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ORIGINAL ARTICLE

Diagnosis of *Trichomonas vaginalis* in Adolescent Females: InPouch TV® Culture Versus Wet-Mount Microscopy

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Purpose: **This study compared the InPouch TV® culture to wet-mount, Diamond's culture medium, and Papanicolaou (Pap) smear for the diagnosis of trichomonas infection in sexually active adolescents.**

Methods: **A total of 467 subjects were recruited among 12–18-year-old girls who received pelvic examinations at two urban adolescent clinics. All girls were tested by wet-mount and InPouch TV®. In addition 339 of 467 had cultures in Diamond's medium and 366 of 467 had Pap smears. Specimens were collected for InPouch TV® and Diamond's cultures and read at 24–48 h and 5 days, and in the case of Diamond's cultures, also at 7 days. In a subset of subjects (268 of 467) who had all four tests done, sensitivities and specificities were calculated using Diamond's culture as the "gold standard."**

Results: **In the 467 subjects, 73 (15.6%) tested positive for trichomonas by at least one method. In the subset with all four tests done, sensitivities of the wet-mount and InPouch TV® were 36% and 81%, respectively; while that of the Pap smear was 56%. The culture media were equally efficient in identifying *Trichomonas vaginalis*. There were no differences found between subjects with or without trichomonas infections in gynecological symptoms, previous history of sexually transmitted diseases, or use of a condom at last intercourse.**

Conclusions: **InPouch TV® culture is a good diagnostic method for *T. vaginalis* because of its long shelf-life, relatively low expense, and high sensitivity (over twice as sensitive as wet-mount).** © ***Society for Adolescent Medicine, 1998***

KEY WORDS:
Trichomonas vaginalis
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In the United States, 1 of every 4 sexually active adolescents is diagnosed with a sexually transmitted disease (STD) each year (1). Reported rates of *Trichomonas vaginalis* infections in teens vary from 8% to 34% (2,3). This infection may be associated with an asymptomatic carrier state or symptoms ranging from acute vaginitis to perinatal complications such as premature rupture of membranes, preterm birth, and postpartum endometritis (4,5). Inflammatory STDs such as trichomonas increase the risk of human immunodeficiency virus (HIV) infection (6). Currently, the standard method of detection of trichomonas is by wet-mount microscopy of vaginal fluid. The sensitivity of this method is reported to be as low as 45–60% (7,8). Culture methods, the "gold standard" for identifying trichomonas, are not used routinely owing to their cost and labor-intensive nature. However, the InPouch TV® culture system (BioMed Diagnostics, Santa Clara, CA) is reported to be equally sensitive, yet more practical than traditional cultures (9,10). This is a culture medium that is specific to *T. vaginalis* contained in a clear plastic envelope. Once inoculated and incu-

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bated, the presence of trichomonas organisms in the culture medium can be directly determined by placing the envelope on the microscope platform. Use of the self-contained InPouch TV® system has not been reported in an adolescent population. The purpose of this study was to compare the performance of the InPouch TV® culture to wet-mount in the diagnosis of trichomonas infection in sexually active adolescent females. In addition, in a subset of the study population, sensitivities and specificities were compared among InPouch TV®, wet-mount, and Pap smear using Diamond's media culture as the "gold standard."

Methods

From February 1994 to July 1995, 467 subjects were recruited among 12–18-year-old sexually active girls who received pelvic examinations at two urban adolescent clinics in St. Louis and Kansas City, Missouri. The clinics provided primary health care to predominately inner-city youth. Patients could only be enrolled once in the study. Consent at all sites was given by the patients themselves in accordance with Missouri law. This study was approved by the institutional review boards of both sponsoring institutions.

A complete medical history was taken for each patient including sexual history, use of contraception, previous history of STDs, and review of current symptoms suggestive of an STD.

During the speculum examination of the vagina, swabs from the vaginal pool and lateral walls were obtained for wet-mount microscopy and InPouch TV® culture. The saline wet-mounts were prepared by placing a sample of vaginal discharge on the slide with one to two drops of saline and read immediately by the supervising physician for the presence of motile trichomonads. The InPouch TV® culture was incubated for 5 days with readings by laboratory personnel at 24–48 h and 5 days. All 467 subjects had both wet-mount and InPouch TV® cultures obtained.

Other tests that detect trichomonas infection, Diamond's media culture (n = 268) and Pap smears (n = 366), were also carried out on some of the study patients. Cultures of vaginal fluid in Diamond's medium, a traditional culture medium for *T. vaginalis*, were performed on the Kansas City clinic patients. At 24–48 h, 5 days, and 7 days, laboratory personnel examined the media for the presence of motile trichomonas organisms. Some patients also had testing for *Neisseria gonorrhoeae* (n = 451) and *Chlamydia tachomatis* (n = 448).

Table 1. Performance of wet-mount versus InPouch TV® for diagnosis of *Trichomonas vaginalis*

		Wet Mount	
		+	–
InPouch TV®	+	35	28
	–	2	402
Total		37	430

Fisher's exact, one-tail, $p < 0.001$ (n = 467).

A trichomonas infection was diagnosed if any of the four detection methods (wet-mount microscopy, InPouch TV®, Diamond's medium culture, or Pap smear) was positive. Any patient who had a positive test was notified of the infection and treated according to Centers for Disease Control guidelines (10).

Results

A total of 467 girls were entered into the study: 346 from the Kansas City clinic and 121 from the St. Louis clinic. The average age for all subjects was 15.5 years, with an average age at first intercourse of 13.7 years. Nearly half of the girls (46%) had had a previous STD; 15% reported a previous infection with trichomonas. Many (56%) reported using a condom at last intercourse. Condoms with or without spermicide was the most common (43%) contraceptive method cited by the teens, followed by DepoProvera (17%) and oral contraceptive pills (13%). Twenty-one percent were not currently using any method of birth control. Reasons for visiting the clinics included routine care and contraceptive services (60%), complaints suspicious for STDs (32%) and tests of cure (8%). Regardless of the reason for the visit, 48% of girls reported gynecologic symptoms including vaginal discharge, dysuria, vulvar itching, vulvar burning, lesions, or abdominal pain.

Seventy-three or 15.6% of the 467 study patients had trichomonas infection diagnosed by any of the four methods. Table 1 shows the performance of wet-mount versus InPouch TV® culture in these subjects. Compared to wet-mount, the InPouch TV® culture identified 70% more cases of trichomonas infections. Eight patients were found to have trichomonas by Diamond's culture but had negative wet-mount and InPouch TV® culture. There were no cases in which the Pap smear was the only test positive for trichomonas.

In a subset of the total population (268 of 467), sensitivities and specificities of the four methods (wet-mount, InPouch TV®, Diamond's media cul-

Table 2. Comparison of wet-mount, InPouch TV®, and Pap smear to Diamond's media culture

	Wet Mount	InPouch TV®	Pap Smear
Sensitivity	13/36 (36%)	29/36 (81%)	20/36 (56%)
Specificity	230/232 (99.1%)	230/233 (98.7%)	231/232 (99.6%)

n = 268.

ture, and Pap smear) for diagnosing trichomonas were calculated. Table 2 shows that InPouch TV® culture was over twice as sensitive as wet-mount. The culture media were equally efficient in identifying *Trichomonas vaginalis*. However, positive results were seen earlier with the InPouch TV® cultures. Trichomonas was identified within 48 h in 87% of positive InPouch TV® cultures versus 71% of positive Diamond's cultures.

Among subjects with or without trichomonas infections, there were no differences in mean age at the visit, age at first intercourse, number of partners, previous history of STDs including trichomonas, reason for visit, gynecological symptoms, or use of a condom at last intercourse.

In all study subjects, the prevalence for *N. gonorrhoeae* was 6.4%, and for chlamydia was 15%. The rates of these infections were not significantly different between patients with and without trichomonas infections. Seventeen of the girls infected with trichomonas (24%) had a coexisting *N. gonorrhoeae* and/or *Chlamydia trachomatis* infection.

Of the 366 girls who had Pap smears done, 8% had reactive or inflammatory cellular changes noted. The association of these cellular changes was found to be significant for those individuals who had trichomonas infections, compared to those without trichomonas infections ($p < 0.001$). There was no association between trichomonas infections and cytology results of low- or high-grade squamous intraepithelial lesions, or atypical squamous cells of undetermined significance.

Discussion

The results of this study show that InPouch TV® cultures have a higher sensitivity than wet-mount microscopy in the diagnosis of trichomonas infections in sexually active adolescent females. This culture system identified 70% more cases of trichomonas than wet-mount alone. These results, showing the increased sensitivity of InPouch TV® compared to wet-mount microscopy, are consistent with previous studies on other populations (9,10).

Wet-mount microscopy is the most common method used to detect trichomonas infections in adolescent clinics. Wet-mount microscopy has the advantage of being inexpensive and readily available and provides a diagnosis within minutes of the examination. Wet-mounts, however, may be misread owing to the clinician's skills, a prolonged period between the examination and the reading, quality of the microscope or a low concentration of the organisms in the sample. The concentration of *T. vaginalis* in a single vaginal specimen varies with the length of infection and degree of inflammation. Wet-mount diagnosis of trichomonas requires a large concentration of organisms (10,000/mL) (12).

InPouch TV® culture appears to be a superior method to diagnosing trichomonas infections in teens. In previous studies, it has been found to produce reliable results in less time than the standard culture method, to have fewer problems with contaminating microorganisms, and to have significant advantages in both sensitivity and specificity. The pouch itself has a long shelf-life and is inexpensive (approximately $2/pouch). Direct inoculation into the pouch avoids the common problem of loss of motility/viability during transport. The InPouch TV® system has the versatility of being stable at room temperature following inoculation. Because InPouch TV® is a closed system, there is little risk for contamination of lab personnel. Positive cultures with InPouch TV® can be achieved with vaginal fluid concentrations of as little as 4–10 organisms/mL (9).

Culturing with traditional medium (e.g., Diamond's), considered the "gold standard" of diagnosis for this infection, is often impractical. Disadvantages include the high cost, strict storage and transport requirements, time-consuming preparation of slides from the incubated culture, and risk of contamination of the culture and exposure of laboratory personnel to biological materials (10). Because of these factors, traditional cultures for *T. vaginalis* are not generally available to clinicians. The advantages of the InPouch TV® culture system itself (cost, ease of use, and containment) suggest that it is superior to Diamond's media. The results presented here show that the sensitivity of the InPouch TV® culture system approaches that of Diamond's media.

Another method of diagnosing trichomonas infection is the Pap smear. In this study, the sensitivity of Pap smear for trichomonas was 56%, identical to the previously reported sensitivities of 56–78% (13). An interesting finding of the study was the association of inflammatory and reactive cellular changes on the Pap smear and trichomonas infections. Although trichomonas may cause cervical inflammation, it is

unknown how much of cervical inflammation can be attributed to trichomonas infections.

There were some limitations to the study. The population studied was composed of a mostly urban, lower-socioeconomic status group of females. Consecutive eligible patients were not always recruited into the study owing to time and staffing problems. This may have skewed the population of subjects; however, the demographic and clinical characteristics of the subjects reflected the clinic populations at both sites. Owing to these factors, the generalization of the conclusions may be limited. In addition, not all tests were performed on all subjects, precluding careful comparisons among them.

Given the prevalence and sequelae of infection, culture for *T. vaginalis* should be considered for sexually active adolescent girls. Diagnosis of trichomonas infections could be more than doubled if culture methods were used in addition to wet-mount microscopy. InPouch TV® is an excellent alternative to traditional culture media in diagnosing trichomonas infection in adolescent females.

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