

# COMPARISON OF CULTURE WITH POLYMERASE CHAIN REACTION FOR DETECTION OF *UREAPLASMA UREALYTICUM* IN ENDOCERVICAL SPECIMENS

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## ABSTRACT

**Background:** *Ureaplasma urealyticum* is known as a sexually transmitted agent, causing mainly urethritis, pelvic inflammatory disease, spontaneous abortion, pyelonephritis, infertility, stillbirth, low birth weight, neonatal meningitis, and neonatal pneumonia. *U. urealyticum* infections not only jeopardize fertility but also pose a risk for infertility treatment and resulting pregnancies. Diagnosis of *U. urealyticum* infections by bacterial conventional methods is very difficult. The aim of this study was to compare culture with polymerase chain reaction (PCR) to determine the prevalence of *U. urealyticum* in endocervical specimens from infertile women.

**Methods:** 312 endocervical swab samples were taken from infertile women, and transported with mycoplasma transport media. The culture was done with liquid-solid methods. DNA was extracted by Cadieux method, and analyzed by PCR protocol with species-specific U4&U5 primers.

**Results:** *U. urealyticum* was detected in 26.2% (82/312) of specimens by both culture and PCR methods. 12.5% (39/312) of samples were PCR positive as well as culture positive, 11.2% (35/312) were positive only by PCR, and 2.5% (8/312) were positive only by culture.

**Conclusion:** A sensitivity of 90% and 57% was found for PCR and culture respectively. PCR is therefore sensitive and more rapid (<24 hour) than culture (2-5 days) for the detection of *U. urealyticum* in endocervical secretions.

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**Keywords:** *Ureaplasma urealyticum*, infertility, PCR, mycoplasma, genitourinary tract diseases.

## INTRODUCTION

The genus *Ureaplasma*, a genus within the family *Mycoplasmataceae*, class *Mollicutes*, has been defined

by its ability to hydrolyze urea.<sup>1</sup> Subdivisions within this genus have been based largely on the host species and antigenic heterogeneity. *Ureaplasma urealyticum* is the designation for all ureaplasmas isolated from humans.<sup>2,3</sup> *U. urealyticum* is an important genital mycoplasma and is found in the cervix or vagina of 40-80% of sexually mature asymptomatic women.<sup>4</sup> The presence of *U. urealyticum* in a large proportion of healthy women complicates the assessment of the pathogenic roles of this

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organism, but several studies have indicated that genital colonization of the *U. urealyticum* can be associated with an increased risk of developing certain pathogenic conditions and pregnancy abnormalities, e.g., pelvic inflammatory disease, premature rupture of membranes, chorioamnionitis, and preterm labor and birth. In addition, it may be acquired by neonates either in utero or by vertical transmission at birth and can cause pneumonia, pulmonary hypertension, chronic lung disease, and meningitis of the newborn.<sup>5,10</sup>

During the past decade, evidence has accumulated for the causative role of *U. urealyticum* in human infertility. *U. urealyticum* was detected at a higher frequency in infertile women.<sup>11,13</sup> Colonization of the upper female genital tract with *U. urealyticum* was found to be associated with adverse pregnancy outcomes.<sup>14</sup> In human in vitro fertilization systems (IVF), the presence of *U. urealyticum* in either semen or the female genital tract resulted in a decrease in pregnancy rate per embryo transfer.<sup>15</sup>

The main method of detecting *U. urealyticum* is by culture, but the organism is difficult to isolate and requires special culture media. Polymerase chain reaction (PCR) is revolutionizing the diagnosis of many infectious diseases, particularly those caused by organisms that are difficult to cultivate.<sup>16</sup> However, there are only a few reports of the detection of *U. urealyticum* by PCR. In this study we compared culture with PCR for detection of *U. urealyticum* in endocervical samples from infertile women.

### MATERIAL AND METHODS

#### Specimens

Duplicate endocervical swab samples were taken from a total of 312 patients, and one was placed immediately in transport medium and the other was placed in a sterilized container with 2 mL of PBS for subsequent PCRs.

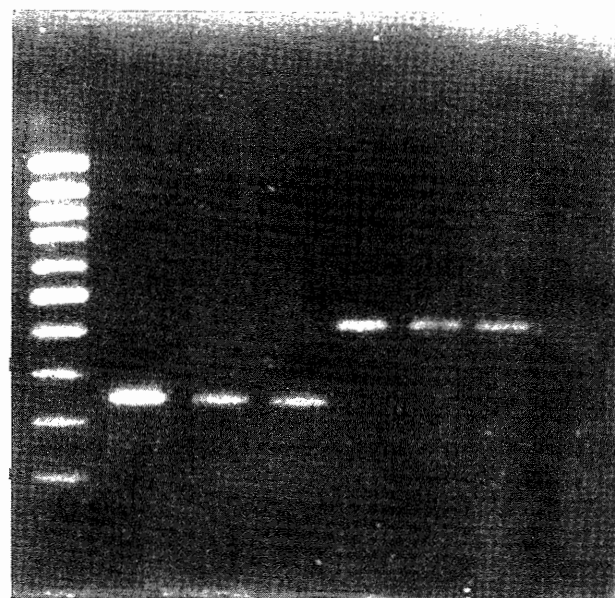
#### Cultivation of *U. urealyticum*

Samples inoculated in liquid medium (0.6% beef heart, 1% peptone, 0.5% sodium chloride supplemented with 20% horse serum, 10% fresh yeast extract, 0.05% HCl-cysteine, 1% urea, 20000U of penicillin G per mL, and 0.125% phenol red, adjusted to pH 6.0), were incubated at 37°C. As soon as the pH of the medium changed, the cultures were centrifuged and the residues were transferred to plates of solid medium, and incubated at 37°C in an atmosphere of 5% CO<sub>2</sub>.

#### PCR

Mycoplasmas have been detected previously with genus-specific primers, followed by amplification of posi-

tive samples with species-specific primers for *U. urealyticum*. DNA was extracted by Cadieux method,<sup>17</sup> and mycoplasma-specific 16S rRNA fragments amplified by use of the published<sup>18</sup> mycoplasma genus specific primers GSO (5-GGGAGCAAACAGGATTAGATACCCCT-3) and MGSO(5-TGCACCATCTGTCACCTCTGTT AACCTC-3). The PCR assay was performed in 50µL of reaction mixture containing 10µL of 10× PCR buffer, 2.5 mM MgCl<sub>2</sub>, 200µM dNTP, 1.25units of Taq polymerase, 20pmol of each primer and 7µL of sample DNA. The reaction mixtures were placed in a thermalcycler (Ependorf, USA). The thermal profile involved an initial denaturation step at 94°C for 3min followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 64°C for 1min, and primer elongation at 72°C for 1 min. The cycling was followed by a final extension step at 72°C for 10 min. The primers U5 (5-CAATCTGCTCGTGAAGTATTAC-3) and U4 (5-ACGACGTCCATAAGC AACT-3) published by Blanchard et al.<sup>19</sup> were used for identification of *U. urealyticum*. The samples were placed in the same thermal cycles and heated to 94°C for 3 min. The cycling profile consisted of 30 cycles of 94°C (denaturation), 52°C (annealing), and 72°C (elongation) for 1 min. The cycling was followed by a final extension step at 72°C for 10 min. Aliquots of amplified samples (10µL) were analyzed by electrophoresis on 1% agarose gel stained with ethidium bromide.



**Fig. 1.** Electrophoretic analysis of PCR products for *Mycoplasma* genus and *U. urealyticum* from endocervical samples; Lane 1, 100bp size marker; lane 2, standard strain (270bp); lane 3&4, patients positive samples for *Mycoplasma* genus; lane 5, standard strain (429bp); lane 6&7, patients' positive samples for *U. urealyticum*, lane 8 negative control (distilled water).

**Table I.** Comparison of endocervical samples culture and PCR.

Culture for <i>U. urealyticum</i>	PCR for <i>U. urealyticum</i>		PCR for <i>Mycoplasma</i> -genus	
	Positive	Negative	Positive	Negative
Positive (n=47)	39	8	46	1
Negative (n=265)	35	230	74	191
Total (n=312)	74	238	120	192

**Table II.** Detection of *U. urealyticum* from patients according to age.

Age (years)	17-27	28-37	38-47	Total
No. of specimens	143	148	21	312
No. positive	43(30%)	33(22.2%)	6(28.5%)	82(26.2%)

**Statistical analysis**

Chi-square ( $\chi^2$ ) test was used for the generation of  $p < 0.05$  values.

**RESULTS****PCR results**

The DNA from each sample was subjected to two PCRs. The first PCR with primers GSO and GMSO, were based on genus-specific 16S rRNA gene sequences. The genus-specific primers reacted with all mycoplasmal species investigated as well as with members of the genera *Ureaplasma*, *Spiroplasma*, and *Acholeplasma*.<sup>20</sup> Of the 312 patients studied, 120 (38.4%) were positive with genus-specific primers. Of these 120 patients, 74 (23.7% of total samples) were PCR positive with species-specific primers for *U. urealyticum* (second PCR).

A photograph of electrophoresis based on bromide-stained agarose gel for PCR-amplified products from the *Mycoplasma* and *Ureaplasma* strains is presented in Fig. 1. DNA from the 16S rRNA sequences, which is amplified by the PCR primers, used in this study shows at 270bp.<sup>20</sup> A 429bp fragment of the urease gene was amplified for identification of *U. urealyticum*. They have been shown previously to be highly specific for *U. urealyticum* and under optimal conditions, to allow detection of  $< 10$ CFU of each serotype of the organism.<sup>21</sup>

***U. urealyticum* isolation from clinical samples and comparison with PCR results**

The prevalence of positive endocervical culture results was 15% (47/312). *U. urealyticum* was detected by PCR assay in 23.7% (74/312). Table I compares the culture and PCR results. Among the 74 patients with a posi-

tive PCR for *U. urealyticum*, endocervical sample culture was negative in 47.2% (35/74). The sensitivity of PCR for *U. urealyticum* to detect a positive *U. urealyticum* culture was 82.9% (39/47), and that of culture to detect a positive PCR assay for *U. urealyticum* was 52.7% (39/74). The sensitivity of PCR for *Mycoplasma* genus to detect a positive *U. urealyticum* culture was 97.8% (46/47).

In total (results of both culture and PCR), *U. urealyticum* was detected in endocervical samples of 82 (26.2%) of 312 infertile women.

The age of the patients who were PCR positive varied from 17-45 years. Distribution of the genital *U. urealyticum* in accordance to patient's age is presented in Table II. No significant difference was found between the age of patients whose sample was PCR positive (positive group) and that of the other patients (negative group). There was also no difference regarding the duration of infertility, vaginal discharge, cervicitis, and abortion, between the positive and the negative group.

**DISCUSSION**

The results indicate that the use of a PCR assay for *U. urealyticum* in endocervical samples results in a higher rate of detection of this microorganism than that observed with standard microbiologic cultures for genital mycoplasmas. 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<sup>16</sup> to detect only 80% of samples infected with *U. urealyticum*. If in our study the 82 positive specimens detected by both culture and PCR represented all infections, then the 47 positive specimens detected by cul-

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ture represent only 57% of the infected samples, whereas the PCR positive samples represent 90% of them. Eight patients in our study had a positive culture for *U. urealyticum* but a negative PCR assay result. Similar observations have been reported by other investigators<sup>25,26</sup> and have been attributed to degradation of bacterial DNA or inhibitor(s) of the PCR reaction in clinical samples. For example, blood is a poor biologic specimen for PCR-based methods, because hemoglobin can inhibit the PCR reaction. Blood contamination of endocervical specimens is therefore a potential source of false-negative results. It is noteworthy that despite these potential problems the overall sensitivity of PCR for *U. urealyticum* in the detection of microbiologically proven infection of the endocervix by *U. urealyticum* was 82% (39/47). In addition to its greater sensitivity and lesser dependence on careful specimen handling between collections and testing, PCR had a further advantage of faster determination. Assay time was reduced from 2 to 5 days for culture to <24 hours for PCR.

In this study, 26% of 312 infertile women were colonized with *U. urealyticum* as detected by culture and/or PCR. Other studies using PCR for *U. urealyticum* in endocervical specimens have reported a prevalence rate as high as 40 to 80%. Since *U. urealyticum* has been found to be significantly associated with low socioeconomic background, such as poverty, number of sexual partners, and use of contraceptive drugs<sup>27,31</sup> it is not surprising that the rate of *U. urealyticum* was lower in our study. Although differences were not statistically significant, but the isolation rate of *U. urealyticum* was higher in women under 30 years of age, that is consistent with that previously described by others.<sup>27,28,30</sup>

The use of PCR methodology is increasing in clinical microbiology laboratories, being useful for agents that are costly, slow, and/or difficult to cultivate. *U. urealyticum* is a good candidate for this technology for all of the above reasons. This study, like others<sup>4,16,19,26</sup> has demonstrated that PCR is at least as sensitive as culture. On the basis of these facts, a case may be made for replacing culture with a PCR-based test.

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