Original article

A comparison of the sensitivity of the InPouch TV, Diamond's, and Trichosel media for detection of *Trichomonas vaginalis*

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Objective: This study compared the ability of three culture media (InPouch TV, Diamond's, and Trichosel) to support the growth of clinical isolates of *Trichomonas vaginalis* and their relative sensitivity for detection of the organism.
Methods: The majority of the clinical isolates were obtained from two San Francisco Bay Area clinics. *T vaginalis* was subcultured in 4 ml of one of the InPouch, Diamond's, or Trichosel media for 24–48 hours before evaluation. Twenty isolates were initially cultured in the InPouch test, 13 with Diamond's, and 10 with Trichosel. A haemocytometer was used to measure the initial concentrations of the organisms. Then serial dilutions were made in saline to yield approximately $2{\cdot}0 \times 10^4$, $2{\cdot}0 \times 10^3$, and $2{\cdot}0 \times 10^2$ motile *T vaginalis* per ml. A 30 µl inoculum from each dilution was transferred into 4 ml aliquots of the three media (387 individual tests, 43 × 3 dilutions × 3 media). Microscopic examinations for viable trichomonads were made at 24, 48, and 96 hours. Microscopy was through the pouch wall for the InPouch medium, and through a cover slipped slide with one drop of Diamond's and Trichosel media.
Results: At 24 hours, the InPouch demonstrated 84/129 positive, Diamond's 23/129, and Trichosel 18/129. At 48 hours, an accumulative positive rate for the InPouch was 98/129, for Diamond's 55/129, and Trichosel 47/129. At 96 hours the total positives for each test were 112/129 for the InPouch, 78/129 for Diamond's, and 74/129 for Trichosel.
Conclusions: The InPouch TV test was significantly more sensitive than either Diamond's or Trichosel (at 0·01 level of significance, $p_{InPouch} > p_{Diamond's}$; $p_{InPouch} > p_{Trichosel}$ on all three dilutions at 24, 48, and 96 hours). This increased sensitivity was the result of either a reduced generation time or the larger volume of media examined microscopically.
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Keywords: *Trichomonas vaginalis*; detection; media

Introduction

The World Health Organisation estimates that 180 million cases of trichomoniasis occur worldwide annually.[1] It may be the most disseminated non-viral infection of all sexually transmitted diseases.[2] Third world countries have reported rates of trichomoniasis that vary between 47% and 19%.[3 4]

Contemporary clinical studies have described the pathology of trichomoniasis. *Trichomonas vaginalis* may be resistant to metronidazole, produce an inflammatory reaction in the host, and increase the predisposition of the host to retrovirus infections.[5 6]

Laboratory methods for identification of *T vaginalis* have included: the wet mount; Papanicolaou (Pap) smear; acridine orange staining; antigen and antibody tests; DNA probe; PCR; and culture.[7] Culture is considered the "gold standard" for diagnosis of *T vaginalis* because of its sensitivity.[8]

The following culture media for *T vaginalis* have been employed: Kupferberg; Trichosel; Kupferberg STS; modified Diamond's; Lash serum; modified Diamond's; and the InPouch TV.[7 8] The purpose of this study was to compare the sensitivity of three media commercially available in the United States, Trichosel and modified Diamond's, with the InPouch TV (InPouch) trichomonas test. The three media were obtained from commercial sources with an appropriate shelf life during their evaluations. Each medium was stored according to the manufacturers' requirements.

Materials and methods

The majority of the clinical isolates of *T vaginalis* were obtained from the City STD Clinic in San Francisco, California, and the Marin County Laboratory in San Rafael, California. When necessary, the clinical isolates were subcultured in 4 ml of InPouch media. This was used because it maintained trichomonad viability between 5 and 6 days, while Diamond's and Trichosel media averaged 3 days. Before evaluation, the isolates were cultured in one of the three media (InPouch, Diamond's, or Trichosel) for 24–48 hours. A 4 ml volume was used for each medium tested. Both Diamond's and Trichosel media were in glass tubes and the InPouch medium in a plastic pouch. The InPouch test employed 20 isolates as the initial test medium, Diamond's 13, and Trichosel 10.

The following procedures were performed for the evaluations. Each trichomonas isolate was incubated at 37°C for between 24 and 48 hours. A haemocytometer was employed for quantitating the original density of trichomonads. Saline was used for making the dilutions of the test specimen. All initial dilutions were selected that could be quantitated with a haemocytometer and were less in final concen-

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tration than the 1×10^4 required to produce a positive wet mount.[9] Each culture was diluted to a concentration of approximately $2{\cdot}0 \times 10^4$. Tenfold serial dilutions were made at $2{\cdot}0 \times 10^3$ and $2{\cdot}0 \times 10^2$. A 30 µl inoculum was taken from each of the three dilutions and added to the three sets of media. Tests were incubated at 37°C and examined microscopically at 24, 48, and 96 hours.

Examination of Diamond's and Trichosel media used one drop of culture medium on a cover slipped glass slide. The InPouch test was examined directly through the wall of the plastic pouch. This is the method required by the instructions for reading the test, which eliminates a slide and coverslip. All tests were evaluated independently by KAB and MZZ.

All results were statistically analysed by using the hypothesis test to test the difference among three media.

Results

A total of 387 tests were performed and reported as positive cumulative cultures (table). Microscopic observation was approximately 2 minutes for the InPouch and 5 minutes for the wet mount tests. Results were recorded as positive when motile trichomonads were observed.

At a trichomonad concentration of approximately $2{\cdot}0 \times 10^4$/ml: the InPouch tests were 97·6% positive in 42/43 samples at 24 hours and the 43rd sample was positive at 48 hours; Diamond's had positive results in 18/43 or 42% samples at 24 hours and 31/43 (72%) at 48 hours, and 33/43 (76·7%) at 96 hours; Trichosel tests were positive in 17/43 (40%) at 24 hours, 29/43 (67%)) at 48 hours, and 33/43 (76·7%) in 96 hours.

The following results were obtained at a concentration of approximately $2{\cdot}0 \times 10^3$/ml trichomonads. At 24 hours the InPouch test was positive in 31/43 (61·5%), 35/43 (81·8%) in 48 hours, and 41/43 (95·3%) at 96 hours. Diamond's medium had 5/43 (11·6%) at 24 hours, 20/43 (26·5%) at 48 hours, and 26/43 (60·4)%) in 96 hours. Trichosel had 1/43 (2·5%) at 24 hours, 16/43 (37·2%) at 48 hours, and 24/43 (55·8%) in 96 hours.

At 24 hours at a trichomonad dilution of approximately $2{\cdot}0 \times 10^2$ /ml the InPouch test had 11/43 (25·5%) positive, while all the tests were negative for both Diamond's and Trichosel media. After 48 hours' incubation the InPouch test was positive in 20/43 (46·5%), Diamond's was positive in 4/43 (9·3%), and Trichosel 2/43 (4·6%). After 96 hours the InPouch test was positive in 28/43 (65·1%), Diamond's 19/17 (44·1%), and Trichosel 17/43 (39·5%).

Statistical analysis demonstrated that at a 0·01 level of significance, $p_{InPouch} > p_{Diamond's}$; $p_{InPouch} > p_{Trichosel}$ on all three dilutions at 24, 48, and 96 hours.

A comparative study of Trichomonas vaginalis using the InPouch, Diamond's, and Trichosel media

	*Accumulative positive cultures**					
	24 Hours		*48 Hours*		*96 Hours*	
Media	*+ve*	*%*	*+ve*	*%*	*+ve*	*%*
At $2{\cdot}0 \times 10^4$/ml dilution:						
InPouch	42	97·6	43	100	43	100
Diamond's	18	24	31	72	33	76·7
Trichosel	17	39·5	29	67	33	76·7
At $2{\cdot}0 \times 10^3$/ml dilution:						
InPouch	31	61·5	35	81·3	41	95·3
Diamond's	5	11·6	20	46·5	26	60·4
Trichosel	1	2·5	16	37·2	24	55·8
At $2{\cdot}0 \times 10^2$/ml dilution:						
InPouch	11	25·5	20	46·5	28	65·1
Diamond's	0	0	4	9·3	19	44·1
Trichosel	0	0	2	4·6	17	39·5
Statistical analysis	At 0·01 level of significance, $p_{InPouch} > p_{Diamond's}$; $p_{InPouch} > p_{Trichosel}$ on all three dilutions at 24, 48, and 96 hours					

*Total isolates: n = 43.

Discussion

In this study the InPouch TV test demonstrated a greater sensitivity at each dilution when compared with either Trichosel or Diamond's media. In a previous evaluation the InPouch TV test was superior to Hollander's medium.[10]

The InPouch TV test serves, firstly for specimen transport, then as a growth chamber during incubation, and finally as a viewing chamber during microscopy. Once the InPouch medium is inoculated the chamber never requires opening to evaluate the test microscopically. The clear plastic pouch enables trichomonads to be readily observable at 100×. The sensitivity of this test may be increased either by a reduction in initial growth generation time or the greater volume of media examined.

Conclusion

The InPouch TV test demonstrated its superiority with more positive results at each dilution and in shorter time periods then either Diamond's or Trichosel. This was particularly evident during the first 24 hours of incubation.

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