Indian J Med Res 125, April 2007, pp 567-571

InPouch TV™ culture for detection of *Trichomonas vaginalis*

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Received March 29, 2006

***Background & objectives*: *Trichomonas vaginalis* accounts for almost half of all curable sexually transmitted infections and has also been associated with adverse outcomes of pregnancy and increased risk of HIV in women. Diagnosis of the condition by direct wet mount examination has a low sensitivity. Herein, we describe our experience with InPouch culture system for the detection of *T. vaginalis*.**

***Methods*: This prospective study was carried out from May 2003 to April 2004 among women presenting with genitourinary symptoms attending a primary health center clinic in Ballabhgarh, India. Two vaginal swabs (cotton tips) were obtained from each woman. The first swab was obtained from the lateral wall of vagina and was used to make a wet mount preparation. The second swab was obtained from the posterior fornix of the vagina and inoculated in the InPouch for culture of *T. vaginalis*.**

***Results*: Of the 601 women, 22 were positive by direct microscopy for *T. vaginalis* while 40 were positive by culture. Overall, *T. vaginalis* accounted for 6.7 per cent of reproductive tract infections.**

***Interpretation & conclusion*: The InPouch TV culture system is a simple, cost-effective and a sensitive method for diagnosing *T. vaginalis* and may be recommended for routine use in diagnosing genital tract infections.**

Key words InPouch culture - reproductive tract infections - sexually transmitted infections - *Trichomonas vaginalis*

Trichomonas vaginalis, a sexually transmitted flagellated protozoan, afflicts an estimated 180 million women per year worldwide[1]. The World Health Organization (WHO) has estimated that this infection accounts for almost half of all curable sexually transmitted infections[2]. Clinical syndrome in females vary from asymptomatic presentations to (more commonly) vaginitis with copious discharge. Infection can be associated with serious sequelae such as preterm labour, premature rupture of membranes and low birth weight as well as increased risk of transmission of other sexually transmitted diseases (STDs), including human immunodeficiency virus (HIV)[3,4].

Associations of trichomoniasis in women with adverse outcomes of pregnancy and increased risk of HIV suggest a need for increased control efforts, and accurate diagnosis is necessary for specific treatment and control of this disease. Diagnosis of trichomoniasis in women is usually accomplished via direct microscopic examination of the vaginal fluid by wet mount preparation; however, the sensitivity of this test is low (overall 60%) and may be lower in asymptomatic women. Culture is clearly the most sensitive diagnostic method and various culture media have been described for cultivation of *T. vaginalis.* Though polymerase chain reaction (PCR) techniques have been found more sensitive than culture, different studies have shown its specificity to be slightly lower than culture[5,6].

In this study, we describe our experience with the InPouch culture system (Biomed Diagnostics, San Jose, California) for the detection of *T. vaginalis*. This is a commercially available system which combines a wet preparation and a culture method to detect *T. vaginalis*.

Material & Methods

We carried out a prospective study from May 2003 to April 2004 among women presenting with genitourinary symptoms attending a primary health center clinic in Ballabhgarh, Haryana, in India. An attempt was made to detect *T. vaginalis* infection in symptomatic women. Informed consent was obtained from each participant woman.

The inclusion criteria for the study were, married and sexually active women between 18 to 49 yr of age with self-reported symptoms of vaginal discharge and/or genital itching and/or genital burning. Pregnant women, women with severe medical disorders requiring immediate referral to higher level of health care, women who were currently menstruating, never been sexually active, who had a hysterectomy, had taken a course of antibiotics within last three weeks and who had been previously enrolled in this study were excluded. This study was a part of a large collaboration study with USA.

Two vaginal swabs (cotton tips) were obtained from each woman. The purpose was to get as much specimen as possible. The first swab was obtained from the lateral wall of vagina and was used to make a wet mount preparation on a glass slide with a drop of normal saline on the site of specimen collection. The slide was initially scanned at 100X, looking for motile trichomonads, and then at 400X to confirm motility, flagellar movement and morphologic features of the organisms. Negative wet mounts were examined for at least two minutes.

The second swab was obtained from the posterior fornix of the vagina and inoculated in the top pouch of InPouch (Biomed Diagnostics, USA) for culture of *T. vaginalis*. InPouch TV is a double pouched container made of soft plastic (Fig.). The specimen was introduced into the bottom pouch immediately after collection; microscopic examination of the bottom pouch was conducted when the specimen arrived in the laboratory (no examination was made of the top pouch because its contents had been immediately pushed into the bottom pouch).

The cultures were incubated at 35°C and examined for motile *T. vaginalis* at 24, 48 and 96 h

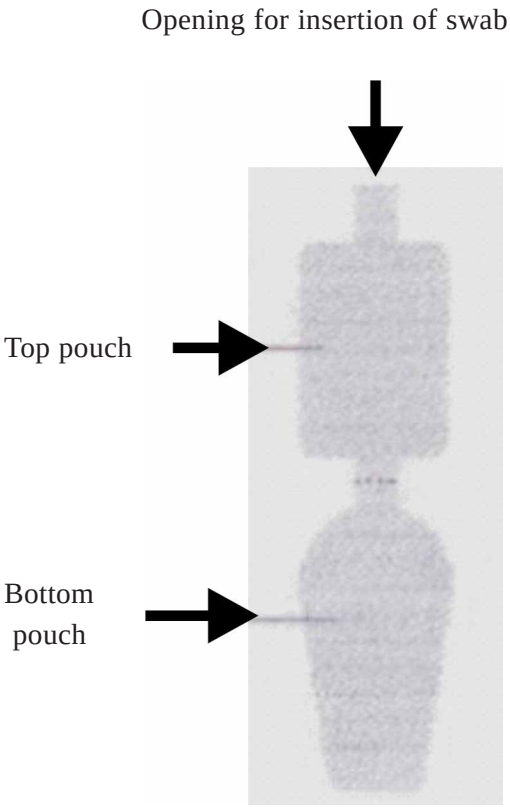

Fig. Diagram of InPouch TV.

of incubation by using a 10X objective directly through the pouch. Internal quality control for *T. vaginalis* InPouch culture was made by incubating one InPouch per batch as a sterility check on reagents and inoculating one pouch with a known culture of *T. vaginalis* to check the quality of the batch of InPouch.

Statistical analysis was done using the Mc-Nemar Chi Square test.

Results & Discussion

A total of 710 women were screened as they were willing to participate in the study, but only 611 were eligible for enrolment. However, data could be collected and analyzed from 601 women as the rest declined internal examination. Of the 601 women, 22 were positive by direct microscopy for *T. vaginalis* while 40 were positive by culture which was found to be statistically significant ($P < 0.001$). All were culture positive within 48 h of incubation. Overall, *T. vaginalis* accounted for 6.7 per cent (40/601) of reproductive tract infections.

The time honoured approach for the diagnosis of trichomonal infection has been microscopic evaluation by the wet mount method, a procedure first described by Donne' in 1836[7]. This procedure, however, detects only 35 to 80 per cent of the cases, depending on the expertise of the microscopist[8,9]. It is already established that a minimal concentration of 10^4 organisms per milliliter of vaginal fluid appears to be necessary for identification of the protozoa by wet mount[8]. The relatively low sensitivity of wet mount examination has been confirmed in this study too.

Because of the limitations of the wet mount, culture remains the most accurate single method for detecting the presence of *T. vaginalis* in patient samples[8-10]. Routinely, 95 per cent of cases are diagnosed by this method. Optimal growth and reproduction of *T. vaginalis* require anaerobic conditions and an unusually large number of essential nutrients, including carbohydrates, amino acids, purines, pyrimidines, fatty acids, vitamins and iron[11]. Although several commercial liquid media are available for this purpose, Diamond's medium is considered the "gold standard". The InPouch TV culture system, the one we have used has been found to be as reliable as Diamond's medium in detecting *T. vaginalis*[12].

We observed that the InPouch TV culture detected 45 per cent more positives than the traditional wet mount. The sensitivity of wet mount preparation in our study was 55 per cent and falls very much within the range documented so far. The specificity of the wet mount preparation was 100 per cent. The presence of trichomonads was determined by its characteristic size, shape and mobility in both tests, the specificity was therefore considered the same for both the wet mount and the InPouch TV method.

Overall, *T. vaginalis* accounted for 6.7 per cent of reproductive tract infections in our study. Worldwide, researchers have reported a prevalence of 1.3 to 16.5 per cent[13,14] of *T. vaginalis* in reproductive tract infections. The prevalence of *T. vaginalis* in India ranges from 0.8 to 14.0 per cent but most studies have relied on the wet preparation alone and there is no "gold standard" confirmation[15-17]. Although the combination of culture and wet mount examination remains the standard approach for detecting *T. vaginalis* in patient samples[12], InPouch offers some distinct advantages. Once the specimen is placed by a clinician into the InPouch chamber, microscopic observation can be made directly through the bag as the bag can be used as a slide on the stage of the microscope. This obviates the need for sampling to examine the culture for growth thereby preventing contamination. These can be conveniently transported from the site of collection to the laboratory and can be stored at room temperature. Other media, once prepared, require refrigeration. Further, its cost is comparable to the ordinary culture tube. Therefore, the InPouch culture system may be used as a routine method of diagnosing trichomoniasis.

Acknowledgment

Authors acknowledge the Global Network for Perinatal and Reproductive Health (GNPRH), USA, for providing the pouches, and cultures were done as a part of a study funded by Population Council, New Delhi, India.

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