Comparison of PCR with Culture for Detection of *Ureaplasma urealyticum* in Clinical Samples from Patients with Urogenital Infections

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PCR was compared with culture for the detection of *Ureaplasma urealyticum* in 50 specimens, including sperm, urine, and prostate secretions, from hospital patients with urogenital infections. Five positive and a further four doubtful diagnoses were made by culture, whereas PCR detected *U. urealyticum* in 12 samples. PCR also was faster than culturing. The increased sensitivity and shorter time requirement of PCR support its further development for the diagnosis of *U. urealyticum* infection.

MATERIALS AND METHODS

Specimens. Specimens were taken from a total of 50 patients, ranging in age from 19 to 45 years, in the Departments of Venereal Diseases, Urology, and Gynecology of the First Affiliated Hospital, Xinjiang Medical College, Urumqi, Xinjiang, People’s Republic of China, from May to July 1993. Urethral swabs (from eight males) or urine samples (from one male and seven females) were taken from 16 patients with symptoms of urethritis (discharge, dysuria, pain, or frequent urination). Endocervical swab samples were collected from 12 female patients with typical symptoms of vaginitis or cervicitis. Prostate secretions were collected by prostate palpation from eight male patients with prostatitis, and semen samples were collected by condom from 14 male patients with infertility problems complicated by prostatitis or orchitis.

Duplicate swab samples were taken, and one was placed immediately in liquid culture medium and the other was placed in a sterilized container with 5 ml of distilled water for subsequent PCR. A subsample of all other specimens was placed immediately in liquid culture medium, and the remainder was placed in sterile containers for PCR. Materials were transported to the laboratory at ambient temperature within 30 min of collection. Culture materials were then placed immediately in an incubator, and PCR samples were stored at 4°C until processing.

Separation and cultivation of *U. urealyticum*. A liquid-solid culture procedure was employed, using media purchased from the Department of Microbiology, Shanghai Secondary Medical University, Shanghai, People’s Republic of China. The media are manufactured to support growth of all ureaplasmal serotypes.

Samples inoculated into the liquid medium (1:1 bovine heart infusion-pig stomach pepsin supplemented with 10% calf serum, 10% fresh yeast extract, 0.15% urea, 2,000 U of penicillin G per ml, and 0.002% phenyl red, adjusted to pH 6.0) were incubated at 37°C. As soon as the pH of the medium changed, usually within 1 to 2 days, the cultures were centrifuged and the residues were transferred to plates of solid medium. Samples that did not show a pH change within 5 days were discarded and recorded as *Ureaplasma* negative.

Cultures transferred to solid medium (made up of the same constituents as the liquid medium with the addition of 1% agar) were incubated at 37°C in an atmosphere of 5% CO₂. Developing colonies were examined microscopically. Colonies with the typical appearance of *U. urealyticum* were classified as positive. Others that did not develop typical colonies within 5 days were classified as doubtful.

DNA extraction from *U. urealyticum* standards. Dried standard strains of *U. urealyticum* (identified as UQ2, USC, and U7C) were purchased from the Department of Microbiology, Shanghai Secondary Medical University. They had originated from the Department of Microbiology, Alberta University, Edmonton, Alberta, Canada. The dried standard strains were suspended in 400 µl of STE (0.1 M NaCl, 0.05 M Tris, 1 mM EDTA) to which were added 25 µl of sodium dodecyl sulfate (10%) and 5 µl of proteinase E (100 mg/ml). After incubation at 30°C for 1 h, the solution was mixed with 400 µl of saturated phenol (pH 8.0) and centrifuged at 2,000 × g for 8 min. The aqueous phase was then mixed with 400 µl of chloroform-isoamyl alcohol (24:1) and centrifuged again at 2,000 × g for 8 min. DNA was precipitated with absolute ethanol at −20°C for more than 2 h and then dissolved in 60 µl of Tris-EDTA solution (pH 7.5). These procedures provided the DNA standards against which assayed specimens were compared.

DNA extraction from specimens. Specimen samples were mixed with distilled water to a volume of 5 ml and centrifuged
at 20,000 × g for 25 min. DNA was extracted from the residue by the method described above for the \textit{U. urealyticum} standards.

**PCR.** Primers were synthesized on an automated DNA synthesizer by the Fuhua Company, Shanghai, People's Republic of China. The published (10) sequences of the primers are as follows: primer 14b, 5'—CCA GGA AAA GTA GTA CCA GGA GC—3'; and primer C72b, 5'—CTC GTA ATC TAA CGC TAT CAC C—3'. The PCR solution (50 μl) contained 10 μl of 5× buffer [25 mM Tris-HCl, 3 mM MgCl₂, 25 mM (NH₄)₂SO₄, 100 μg of gelatin per ml, 5% formamide], 20 pmol of each primer, 400 μM each deoxynucleotide triphosphate, 2 U of Taq polymerase (Fuhua Company, Shanghai, People's Republic of China), and 2 μl of template DNA. \textit{U. urealyticum} DNA was amplified in a DNA thermal cycler (Perkin-Elmer Cetus Corporation, Norwalk, Conn.) during 30 thermocycles, each consisting of a 30-s denaturation step at 94°C, a 30-s annealing step at 55°C, and a 90-s elongation step at 68°C. The amplified product was visualized and photographed under UV light after electrophoresis for 30 min at 110 V through a 2.0% agarose gel containing ethidium bromide. Fragment sizes relative to marker pGEM7Zf(+) Healll (New England Biolabs) and an unnamed marker supplied by Fuhua Company were determined.

**RESULTS**

A photograph of electrophoresis bands on bromide-stained agarose gel for two markers and PCR-amplified products from the \textit{U. urealyticum} standard and five specimens is presented in Fig. 1. DNA from the urease gene in \textit{U. urealyticum} which is amplified by the PCR primers used in this study shows at 460 bp (10). The two markers at 458 bp confirm the PCR-amplified \textit{U. urealyticum} standard DNA at 460 bp. By comparison with the standard, four of the specimens are clearly positive for \textit{U. urealyticum} and one is negative.

A comparison of all results from the PCR procedure with those from the culture method is shown in Table 1. Five of the 50 cultured specimens were positive for \textit{U. urealyticum}, and a further four were doubtful (positive identification on liquid medium but no positive identification on solid medium). PCR showed positive test results for 12 specimens, which included all of the positive cultured specimens plus the four doubtful cultured semen specimens and one prostate secretion and two cervical secretion specimens that failed to culture.

On average, only 24% of the 50 clinical diagnoses, ranging from 12% of the urinary tract infections to 43% of the infertility cases, were associated with positive \textit{U. urealyticum} tests.

**DISCUSSION**

Although the combined use of liquid and solid media is thought to be the most sensitive culture method available, it has been shown in other studies (9) to detect only 80% of samples infected with \textit{U. urealyticum}. If in our study the 12 positive specimens detected by PCR represented all infections, then the 5 positive and 4 doubtful specimens detected by culture similarly represent only 75% of the infected samples.

The lower level of detection by culture may be attributed at least in part to the generally recognized difficulties of culturing and isolating \textit{U. urealyticum} (3). The difference also may be related to possible effects of uncontrolled ambient temperature between collection and the beginning of incubation in the present study or to effects of other organisms during incubation or material present in the specimens. Organisms that die before incubation of course cannot be cultured, whereas the DNA of dead organisms can still be detected by PCR. The reason four semen specimens detected positively by PCR were detected only doubtfully by culture in the present study is a matter for conjecture, but it may have been a consequence of other organisms or material in the specimens that progressively inhibited \textit{U. urealyticum} during incubation in the liquid medium to the extent that it could not be cultured in the solid medium.

In addition to its greater sensitivity and lesser dependence on careful specimen handling between collection and testing, PCR had a further advantage of faster determination. Assay time was reduced from 2 to 3 days for culture to 1 to 2 days for PCR. On the basis of these facts, the PCR shows considerable promise for the rapid and specific diagnosis of \textit{U. urealyticum} infection, provided that there is sufficient demand to justify the cost of a thermal cycler and the requirements for more costly reagents than are needed for culture.

The detection of \textit{U. urealyticum} in 24% of the specimens tested leaves its clinical significance open to question. It is known that other organisms can cause the clinical disorders studied and also that not all serotypes of \textit{U. urealyticum} are pathogenic. The PCR primers used for this study amplify a DNA sequence from all \textit{Ureaplasma} strains that have been tested (10) but do not distinguish between biovars or serotypes. Investigations using PCR primers that distinguish between biovars (6) should provide further information about the pathogenicity of \textit{U. urealyticum}.
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REFERENCES


