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Modified Thioglycolate Medium: a Simple and Reliable Means for Detection of *Trichomonas vaginalis*

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Despite the declining rate of sexually transmitted diseases in developed countries, trichomoniasis is still one of the most common venereal infections. While diagnosis of this condition is commonly based on the microscopic wet-mount method, culture remains the most accurate single procedure for detecting the presence of *Trichomonas vaginalis* in clinical samples. In the present study, the efficacy of a modified formula of the commonly available thioglycolate medium was compared with that of the standard Diamond's medium for detection of *T. vaginalis* in samples from 176 women with vaginal symptoms. Thioglycolate medium supplemented with yeast extract, horse serum, and antimicrobial agents was as reliable as Diamond's medium for detection of *T. vaginalis* in vaginal fluid samples. Modified thioglycolate medium may be used as a readily available, low-cost substitute for the standard medium for culturing *T. vaginalis*.

Trichomoniasis is a widespread sexually transmitted disease, with prevalences of 5% in asymptomatic subjects attending family planning clinics and up to 40% in patients at sexually transmitted disease clinics (10). Ten to fifty percent of women infected with *Trichomonas vaginalis* are asymptomatic (10). Clinical manifestations usually associated with trichomoniasis, such as a yellow-green discharge, pruritus, vaginal malodor, and abdominal pain, are nonspecific and cannot be relied on for an accurate diagnosis (4, 10, 13). Clinic-based laboratory diagnosis of *T. vaginalis* is equally unsatisfactory. The vaginal pH is elevated above 4.5 in as many as 90% of cases (9). This finding, however, is nonspecific, as 90% of women with bacterial vaginosis also have an elevated vaginal pH (12). A fishy odor after application of 10% potassium hydroxide is present in 50% of patients (2), but this test too is nonspecific for trichomoniasis (12). The time-honored approach for the diagnosis of trichomonal infections has been microscopic evaluation by the wet-mount method. This procedure, however, detects only 35 to 80% of the cases, depending on the expertise of the microscopist (6, 8). Thus, culture remains the most accurate single method for detecting the presence of *T. vaginalis* in patient samples (6, 8, 11); routinely, 95% of cases are diagnosed by this method. Several commercial liquid media are available for this purpose, with Diamond's medium being considered the "gold standard" (5). Among the disadvantages of these media are that they are expensive and not readily available, especially in developing countries.

In the present study, the usefulness of a new culture medium based on the commonly available thioglycolate medium was compared with that of the standard Diamond's medium (Remel, Lenexa, Kans.). The study was conducted in an urban gynecologic clinic attended mainly by lower-middle-socioeconomic-class patients in greater Tel Aviv, Israel.

The test medium consisted of fluid thioglycolate medium (Difco Laboratories, Detroit, Mich.) enriched with yeast extract and supplemented with inactivated horse serum, amphotericin B, penicillin G, and gentamicin (Table 1). Yeast extract was added before the medium was autoclaved, and antimicrobial agents and horse serum were added aseptically afterward. The fluid medium was dispensed in 10-ml screw-cap tubes. Both thioglycolate and Diamond's media were stored in a refrigerator at 4°C.

Women with vaginal symptoms attending the clinic from October 1994 to December 1995 were examined for *T. vaginalis* infection. Two vaginal fluid samples were obtained from the posterior fornix with cotton swabs and used to inoculate modified thioglycolate medium and Diamond's medium. The inoculated tubes were incubated at 35°C for 7 days. For detection of growth, 10 μl of each culture was sampled daily for 7 days from the bottoms of the tubes and examined microscopically. The presence of *T. vaginalis* was diagnosed by the characteristic morphology and motility of the protozoa observed under microscopy.

A total of 176 women were studied; 24 (13.6%) were infected with *T. vaginalis*. Full concordance was observed between the culture media, both of which detected the parasite in the same patients. The times to identification of the protozoan were also similar, with the modified thioglycolate medium being somewhat better: in 15 of 24 Diamond's medium samples and 17 of 24 modified thioglycolate medium samples, the parasite was detected 18 to 24 h after inoculation, while in 7 of 24 and 4 of 24 samples, respectively, it was diagnosed after ≥72 h (Table 2). On the other hand, overgrowth of *Candida* spp. was observed earlier in Diamond's medium than in modified thioglycolate broth: after 18 h in the former and after 48 to 72 h in the latter.

Most importantly, the cost of purchasing Diamond's medium was six times higher than the cost of materials and labor involved in preparing the same amount of modified thioglycolate medium.

Optimal growth and reproduction of *T. vaginalis* require anaerobic conditions. For appropriate in vitro cultivation, an unusually large number of essential nutrients, including carbohydrates, amino acids, purines, pyrimidines, fatty acids, vitamins, and iron, is mandatory (7). Although the optimal medium for culturing of *T. vaginalis* has yet to be defined, we thought that thioglycolate medium, the standard broth for anaerobic cultures, might be a suitable alternative to Diamond's medium. Furthermore, these two media have in common several ingredients, such as casein, yeast extract, carbohydrates (maltose or glucose), and L-cysteine. With minor modifications in the thioglycolate formula, such as adjustment of the yeast extract content and addition of horse serum and antimicrobial

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TABLE 1. Compositions of modified thioglycolate medium and Diamond's medium

Modified thioglycolate medium[a]		Diamond's medium	
Ingredient	Amt	Ingredient	Amt
Casitone	15 g	Casein peptone	24 g
Yeast extract	5 + **7 g**	Yeast extract	12 g
Glucose	5.5 g	Maltose	6 g
Sodium chloride	2.5 g		
L-Cystine	0.5 g	Cysteine L-hydrochloride	1.2 g
Sodium thioglycolate	0.5 g		
Agar	0.75 g		
Resazurin	0.001 g	L-Ascorbic acid	0.24 g
Horse serum	**120 ml**	Horse serum	120 ml
Amphotericin B	**2 mg**	Amphotericin B	2 mg
Penicillin G	**1,000,000 U**	Penicillin G	1,000,000 U
Gentamicin	**80 mg**	Streptomycin	1.5 g
Distilled water	900 ml	Distilled water	900 ml
pH	7 ± 0.2	pH	6.5 ± 0.2

[a] Modifications to Difco thioglycolate medium are shown in boldface type.

agents, we have obtained a medium equivalent to Diamond's medium.

In the present study, we have demonstrated that modified thioglycolate medium performed as well as Diamond's medium. Interestingly, *Candida* spp. were slower to appear in the modified thioglycolate medium despite the similar concentrations of amphotericin B in both media. In addition to its efficacy, modified thioglycolate medium has the advantage of being easily prepared from readily available components at a lower cost than Diamond's medium. This consideration is particularly relevant for laboratories with budgetary restraints, a common situation in developing countries.

Moreover, modified thioglycolate medium allows more flexibility in providing a supply of culture medium for *T. vaginalis* and eliminates concerns about expiry dates. Thioglycolate medium is used on a routine basis with high turnover in most bacteriology laboratories, and the modified formula can be readily prepared according to actual needs. Thus, the necessary supply of culture medium can be easily tailored to the specific needs of a laboratory. This is particularly advantageous for laboratories with small and/or irregular demands for trichomonal culture medium.

Two other systems have been recently described for the detection of *T. vaginalis*. The In Pouch TV system (Biomed Diagnostics, Santa Clara, Calif.) allows direct, rapid microscopic examination of a culture pouch without daily sampling, and it was found to be as reliable as Diamond's medium in detecting *T. vaginalis* (3). The Affirm VP III system (Micro Prob Corporation, Bothell, Wash.) is a rapid, semiautomated commercial system that uses synthetic oligonucleotide probes for the simultaneous detection of *T. vaginalis*, *Gardnerella vaginalis*, and *Candida* spp. from a single vaginal swab. In comparison with the standard methods, this probe system was positive for 12 of 12 specimens positive by wet mount and 12 of 15 specimens positive by culture on Diamond's medium; there were no false positives (1). The obvious advantage over culture is that the probe assay takes only 40 min to complete.

The combination of culture and wet-mount examination remains the standard approach for detecting *T. vaginalis* in patient samples. Modified thioglycolate medium was found to be as efficient as Diamond's medium in recovering the parasite from clinical specimens and may provide a readily available, low-cost substitute for the standard medium.

TABLE 2. Timing of *T. vaginalis* detection

Medium	No. of specimens in which *T. vaginalis* was first detected at:				
	18–24 h p.i.[a]	48 h p.i.	72 h p.i.	96 h p.i.	120 h p.i.
Modified thioglycolate	17	3		1	3
Diamond's	15	2	1	4	2

[a] p.i., postinoculation.

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