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The Plastic Envelope Method, a Simplified Technique for Culture Diagnosis of Trichomoniasis

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Although culture of *Trichomonas vaginalis* is more sensitive than wet mounts in the diagnosis of trichomoniasis, the lack of convenience of culture prevents it from being widely used. To improve the acceptability of diagnosis by culture, a plastic envelope method (PEM) was devised. PEM permits both immediate examination and culture in one self-contained system. The medium consists of dry ingredients that are reconstituted with water before use. The effectiveness of immediate examinations by PEM was compared with that of wet mounts, and the effectiveness of culture by PEM was compared with that of culture in Trichomonas Medium No. 2 (Oxoid). Of 710 vaginal secretion specimens from symptomatic and asymptomatic women that were tested by the four methods, 62 (9%) were positive for *T. vaginalis*. The sensitivity was 66% by wet mount, 66% by immediate examination by PEM, 89% by cultures in Oxoid medium, and 97% by culture by PEM. The two culture methods had equivalent sensitivities but were significantly ($P < 0.0001$) more sensitive than the two immediate methods. The combined immediate examination by PEM plus culture was more convenient to use than wet mounts plus culture in Oxoid medium. The long shelf-life of PEM's dry medium and its anticipated low cost are additional advantages.

In the diagnosis of *Trichomonas vaginalis*, culture of the organism has been shown to be more sensitive than immediate examination of glass slide wet mount preparations (2, 8, 10, 11, 13, 15, 17). Several investigators reported superior results (2, 10, 11, 15) when both methods were used to make a diagnosis. Nevertheless, culture is not widely used in routine office or clinic diagnosis of trichomoniasis because it is an inconvenient procedure. Compared with wet mounts, culture requires more time and effort, and because the medium is complex, it is more expensive, may not be widely available, and generally has a limited shelf-life. Thus, there is a need for a technically simple, self-contained, and accurate culture method that provides dry ingredients with a long shelf-life (12).

A simplified method for the diagnosis of *T. vaginalis* infection by culture was previously developed at International Health Services. By this method—designated the plastic envelope method (PEM)—a clear, flexible plastic pouch that contains liquid medium is used. In an evaluation of PEM, Brady et al. (3) reported that of 19 persons with known infections, 74% were identified by culture by PEM, whereas 68% were identified by wet mounts, a difference that was not significant.

The pouch that is used in the PEM has since been revised to improve its anaerobic qualities and ease of use. The shelf-life of the medium has been lengthened through the use of dry ingredients in tablet form. The dry ingredients are reconstituted when distilled water is added to the pouch. The resultant solution, when inoculated with vaginal secretions, is selective for *T. vaginalis*. The organisms can be identified microscopically directly through the plastic film in an initial immediate examination and at intervals thereafter during incubation.

In this study, we compared the effectiveness of the revised PEM, a standard culture medium (Trichomonas Medium No. 2 [Oxoid]) (7), and conventional slide wet mounts in diagnosing trichomoniasis.

MATERIALS AND METHODS

Study population. Consecutively examined women who attended the Outpatient Obstetric and Gynecology Clinic at El-Hussein University Hospital, Al-Azhar University, Cairo, Egypt (December 1989 to January 1991) and who agreed to participate in the study were enrolled in the study. Excluded were women with current menses and those who had had a vaginal douche or sexual intercourse in the previous 24 h or who had used an antibiotic or a vaginal cream in the previous 2 weeks. A total of 710 women were included in the study; 320 women had symptoms of vaginitis, and 390 women were free of these symptoms but underwent pelvic examinations for other reasons.

History taking, physical examinations, collection of specimens, and immediate PEM and slide wet mounts were done by one clinician (M.K.). Examination (not blindly) of PEM and Oxoid cultures were done on some days by M.K. and on other days by M.S.

Description of the PEM pouch. In its present form, the PEM pouch (Fig. 1 and 2) consists of upper and lower chambers that are nearly equal in size and that are separated by a narrow channel. The film of the pouch, which is soft, clear, and transparent, minimizes oxygen and water vapor transmission. The oxygen content within the medium is

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FIG. 1. PEM pouch showing a nutrient tablet within the upper chamber and the viewing frame positioned across the lower chamber.

lowered further by the reducing action of the ascorbic acid and the cysteine in the medium. To facilitate microscopic examination of the chamber contents, a small rigid plastic frame holds the pouch in a horizontal position.

The constituents of the dry PEM medium were a modification of the medium described by Hollander (9). The modifications were the addition of tioconazole hydrochloride (0.01 mg/ml; Pfizer), vitamin B_{12} (8 μg/ml), L-cysteine (0.1 mg/ml), and chloramphenicol (0.16 mg/ml). Agar was omitted.

To reconstitute the medium, 4 ml of distilled water was added to the upper chamber; the water dissolved the tablet within 1 min. The pouch was then closed and the medium was pushed through the narrow channel into the lower chamber. Next, the small amount of fluid remaining in the upper chamber was inoculated with vaginal secretion, the solution was mixed by manipulating the pouch with the fingers, and then the pouch was examined microscopically. To initiate the culture, some medium in the lower chamber was pushed into the upper chamber and mixed with the specimen; the upper chamber contents were then pushed back into the lower chamber. In the process, if any bubbles formed in the lower chamber, they were expressed out. During incubation, the pouch was held in the vertical position. In subsequent microscopic examinations, the bottom portion of the lower chamber was examined microscopically, but with care not to agitate the chamber contents.

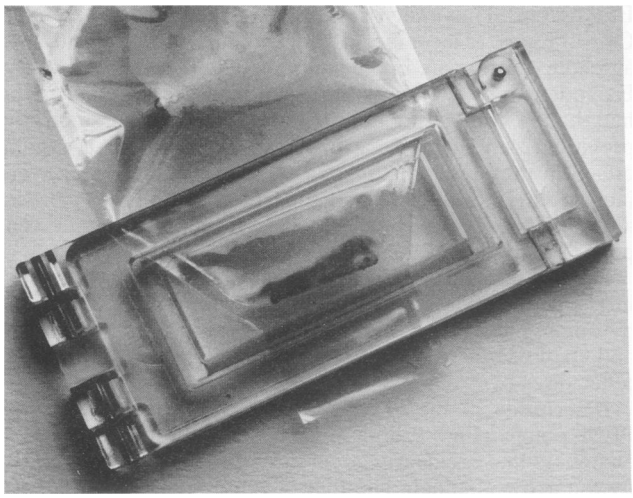

FIG. 2. Viewing frame enclosing a mass of trichomonads and epithelial cell debris in the bottom portion of the lower chamber.

Specimen collection and processing. By using a nonlubricated speculum, samples of vaginal secretions from the posterior fornix were collected on three sterile, cotton-tipped swabs. The first swab was rotated in a drop of physiologic saline on a microscopic slide. The second swab was used to inoculate 10 ml of Trichomonas Medium No. 2 (Oxoid) (7) contained in rubber-stoppered test tubes; the medium was stored at 34°C and was used within 7 days of preparation. The third swab was used to inoculate the PEM medium. The PEM pouches were stored at ambient temperatures until they were used; distilled water was added about 2 h before the medium was inoculated with vaginal secretions.

Observations of slide wet mounts (covered with cover slips [20 by 20 mm]) and the PEM chambers were done by conventional light microscopy at low power (×100) to scan for motile trichomonads and then at high power (×400) to confirm the characteristic motility and morphology of trichomonads. The examinations were started immediately and continued for 3 min or until they became positive. Inoculated PEM and Oxoid cultures were incubated aerobically at 34°C. The lower chambers of PEM cultures and wet mounts of a drop of fluid from shaken Oxoid culture tubes were examined at 24-h intervals for 5 days or until they became positive. Although yeasts were occasionally found, records of their presence were not made.

The Fisher exact test was used to test statistically significant differences in positivity between the diagnostic methods.

RESULTS

Of the 710 women tested for *T. vaginalis*, 62 (8.7%) were positive for *T. vaginalis* by one or more of the tests; 41 (5.8%) of the 62 infections were detected by both slide wet mounts and immediate PEM examinations, 55 (7.7%) were detected by Oxoid culture, and 60 (8.5%) were detected by PEM culture (Table 1). The differences in positivity between the PEM culture and the immediate methods or the Oxoid culture and the immediate methods were significant ($P < 0.0001$).

Immediate examinations by using both wet mounts and PEM pouches gave identical results; all specimens that were positive by these tests were also positive by one or both culture methods. The immediate methods, however, detected no positive specimens that were not positive by the culture methods.

PEM culture detected seven positive specimens that were missed by Oxoid culture, and Oxoid culture detected two specimens that were missed by PEM (Table 1). The concor-

TABLE 1. Results of testing for *T. vaginalis*

No. of specimens positive by one or more tests	Immediate methods		Culture methods	
	Slide	PEM	Oxoid	PEM
41	+	+	+	+
12	−	−	+	+
2	−	−	+	−
7	−	−	−	+
0	+	+	−	−
No. (%) positive[a]	41/710 (5.8)	41/710 (5.8)	55/710 (7.7)	60/710 (8.5)

[a] Of 710 specimens tested, 62 were positive by all methods.

dance between the two culture methods for positive and negative findings was 701 of 710 specimens (98.7%); the difference between the two methods was not significant. With regard to the rates at which culture methods detected the organism, of the 21 positive specimens detected only by culture, at 24 h PEM detected 8 positive specimens (38.1%), whereas Oxoid culture detected 4 (19.0%) positive specimens ($P > 0.05$). Of the 60 positive specimens detected by PEM culture, 47 were found on day 1 and 13 others were found on days 2 to 4. Of the 55 positive specimens detected by Oxoid culture, 42 were found on day 1 and 13 others were found between days 1 and 4.

DISCUSSION

Although culture of *T. vaginalis* is the most sensitive means of diagnosing trichomoniasis (2, 8, 10, 11, 13, 15, 17), the lack of convenience has prevented it from being widely used as an office or clinical laboratory procedure. PEM was devised to improve the acceptability of diagnosis by culture. The plastic pouch developed by Brady et al. (3) contained a single elongated chamber that was analogous to a test tube. The new PEM substitutes a plastic film that is less permeable to oxygen, uses two chambers with a narrow connecting channel, incorporates within the pouch a dry medium that is reconstituted with water before use, and uses tioconazole to inhibit yeast growth.

In this study, PEM was as effective as established methods for the detection of *T. vaginalis* infections in females. PEM culture detected a few more positive specimens than did Oxoid culture, and it did so somewhat sooner than Oxoid culture did, while the results of immediate examinations by PEM were identical to those of wet mounts.

Our findings that wet mounts were significantly less sensitive than PEM or Oxoid culture are similar to other comparisons of wet mounts with culture (2, 8, 10, 11, 15, 17). In Iran, Imandel et al. (10) reported that wet mounts detected 60% of the positive specimens that were detected by culture. In the United States, positive findings by wet mount compared with those by culture were 64% by Schmid et al. (17), 60% by Krieger et al. (11), and 50% by Fouts and Kraus (8).

The modified PEM proved to be easy to use, reliable, and sensitive. The advantages of PEM culture over Oxoid culture are as follows. (i) In our experience, it was more convenient to use the self-contained PEM culture system than a standard test tube culture method. (ii) The PEM incorporates dry medium ingredients that can be held at ambient temperatures until they are used. Results of our experiments (1) indicate that the shelf-life of the PEM dry medium at ambient temperatures is at least 1 year; its total shelf-life has not been fully evaluated. The long PEM shelf-life is preferable to that of commercial liquid medium, which must be stored frozen or refrigerated. Outside of the United States commercial media, including Oxoid Trichomoniasis Medium No. 2, are sometimes available as dry ingredients (12). (iii) PEM is inexpensive to manufacture, which should result in a low cost to the user. (iv) Because PEM is inoculated with specimen at the time that the specimen is collected, no transport medium is needed. (v) An additional feature of PEM is that it incorporates a convenient method for immediate diagnosis, has a sensitivity equivalent to that of the wet mount method, and is as easy and rapid to use as wet mounts.

A good culture medium for *T. vaginalis* should produce a flourishing colony in 3 to 4 days when one starts with an inoculum of 10 or fewer parasites (12). To accomplish this, modern trichomoniasis media contain a serum (often horse serum), glucose or maltose, a protein hydrolysate, yeast extract, reducing agents, electrolytes, and antibiotics (12). Some media also contain phosphate buffers, vitamins (particularly vitamin B_{12}), an iron source, and nystatin or amphotericin B to control yeast overgrowth. The several media which have been evaluated are comparable in their effectiveness (10–12, 17). The unique constituent in the PEM medium is tioconazole, an imidazole that dissolves readily in water. Although imidazoles are generally toxic to trichomonads, when a low concentration of tioconazole was tested for its effect on suppressing yeasts, it had no measurable effect on *T. vaginalis* (1).

When two media were used concurrently for diagnosis by the culture method, Krieger et al. (11) reported a 7% increase in sensitivity compared with that when a single culture was used. Similarly, when the results obtained by using two media were combined, we increased the sensitivity by 3%. Whether examination of PEM cultures for additional days might increase the number of positive specimens remains to be determined. In other PEM evaluations by us (1), we rarely found that cultures became positive on day 6 or 7 postinoculation. However, when Schmid et al. (17) used light inocula to initiate infections, they found that maximum yields with five different media occurred when they continued observations for 7 days.

The 8.7% frequency of trichomoniasis infection in an outpatient gynecologic population in this study in Egypt compares with rates of 16 to 33% in previous studies in that country (4–6, 14) and with highly variable rates from nil (18) to 75% (16) in reports from other countries.

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