of three negative control determinations plus 0.100.

Cell culture isolation method. Specimen extracts (2SP) were inoculated into two glass vials containing McCoy cells seeded on cover slips. After 48 h of incubation at 36°C, one cover slip was stained with iodine and examined for the presence of typical chlamydial inclusions. Cells on the other cover slip were disrupted with glass beads and then passed to fresh cell cultures, incubated for another 48 h, and then stained with iodine (7). Cultures were considered positive if one or more inclusions were observed in either culture. Chlamydiazyme and cell culture procedures were performed by separate technologists, and the results were determined independently.

RESULTS

Two phases of the study were done. In the first phase of the study, 215 specimens were submitted (111 males, 104 females). C. trachomatis was isolated in cell cultures from 35 specimens (16.3%); in 5 of these instances (4 from males) the Chlamydiazyme test was negative (specificity, 85.7%) (Table 1). On the other hand, there were 18 instances in which the Chlamydiazyme test results could not be confirmed by recovery of the organism (specificity, 90.6%). Interestingly, 15 of these specimens (83%) were endocervical specimens. Repeat specimens from seven of these patients (cell culture negative, Chlamydiazyme positive) were obtained. These same results were obtained from three patients, whereas determinations from the two assay methods were negative with specimens from three others and co-positive in only one instance. All but 4 of the 18 false-positive results had OD readings of between 0.1 and 0.2. Therefore, arbitrarily using this upper limit cutoff, the specificity of the test in the phase I study could be increased to 97.8% with no loss in the sensitivity of the test (Fig. 1). In addition, the negative predictive value remained unchanged between the OD cutoff levels of 0.1 and 0.2; however, the positive predictive value increased from 62.5% (OD ≥ 0.1) to 88.2% (OD ≥ 0.2) owing to the elimination of 14 of the 18 false-positive results.

The second phase of the study used a modified Chlamydiazyme kit provided by Abbott Laboratories. From 201 specimens (95 males, 106 females), 41 chlamydial isolates were obtained (20.4%) (Table 2). Eight of these were not detected by the Chlamydiazyme assay (sensitivity, 80.5%). Only three positive Chlamydiazyme test results (two specimens from females) could not be confirmed by culture (specificity, 98.1%).

DISCUSSION

The high number of false-positive results (18) obtained in the phase I study was of concern scientifically, but also of importance because of the possible social effect involved in the medical management of such patients and their contacts subsequent to laboratory results indicating the presence of a sexually transmitted disease. With the modified assay, only three specimens in phase II studies were Chlamydiazyme positive without culture confirmation. All three of these samples had OD readings of ≥0.8. Collectively (both studies), 17 of 21 (81%) of the false-positive results came from assays of endocervical specimens, suggesting the possibility of some interfering substance with the Chlamydiazyme test. Alternatively, this lack of test specificity with specimens from females may actually occur from reduced sensitivity of culture methodology in that the overall isolation rate for men was 50 of 206 (24.3%) and that for women was 26 of 210 (12.4%). Higher isolation rates of C. trachomatis have been consistently noted by others (5).

Calculations of the sensitivity of the Chlamydiazyme test included results obtained after initial inoculation and after passage to fresh cell cultures. Thus, if culture results for only the initial inoculation were considered, as would be the case for most clinical practices, the sensitivity of the test would be increased to 87.8 and 84.6% for phase I and II studies, respectively.

Interestingly, only 13 specimens were culture positive in both studies when the Chlamydiazyme test was negative. Thus, the high negative predictive value of the assay (97%, phase I; 95.2%, phase II) should allow the physician to confidently exclude C. trachomatis in the differential diagno-
sis of a sexually transmitted disease when the Chlamydiazyme test is negative (Fig. 1).

Overall, Chlamydiazyme assay provides for a rapid (4 h), sensitive, and specific assay for the detection of chlamydial antigens.

LITERATURE CITED