Original Article

Diagnosis of *Trichomonas vaginalis* Infection by PCR

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Abstract
Objective: To compare the sensitivity of PCR, wet preparation and culture in detecting *Trichomonas vaginalis* in urine and vaginal fluid.
Methods: A PCR targeting the beta-tubulin genes of *T. vaginalis* was used for the detection of the organism in both vaginal swab and urine specimens from infected patients. Random urine samples were collected from 30 patients (23 females and 7 males), and tested for *T. vaginalis* by wet preparation and the Inpouch *T. vaginalis* culture systeme. Two vaginal swabs were collected by each woman. PCR detection. was carried out on samples negative by first methods.
Results: The positive result was found in 28.57% in male urine and 39.13% in female urine samples, 65.21% in 1st swab and 78.26 % in 2nd swab by wet preparation. By culture, the male urine samples showed 42.85% positive, female urine 69.56% while 1st swab showed 86.95% positive and 2nd swab 91.30% positive. All negative cases by culture in urine and vaginal samples were tested by PCR, which showed 2 cases to be positive in male urine samples and 5 cases positive in female urine sample.
Conclusion: PCR assay was as good as or more sensitive than wet preparation and culture and resulted in practical advantage of providing results in shorter time. However, PCR test is still very expensive. (Rawal Med J 2007;32:36-38)

Key Words: Trichomonas, vaginitis, STD, PCR.

INTRODUCTION

Trichomonas vaginalis is a sexually transmitted infection that can result in vaginitis, urethritis, and preterm birth. It has been associated with nongonococcal urethritis in men. Many individuals are asymptomatic. Traditional methods of diagnosis, including wet preparation, can be unreliable because of poor sensitivity. Developing a urine assay would confer the advantages of easy procurement, transport, and storage of patient samples.[1] Other diagnostic techniques, such as fluorescent antibody,[2] enzyme-linked immunosorbent assay[3] and a hybridization test[4] have been used to detect *T. vaginalis*. The targeted genes encode the amino acid sequence of beta-tubulin protein, a major component of the *T. vaginalis* cytoskeleton. The method of PCR allows the selective amplification from DNA, a particular fragment is selected from a complex genome by enzymatic amplification in vitro. In this study, a PCR targeting the beta-tubulin genes of *T. vaginalis* were developed for the detection of the organism in both vaginal swab and urine specimens from those patients who were negative in culture.

MATERIALS AND METHODS

Random urine samples were collected from 30 patient (23 females and 7 males), tested positive for *T. vaginalis* by wet preparation and the Inpouch *T. vaginalis* culture system (Biomed Diagnostic, san Jose, Calif.). Of the concentrated culture, a 25-µl aliquot was diluted with Evans blue, and *T. vaginalis* organisms were counted in a hemocytometer to determine the organism concentration (number per milliliter).[5] Two vaginal swabs were collected from each woman, 1st, before insertion of the speculum, were placed in 1 ml of a commercial PCR transport medium (Amplicor; Roche Diagnostic systems, Branchburg, NJ) and kept at 4C. until arrival at the laboratory within 4 days of collection. A 2nd vaginal swab sample was obtained after the insertion of the speculum, it was immediately touched to glass slide together with a drop of normal saline for microscopic (X100) wet examination for *Trichomonas* in vaginal fluid. After the wet preparation was made, the swab was immediately inoculated into the Inpouch *T. vaginalis* culture system.[6] First swab was taken to detect superficial and second to detect cervical parasite.

PCR was per formed with 10µl of cultures processed by the Chelex method using a set of primers targeting a conserved region of the beta-tubulin genes of *T. vaginalis* based on FRET (Fluorescence Resonance Energy Transfer) probe chemistry.[7] PCRs were performed in an automated thermocycler (Perkin-Elmer Cetus, Norwalk, Conn.). Negative controls included uninoculated transport media, throughout the specimen preparation and PCR process. A low copy number of *Trichomonas* as positive control was included in every PCR run.

RESULTS

Urine samples in male showed positive in 28.57%, female urine samples positive in 39.13%. 1st swab of vaginal samples positive in 65.21% while 2nd swab was positive in 78.26% in wet preperation. The culture of male urine samples was positive in 42.85%, female urine samples positive in 69.56%. Also, 1st swab of vaginal sample positive in 86.95% and 2nd swab of vaginal sample was positive in 91.30% (table 1). All negative cases by culture in urine and vaginal samples were tested by PCR which resulted as 2 cases positive in male urine samples and 5 cases were positive in female urine samples.

Table 1. Results for vaginal and urine samples tested for *T. vaginalis* with wet preparation, culture and PCR.

No. Examination		Wet preparation		Culture		PCR	
Urine Samples		positive	%	positive	%	No. examination *	positive
Males	7	2	28 .6	3	42.9	4	2
Females	23	9	39.1	16	69.6	7	5
Total	30	11	36.7	19	63.3	11	7
Vaginal samples							
1st swab	23	15	65.2	20	86.9	3	1
2nd swab	23	18	78.3	21	91.3	2	2

* Samples negative by culture

DISCUSSION

Trichomonas PCR with primer set BTUB 9/2 was 100% sensitive and specific. It was more sensitive than wet preparation or culture. A recent study utilizing TETR PCR for analysis of male urine specimens found that the sensitivity of TETR PCR was superior to that of culture.[8] A real-time PCR assay tested vaginal samples, at 40 cycles, with Taq Man PE thermocycler-based system and took about 2.5 h to complete.[9] By using lightCycler technology, runs of the BTUB FRET PCR assay were completed in under 30 min. at 50 cycles. A sensitivity and specificity of the BTUB FRET PCR in urine was reported as 97.8% and 97.4% respectively.[5]

Culture may be the gold standard, but it is inherently limited because it relies on the organism to be viable for proper detection. Additionally, culture results can be subject to interpretation by the viewer, whereas PCR offers a more definitive result. Traditional gel-based PCR assays have been found to be less accurate with urine samples than with vaginal swabs.[10] Number of confirmed *T. vaginalis* positive samples has been reported to be 39% by PCR with compared to culture 9.1%.[7]

Primer set BTUB9/2 was designed to target a well-conserved region in all three bet-tubulin genes, thus improving sensitivity because of increased number of DNA target copies available for amplification.[11] Although wet preparation had minimal cost, its sensitivity is highly dependent on the expertise of the microscopist, prompt transport and laboratory processing before the organism lyses or loses motility.[12] Even when the wet preparation was performed at the collection site, its sensitivity of 36% was suboptimal compared to PCR.[7] Culture had a better sensitivity (70%) in their study than wet mount examination but required more time for laboratory turnaround since cultures are held for 1 week.[7] PCR results are available in 2 to 3 days and provid the highest sensitivity. The cost of PCR testing comes mainly from the cost of reagents. Although *Trichomanas* PCR requires more technical skill, molecular amplification techniques are currently in use in many laboratories for the detection of *C. trachomatis* and *N. gonorrhoeae* infections. Thus, Trichomonas PCR could easily by incorporated into the work flow of other diagnostic amplification procedures.[7] In conclusion, the performance of the PCR assay was as good as or more sensitive than wet preparation and culture and afforded the practical advantage of providing results in a more timely fashion. However, PCR remains an expensive test.

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