

Detection of *Trichomonas vaginalis* in Pregnant Women with the InPouch TV Culture System

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***Trichomonas vaginalis* causes a common genitourinary infection which is frequently asymptomatic. At present, pregnant women are not usually screened for the infection unless they are symptomatic. In the present study, we screened and obtained samples for culture from all pregnant women attending a prenatal clinic with the InPouch TV culture system and compared results with those of standard culture in Diamond's medium and slide wet mount examination. The InPouch TV culture system was as reliable as Diamond's medium in detecting *T. vaginalis* and may be useful and effective in a pregnancy clinic setting.**

Trichomoniasis is one of the most common sexually transmitted diseases, with an estimated 7 million cases annually in the United States and 180 million cases annually worldwide (2). In the United States, it is not a reported sexually transmitted disease and the true volume of cases may be underestimated. In a recent study on vaginitis during pregnancy sponsored by the National Institutes of Health, an estimated prevalence rate of trichomoniasis was 12.5% in 12,281 pregnant women (3, 4). Those studies demonstrated the independent association between *Trichomonas vaginalis* infection and preterm birth. *T. vaginalis* infection emerged as a significant risk factor for premature rupture of membranes. In light of this newly emphasized association and the frequency of asymptomatic infections, the importance of culturing for and successfully detecting *T. vaginalis* in pregnant women becomes significant. However, the current medical practice is to screen pregnant women only if they have vaginal complaints and are symptomatic. The current technique is to screen vaginal fluid by the microscopic wet mount method. The method detects only 35 to 80% of cases, depending on the expertise of the microscopist (8). Thus, culture with wet mount is the standard method for detecting the presence of trichomoniasis in patient samples (6, 8, 12). One disadvantage is that culturing is not readily available and it requires daily sampling and microscopy for the detection of growth (5, 7). This is a personnel cost consideration for many smaller laboratories.

In the present study, the usefulness of a new culturing system, InPouch TV, was compared with the standard culture technique with Diamond's medium. The study was performed in an urban clinic with a pregnant population of patients. The InPouch TV system allows direct, rapid microscopic examination of the culture pouch without daily sampling and is more time efficient (1). Findings revealed that this system is as sensitive as the standard culture technique.

Pregnant women attending the Denver General Hospital antenatal clinic from September 1991 to January 1992 were screened for *T. vaginalis* infection by microscopic wet mount examination of vaginal fluid, and samples for culture

were obtained for examination by the InPouch TV culture system (BioMed Diagnostics, Santa Clara, Calif.) and with modified Diamond's medium (Remel Media; Remel, Lenexa, Kans.). Three vaginal fluid samples were randomly obtained from the posterior fornix by using cotton swabs. One swab was prepared for wet mount examination, and the other swabs were used to inoculate the InPouch TV bag (Fig. 1) and Diamond's medium.

The InPouch TV culture bag was inoculated by swirling the swab sample in the upper chamber and forcing the inoculum into the lower chamber. The bags were sealed with the metal tabs and were transported to the laboratory within a few hours. All bags were held at 35°C if there was a delay in transport.

The presence of *T. vaginalis* was diagnosed by appropriate morphology and motility of the protozoa at the time of microscopic examination of the vaginal fluid or culture medium. Ten microliters of Diamond's medium cultures was examined microscopically with a hemacytometer. To examine the InPouch TV bag, a plastic microscope slide mount was clipped in place onto the lower chamber and the contents of the bag were read without opening the culture or sampling the contents. Approximately 100 to 200 µl of culture fluid can be examined in this fashion.

A total of 232 pregnant women were screened, and a total of 34 (14.66%) patients were found to have *T. vaginalis* infection. The InPouch TV system detected *T. vaginalis* isolates in 30 of 34 (88.25%) patients and the Diamond's medium detected *T. vaginalis* isolates in 31 of 34 (91.12%) patients. There was no significant difference between the culture methods. The wet mount examinations were able to detect *T. vaginalis* isolates in 29 of 34 (85.3%) patients. This high detection rate was a function of careful microscopy.

There were six discrepancies in the culture results (Table 1). Three strains grew in Diamond's medium but did not grow in the InPouch TV system. Two strains grew in the InPouch TV system but did not grow in Diamond's medium. One strain did not grow in either culture system but was detected by wet mount examination of the vaginal fluid.

The standard method of culturing with Diamond's medium and the required microscopic examination of the culture fluid is considered time-consuming and laborious. Commonly,

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FIG. 1. InPouch TV culture system. An uninoculated bag is on the left and an inoculated bag is on the right. A microscope viewing clip is on the bottom of the right-hand bag. This facilitates microscopy of the sample immediately after inoculation and can serve as a wet mount preparation. The clip is also used to view culture contents daily.

samples for culture are sent to centralized laboratories for processing, and frequently, results are not available for a week or more. In some large clinic settings where the prevalence rate of trichomoniasis is low, culturing of samples from all patients would not be considered cost-effective. Thus, an improved method of culturing which is sensitive, cost-effective, and efficient would be useful (8).

Recently, the commercial availability of enzyme-linked immunosorbent assays and fluorescent-antibody diagnostic kits for trichomoniasis has ended. The wet mount examination, while very rapid and popular, has a reported sensitivity of only 35 to 80%, depending on the skill of the observer (8). However, when coupled with culture, a significantly higher detection rate of disease is attained. Thus, the standard for diagnosis of *T. vaginalis* infection remains culture and wet mount examination.

In the present study, we demonstrated that the InPouch TV system performed as well as the Diamond's medium culture technique. In another report of the InPouch TV system, the pouch was found to be superior to Hollander's culture in a venereal disease clinic population (1). In that study, there was a positive rate of 14.7% with the InPouch

TABLE 1. Culture results and discrepancies by method^a

Wet mount examination	Diamond's culture	InPouch TV culture	No.
+	+	+	27
+	+	-	1
+	-	+	0
+	-	-	1
-	+	+	1
-	+	-	2
-	-	+	2
-	-	-	198

^a +, motile trichomonads; -, no trichomonads seen.

TV system compared with a positive rate of 11.7% with Hollander's medium. In that study, the InPouch TV system was found to be superior to culture in Hollander's medium.

Although the detection rates of each culture system are similar, there are several differences from the InPouch TV system which may be important. One consideration is that microscopy can be performed on the bag itself, which alleviates the need to enter the broth culture. This decreases contamination problems and speeds up the examination time. Another consideration is that 200 µl of culture is screened with the clip-on microscopy mount, whereas only 10 µl from the Diamond's medium culture can be examined at a time with a hemacytometer. This suggests that a positive culture would be found sooner with lower organism counts with the InPouch TV system. The pouch system also maintains viability of the organisms longer than Diamond's medium does (1, 5a).

The discrepancies between the InPouch TV system and Diamond's medium in the recovery of isolates are interesting. It suggests that there is some strain variability. The facts that the InPouch TV system recovered some isolates which Diamond's medium did not and that there was one isolate which neither culture system detected suggest that isolates may have nutritional variabilities.

The presence of *T. vaginalis* infection in women frequently can be overlooked. In women, as many as 15 to 50% of infections may be asymptomatic (11). The range of presenting symptoms may be from no evidence of clinical disease to florid vaginitis with a profuse smelly discharge. However, it is estimated that 30% of these asymptomatic infections become symptomatic within 3 to 6 months. This suggests that a significant number of women would go undiagnosed and untreated if only symptomatic patients were screened and their samples cultured.

Infections in pregnant women are particularly troublesome in that treatment is not recommended in the first trimester because of the possible mutagenic effects of the only available antibiotic for treating *T. vaginalis* infections, metronidazole. Metronidazole has been found to cause single-stranded breaks in human lymphocyte DNA (9), and in one study it was found that there were increased rates of birth anomalies in women treated with metronidazole in the first trimester (10). Although that study was repeated and different results were obtained, there still exists a sense of caution in prescribing the drug. In fact, some physicians are reluctant to treat a pregnant woman unless she is symptomatic and uncomfortable. However, with the emerging information of the link between *T. vaginalis* infection and increased risk of preterm birth, it is becoming increasingly important to diagnose this infection. Thus, we suggest that

all pregnant women be screened by wet mount and a sample be obtained for culture for *T. vaginalis*.

In the present study, we found that the InPouch TV culture system performed as well as Diamond's medium in recovering *T. vaginalis* isolates and may provide a suitable substitution for the culture method.

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