JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1997, p. 3308–3310 Vol. 35, No. 12
0095-1137/97/$04.00+0

Comparison of the InPouch TV Culture System and Diamond's Modified Medium for Detection of *Trichomonas vaginalis*

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Received 7 July 1997/Returned for modification 20 August 1997/Accepted 24 September 1997

This study compared the use of Diamond's modified medium to InPouch for the culture of *Trichomonas vaginalis* from pooled vaginal secretions. The sensitivity for InPouch was 82.4% (61/74) versus 87.8% (65/74) for Diamond's modified medium. There were no significant differences in the sensitivity and negative predictive value of InPouch compared to Diamond's modified medium.

Trichomoniasis occurs worldwide with an incidence of approximately 180 million infections per year, including about three million new cases per year in the United States (3a, 21). Trichomoniasis is caused by *Trichomonas vaginalis*, a sexually transmitted flagellated protozoan that produces vaginal infections that range from asymptomatic to symptomatic, with inflammation, irritation, and foul-smelling discharge (14). *T. vaginalis* infections are not self-limited and produce nonulcerative inflammation of the genital epithelium that can progress to necrosis and hemorrhage. This inflammatory response has been associated with an increased risk of human immunodeficiency virus (HIV) seroconversion in women (11, 16, 20). Women infected with *T. vaginalis* and *Chlamydia trachomatis* have an increased risk for pelvic inflammatory disease (13), and pregnant women infected with *T. vaginalis* may be at increased risk of preterm delivery (12, 15). Therefore, the magnitude of *T. vaginalis* infections and their associated morbidity makes accurate diagnosis important and cost-effective.

Direct microscopic examination of vaginal secretions is the most common and rapid method used to diagnose trichomoniasis. Culture of vaginal and urethral specimens is the most sensitive, although a slower diagnostic technique (8, 10). In this study we compared the use of Diamond's modified medium (6, 7, 17) to a newer diagnostic culture system, InPouch (Biomed, San Jose, Calif.). The latter is a plastic bag culture system that provides an upper chamber for an immediate exam of the specimen plus a lower chamber for a trichomonas culture (1, 5).

(These results were reported in part at the 96th General Meeting of the American Society for Microbiology, New Orleans, La., 19 to 23 May 1996.)

Study participants. Women studied were recruited from participants in one of two prospective studies. One group of women was enrolled in the Bronx site of the HIV Epidemiology Research Study (19), a multicenter study of HIV infection in women. The second group was from a longitudinal study of HIV in Bronx drug users (9, 18). The racial and ethnic distribution was 50% Latino, 34% African-American, 11% Caucasian, and 5% other. The median age was 38 years (range, 20 to 57).

Culture. Study participants had a *T. vaginalis* culture performed with each pelvic examination. All women were seen at semiannual scheduled research visits (i.e., not symptom driven) at which they had pelvic examinations. Two Dacron swabs were inserted into the pooled vaginal secretions and also touched both fornices and the mid-third wall of the vagina. One swab was immediately placed into a screw-cap tube of Diamond's modified medium (Remel, Lenexa, Kans.). Diamond's modified medium was stored at 4°C and allowed to reach room temperature prior to use. The second swab was used to inoculate the upper chamber of the InPouch bag. Within several hours (≤6 h) of obtainment of a specimen the cultures were sent to the parasitology laboratory, where they were received coded so that the Diamond's modified medium and InPouch cultures from the same patient were unknown to the parasitologist. The upper chamber of the InPouch was immediately examined for the presence of motile *T. vaginalis* by using the 10× objective. InPouch cultures which demonstrated motile trichomonads were considered positive and not further examined; all other cultures were incubated at 35°C. For the HIV Epidemiology Research Study participants, trained research clinicians performed a direct microscopic examination of vaginal secretions mixed with 0.9% saline (traditional wet preparation ["wet prep"]). In the parasitology laboratory Diamond's modified medium and InPouch cultures were examined for motile *T. vaginalis* at 24, 48, and 96 h of incubation by using a 10× objective. Diamond's modified medium cultures were examined by preparing wet preps from the tube sediment, while the sediment of the InPouch was examined directly through the pouch.

Statistical analysis. Any culture reported positive for *T. vaginalis* was considered a true positive; no cultures were considered false positive. False negatives were defined as *T. vaginalis* cultures negative in one culture medium which were positive in the other. Comparison of the two culture methods was done by using Fisher's exact test.

From 30 March 1995 through 30 December 1996, 715 pairs of specimens for culture by each method were obtained from 395 subjects. A total of 74 specimen pairs were positive for *T. vaginalis* (10.3%) (Table 1). By definition there were no false-positive cultures, but each method had some false-negative cultures (13 for InPouch and 9 for Diamond's modified medium). The sensitivity and negative predictive values for each method did not differ significantly. The time to detection of a positive culture in either medium did not differ significantly, and 80% of the positives were detected within the first 24 h.

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TABLE 1. Comparison of *T. vaginalis* cultures performed with Diamond's modified medium versus InPouch

Method of detection	No. (%) of positive specimens (n = 715)	Sensitivity (%)[a]	Negative predictive value (%)[b]
Diamond's modified medium	65 (9.0)	87.8	98.6
InPouch	61 (8.4)	82.4	98.0
Both methods	74 (10.3)		

[a] The number positive in each method/total number positive (n = 74).
[b] False negatives were defined as being negative in one method but positive in the other.

The direct wet prep was compared to the direct exam in the upper chamber of the InPouch for only 15 culture-positive specimens. Standard wet prep was positive for 11 (73%), and direct examination of the InPouch was positive for 8 (53%) (*P* value, not significant). When all specimens examined were included, 46 (75%) of 61 culture positive in InPouch were positive on direct exam in the upper chamber.

T. vaginalis infections are regarded as the most prevalent nonviral sexually transmitted disease and are similar to other sexually transmitted diseases in that the prevalence increases with increased numbers of sexual partners, presence of other sexually transmitted diseases (especially gonorrhea), and failure to use barrier or hormonal contraceptives (8). In the same report, asymptomatic patients attending a family planning clinic had infection rates of about 5% compared with 50 to 75% in female sexual workers. In our study sample the infection rate was 10%. This is slightly lower than the infection rate reported in a multicenter study of women with vaginitis, where the *T. vaginalis* infection rate was 15.1% (4). Interestingly, at the New York City site of the previous study, the *T. vaginalis* infection rate was 49.2%. Since *T. vaginalis* has been associated with more serious medical conditions, it is important that microbiologists inform clinicians about the most sensitive and cost-effective diagnostic techniques for *T. vaginalis.*

In most settings the microscopic evaluation of vaginal discharge (wet prep) has been the standard method used to diagnose *T. vaginalis* infections. The wet prep is fast and convenient for clinicians, but in symptomatic women the sensitivity of the wet prep in demonstrating motile trichomonads (definitive diagnosis) is only 60 to 80% (8). Other stains, such as Giemsa, Papanicolaou, and acridine orange, have similar sensitivities and are more labor intensive. DeMio et al. found that in some cases the sensitivity of the wet prep was equivalent to that of Diamond's modified medium culture (4). Our data for direct examination of the upper chamber of InPouch were consistent with the overall sensitivities cited in the literature for standard wet prep.

Diamond's modified medium has been shown to be the most sensitive medium for the culture of *T. vaginalis* (6, 7, 17). The main purpose of our study was to compare the positivity rate in Diamond's modified medium with that obtained using the InPouch. Culture of specimens in either medium did not detect all positive specimens. Overall, 74 of 715 specimens were culture positive for *T. vaginalis*, but only 65 (88%) were detected in Diamond's modified medium and 61 (82%) in InPouch. Previous studies have also noted an ability of certain strains to grow in one medium or another, but not both (2, 5). The nature of this strain variability is unknown. Overall, culture in either medium only increases the positivity rate about 10% over direct observation, so that a better medium for *T. vaginalis* would be of value.

InPouch offers some distinct advantages when compared to Diamond's modified medium culture. Once the specimen is placed by a clinician into the InPouch chamber, microscopic observation can be made directly through the bag and does not require sampling to examine the culture for growth. Additionally, in medical centers where satellite laboratories have been removed to comply with the Clinical Laboratory Improvement Act regulations, InPouch provides a convenient method for physicians to obtain direct specimen and culture results rapidly. Cost for the InPouch is comparable to that of a tube of Diamond's modified medium, and the former may be stored for up to 1 year at room temperature. The time required for a technologist to perform each test is equivalent. Diamond's modified medium has a shorter expiration date and requires refrigeration. Finally, commercial DNA probes for *T. vaginalis* have recently become available for rapid diagnosis of trichomoniasis, but test sensitivity has not equaled that of culture (3, 4).

In conclusion, the InPouch was similar to use of the wet prep and culture in Diamond's modified medium in sensitivity and is a reasonable alternative method for the direct observation and culture of *T. vaginalis* from patient specimens. Additional studies may address issues of sensitivity and specificity in high-risk populations and men with urethritis where the definitive role of *T. vaginalis* in nongonococcal urethritis is still unclear (9, 10, 21).

This study was supported in part by a cooperative agreement with the Centers for Disease Control and Prevention (U64/CCU206798), a grant from the National Cancer Institute (R01 CA59140), and by Biomed, San Jose, Calif.

We also thank Hector D. Rivas for his assistance in preparation of the manuscript, John C. McKitrick for his critical review, and Anna L. Winston, Shoshanna Silberman, and Eileen Dolee for obtaining clinical specimens.

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