

Assessing feasibility of urine samples for diagnosis of trichomonas vaginitis by both wet mount and nested PCR

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ABSTRACT

Background - Aim

Unlike the conventional methods for diagnosis of *Trichomonas vaginalis* infection in women, based on microscopic examination of vaginal smears as direct wet mount or after culture, the aim of the present study was to assess the feasibility of urine samples for diagnosis of *Trichomonas vaginitis*, using wet mount microscopy and nested PCR.

Material- Methods

Wet mount microscopy and nested PCR, using 4 primers chosen to target the Tv-E650 gene were performed on urine samples that were collected from 21 women of child-bearing age, in whom trichomoniasis was demonstrated by culture of vaginal swabs on modified Diamond's medium.

Results

Wet mount examination of urine samples revealed *T. vaginalis* in only 7 cases (33.3%). Nested PCR resulted in DNA amplification in urine samples of the same 7 cases and one additional case (8 out of 21 cases, 38.1%). By performing inhibition assays for the remaining 13 PCR-negative urine samples, the presence of inhibitors to amplification was suspected in 6 samples, out of which 4 samples converted to PCR-positive on repeating the PCR run after eliminating these inhibitors by using a threefold increase in Taq. Accordingly, the sensitivity of PCR in the present study has risen up to 57.14%. A statistically significant difference between nested PCR and wet mount diagnosis was only achieved after inhibitor elimination.

Conclusions

The limited diagnostic performance of both wet mount and nested PCR for urine samples in the present study denote that urine samples, although providing convenience in sampling and transport are hardly feasible for diagnosis of trichomoniasis in women. Furthermore, whenever doing PCR for urine samples, the possibility of the presence of inhibitors of DNA amplification should be considered (in this study as high as 28.57% of cases) in order not to miss diagnosis on this grounds.

Keywords: Polymerase Chain Reaction, trichomoniasis, trichomonas vaginitis, urine samples

INTRODUCTION

Trichomonas vaginalis is a parasitic protozoan that causes trichomoniasis, a sexually transmitted disease (STD) of worldwide importance.¹ The conventional methods for diagnosis involve the direct microscopic examination of wet-mount or culture-based systems for vaginal smears.² On the other hand, the Food and Drug Administration (FDA), approved nucleic acid amplification tests as a substitute of culture for *T. vaginalis*.³ Developing an assay for urine would be desirable because such a method would confer the advantages of easy collection, transport and storage of samples. The aim in this study was to assess the feasibility of urine samples in the diagnosis of trichomoniasis in women by microscopic examination of wet mount and PCR.

MATERIAL AND METHODS

The study group included 21 women at child-bearing age, attending the Maternity and Children's Hospital, Al-Madina, Saudi Arabia. *Trichomonas vaginitis* was demonstrated by culture of vaginal swabs on modified Diamond's medium.⁴ First-voided urine was collected in urine containers. Samples were centrifuged at 37°C for 5 min at 2,000 rpm. For DNA isolation, the resulting sediment was washed once with 800 µl of PBS and was directly immersed in lysis buffer and processed as described below. Wet-mount preparations for the centrifuged urine samples were examined microscopically, using low (x100) and high power (x400) magnification.

DNA extraction:

DNA extraction was done using the Proteinase K-Phenol/Chloroform method according to Rawal et al. (1994).⁵ Here, the urine samples were washed, followed by high speed centrifugation at 12,000 rpm, after which the DNA could be extracted from the resulting pellet, using proteinase K treated nuclei lysis buffer and a series of steps involving repeated vortexing and centrifugation and resulting finally in a dried pellet. This was dissolved in 20 µl Tri-EDTA (TE) and/or kept at -20°C until PCR amplification would be done.⁶ All chemicals were purchased from Sigma pharmaceuticals.

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