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Evaluation of Six Media for the Growth of *Trichomonas vaginalis* from Vaginal Secretions

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Many media have been formulated for the growth of *Trichomonas vaginalis*, but the relative sensitivities of these media have not been determined. We evaluated the ability of six media, including all five media commercially available in the United States, to grow *Trichomonas vaginalis* from vaginal secretions. In a first experiment, we evaluated the ability of five media to grow *T. vaginalis* from vaginal secretions of 375 women and determined the optimal days on which to read culture tubes, by inoculating aliquots of secretions into each medium and reading the tubes 1, 2, 3, 4, and 7 days later. Sixty-five patients (17%) had a positive wet-mount examination for *T. vaginalis*, and all the positive results were confirmed by growth in at least one medium. Of 310 wet-mount-negative specimens, 37 (12%) grew *T. vaginalis*; overall, 102 women (27%) had a positive culture. Diamond and modified Diamond media (the latter being the only medium not commercially available) detected 99 (97%) and 92 (90%) isolates, respectively, compared with three formulations of Kupferberg medium, which detected 77 (75%), 50 (49%), and 43 (42%) isolates. The optimal single day to read wet-mount-negative cultures was day 7, but 4 (11%) of the 37 positive specimens were positive only before day 7. In a second study, we compared the ability of modified Diamond medium with that of a sixth medium, Lash medium, to grow *T. vaginalis* from 48 wet-mount-positive specimens; modified Diamond medium supported growth in all cases, whereas Lash medium supported growth in only 26 (54%) cases. We conclude that formulations of Diamond medium are superior to formulations of Kupferberg or Lash medium for growth of *T. vaginalis*.

The diagnosis of trichomoniasis in women is most commonly made by wet-mount examination or culture of vaginal secretions. Because of ease, rapidity, and cost, wet-mount examination is most commonly used, but estimates of its sensitivity are as low as 51% (1). Its sensitivity depends, however, upon the diagnostic adequacy of what it is compared with. We evaluated the ability of six culture media, including all media commercially available in the United States, to grow *Trichomonas vaginalis* from vaginal secretions, evaluated the sensitivity of the wet mount to detect *T. vaginalis*, studied the optimal time for examining media over a 1-week period, and examined the variation among technicians in the reading of wet mounts of vaginal secretions.

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MATERIALS AND METHODS

The commercial media we evaluated were Kupferberg Trichosel medium (BBL Microbiology Systems, Cockeysville, Md.), Kupferberg STS medium (Remel Laboratories, Lenexa, Kans.), Difco Kupferberg medium (Difco Laboratories, Detroit, Mich.), Diamond medium (Carr-Scarborough Microbiologicals, Stone Mountain, Ga.), and Lash serum medium (Difco). We also evaluated a modification of Diamond medium (1). Kupferberg STS medium from Remel Laboratories, Diamond medium from Carr-Scarborough, and Lash medium were available only already formulated in individual tubes. The remaining commercial media were prepared as specified by the manufacturers, by using bovine serum, the only serum recommended by all manufacturers. Batches made by us were tested for sterility (5% sheep blood agar and enriched thioglycolate broth at 35°C for 48 h and at room temperature for 7 days), stored refrigerated, and used within 3 months of preparation; commercially prepared media were used before the expiration date (Diamond medium was frozen). Culture tubes contained various amounts of media: 10 ml (Trichosel, Difco Kupferberg, and STS media), 5 ml (modified Diamond medium), 4.2 ml (Diamond medium), or 10.4 ml (Lash medium).

Each Monday, we enrolled in our study all women who attended the DeKalb County Sexually Transmitted Diseases Clinic. A Dacron-tipped swab was used to obtain vaginal secretions, placed into a tube containing 1.5 ml of nonbacteriostatic normal saline, swirled, and discarded. A wet mount of vaginal secretions was made by placing a drop (0.05 ml) of the mixture under a cover slip and examining it under low power (100×) for at least 15 s; this was done within 15 min of obtaining the specimen. If suspicious-looking organisms were found, they were examined under high power (400×) for confirmation as *T. vaginalis*. The tubes with inoculum were left at room temperature for up to 3.5 h, when they were vortexed, and 0.2-ml aliquots were used to inoculate the culture tubes.

Culture tubes with tight caps were incubated in an aerobic atmosphere at 35°C, the only temperature common to the instructions of all manufacturers. A 0.05-ml sample of medium from the bottom of each tube was examined for 15 s at 100× on day 1 (Tuesday), day 2 (Wednesday), day 3 (Thursday), day 4 (Friday), and day 7 (Monday); the examiner was blinded to previous results. Tubes were examined for trichomonads, yeasts, and bacteria.

Two experiments were performed. In the first, we evaluated all media except Lash medium, which we were initially unaware of. In the second, we compared Lash medium with the best-performing medium from the first study.

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TABLE 1. Culture results of wet-mount-positive and wet-mount-negative specimens in different media

Specimen type and day of reading	No. (%) of positive cultures in following medium:				
	Diamond	Modified Diamond	Trichosel Kupferberg	STS Kupferberg	Difco Kupferberg
Wet mount positive (n = 65)					
Tuesday (day 1)	42 (64.6)	45 (69.2)	8 (12.3)	11 (16.9)	17 (26.2)
Wednesday (day 2)	60 (92.3)	61 (93.9)	24 (36.9)	14 (21.5)	16 (24.6)
Thursday (day 3)	59 (90.8)	62 (95.4)	43 (66.2)	19 (29.2)	21 (32.3)
Friday (day 4)	61 (93.9)	60 (92.3)	54 (83.1)	25 (38.5)	32 (49.2)
Monday (day 7)	53 (81.5)	56 (86.2)	48 (73.9)	27 (41.5)	28 (43.1)
Wet mount negative (n = 310)					
Tuesday (day 1)	1 (0.3)	4 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
Wednesday (day 2)	8 (2.6)	9 (2.9)	1 (0.3)	0 (0.0)	0 (0.0)
Thursday (day 3)	18 (5.8)	19 (6.1)	4 (1.3)	0 (0.0)	0 (0.0)
Friday (day 4)	26 (8.4)	22 (7.1)	8 (2.6)	0 (0.0)	1 (0.3)
Monday (day 7)	32 (10.3)	25 (8.1)	17 (5.5)	2 (0.7)	3 (1.0)

We also evaluated possible differences in the sensitivity of wet-mount reading among laboratory technicians by determining for each technician the percentage of culture-positive specimens that had positive wet-mount results.

The chi-square test (without Yate's correction) was used to statistically test differences in positivity among the media.

RESULTS

One manufacturer (Carr-Scarborough) recommends that tubes be incubated anaerobically. Initially, to determine whether anaerobic incubation differed from aerobic incubation, we inoculated two tubes of Carr-Scarborough medium, with loose caps, with identical samples of secretions from 16 women with positive wet-mount results and incubated one tube aerobically and one anaerobically (in a GasPak jar [BBL]). The following day, all tubes had approximately equal growth of trichomonads; therefore, all subsequent cultures were incubated aerobically.

We enrolled 375 women in the first study. Sixty-five patients (17%) had positive wet-mount examinations, and all were confirmed by growth of *T. vaginalis* in at least one medium. Of the 310 patients with negative wet-mount examinations, 37 (12%) were positive for *T. vaginalis* by growth of the organism in at least one medium. Hence, 102 (27%) of the 375 women were infected with *T. vaginalis*, and the vaginal wet mount detected 65 (64%) of them.

Because cultures are done only for patients who have a negative wet-mount examination, we analyzed our culture results by wet-mount positivity (Table 1). No statistically significant differences were found between Diamond and modified Diamond medium, but each of these two formulations of Diamond medium significantly ($P < 0.01$) outperformed each of the three formulations of Kupferberg medium. Within the latter group, Trichosel medium was significantly ($P < 0.05$) superior to the other two formulations. Among the 102 positive cultures, 7 (7%) were positive in only one medium: 4 in Diamond medium, 2 in modified Diamond medium, and 1 in Trichosel medium.

Culture results markedly affected the apparent sensitivity of the wet-mount examination. A total of 99 of the 375 cultures were positive in Diamond medium, 92 in modified Diamond medium, 77 in Trichosel medium, 50 in Difco Kupferberg medium, and 43 in STS medium. The wet-mount sensitivities (i.e., the number positive by wet mount as a percentage of the number positive by culture) for each medium were as follows: Diamond medium, 65 of 99 (66%); modified Diamond medium, 64 of 92 (70%); Trichosel medium, 60 of 77 (78%); Difco Kupferberg medium, 47 of 50 (94%); and STS medium, 41 of 43 (95%).

The day of maximal yield depended on the vaginal wet-mount results and the specific media (Table 1). Among wet-mount-positive specimens for four media, the maximal yield was reached at day 3 or 4 and declined at day 7; the maximal yield for the fifth medium (STS) was reached at day 7. Among wet-mount-negative specimens, the maximal yield was achieved at day 7 for all media, when 33 (89%) of the 37 total positive specimens were positive; therefore 4 specimens were positive only before day 7.

Of the wet-mount-negative specimens, 2 of the 34 (6%) positive cultures in Diamond medium and 3 of the 28 (11%) positive cultures in modified Diamond medium were negative on the last day of reading but positive before then. In four of these five instances, the culture was positive on only one day (day 1 twice, day 2 once, and day 4 once). This phenomenon did not occur with the three Kupferberg media, for which no culture was negative on the last day but positive before then.

All three formulations of Kupferberg media suppressed the growth of bacteria better than did Diamond or modified Diamond medium, with modified Diamond medium allowing by far the greatest contamination. The percentages of 1,875 daily observations for each medium (375 cultures read on each of the five days) that showed contamination with bacteria were as follows: Diamond, 15.0%; modified Diamond, 31.2%; Trichosel, 14.5%; STS, 4.5%; and Difco Kupferberg, 12.3%. The presence of bacteria in the media did not appear to affect the recovery of *T. vaginalis*. For each medium, of the 102 vaginal specimens that yielded *T. vaginalis*, bacteria were present in similar percentages of tubes whether the tube contained *T. vaginalis* or not. Also, modified Diamond medium had the largest number of bacteria, yet performed nearly as well as Diamond medium in yielding *T. vaginalis*.

Unlike bacterial contamination, yeast contamination was equal in all five media. The percentages of the 1,875 observations showing contamination with yeasts were as follows: Diamond, 32.0%; modified Diamond, 30.6%; Trichosel, 32.4%; STS, 31.5%; and Difco Kupferberg, 28.2%. As with bacteria, the presence of yeasts did not appear to influence the recovery of *T. vaginalis*.

To determine whether the smaller amounts of media in the tubes containing Diamond medium (4.2 ml) or modified

TABLE 2. Culture results of wet-mount-positive and wet-mount-negative specimens in different volumes of modified Diamond medium

Specimen type and day of reading	No. (%) of positive cultures in following vol:		Significance[a]
	5 ml	10 ml	
Wet mount positive (n = 56)			
Tuesday (day 1)	37 (66.1)	31 (55.4)	$P < 0.05$
Wednesday (day 2)	52 (92.9)	44 (78.6)	$P < 0.05$
Thursday (day 3)	53 (94.6)	48 (85.7)	NS
Friday (day 4)	51 (91.7)	50 (89.3)	NS
Monday (day 7)	47 (83.9)	48 (85.7)	NS
Wet mount negative (n = 280)			
Tuesday (day 1)	2 (0.7)	0 (0.0)	NS
Wednesday (day 2)	7 (2.5)	6 (2.1)	NS
Thursday (day 3)	16 (5.7)	10 (3.6)	NS
Friday (day 4)	19 (6.8)	19 (6.8)	NS
Monday (day 7)	22 (7.9)	22 (7.9)	NS

[a] P values are shown for significant results. NS, Not significant.

Diamond medium (5.0 ml) were responsible for the greater yield of *T. vaginalis* in these media, we added a tube of modified Diamond medium containing 10 ml of medium to the protocol for examination of the final 336 specimens (Table 2). There were no significant differences in the wet-mount-negative specimens between the two media, although cultures became positive significantly faster ($P < 0.05$) in the 5-ml volumes among the wet-mount-positive samples.

In the second experiment, we compared the efficacy of Lash medium with that of modified Diamond medium. For efficiency, we studied only women with wet-mount-positive samples. All 48 women studied had a positive culture in modified Diamond medium, but only 26 (54%) had a positive culture in Lash medium.

To determine whether some laboratory technicians might be better readers of wet-mount specimens than others, we analyzed the positivity rates recorded by six laboratory technicians who examined at least eight vaginal wet-mount specimens with positive cultures (Table 3). There was considerable intertechnician variability, but the numbers were small and the differences were not statistically significant.

DISCUSSION

We found significant differences in the ability of different variations of culture media to support the growth of *T. vaginalis*. The performances of the two Diamond media were similar but superior to that of Kupferberg and Lash media. Among the three Kupferberg media, however, Trichosel was superior to STS or Difco Kupferberg. The formulations of STS and Difco Kupferberg are the same, whereas Trichosel contains yeast extract, but we performed no experiments to determine whether addition of yeast extract to the other two media would improve their performance. Yeast extract has been found to enhance the growth of *T. vaginalis* in serum-free medium, although it appears to offer no advantage in media containing serum (6). Lash medium compared poorly with modified Diamond medium, and we suspect that it would have compared poorly with the Kupferberg media, had we performed this direct experiment. Lash medium, which is quite different from formulations of Diamond and Kupferberg media, is based on casein hydrolysate and a high concentration of serum (3).

TABLE 3. Wet mount results, by technician,[a] for patients with positive cultures

Technician	No. positive/ total no. (%)[b]
A	3/8 (38)
B	4/10 (40)
C	23/36 (64)
D	12/18 (67)
E	7/9 (78)
F	11/14 (79)

[a] Including only technicians examining wet mounts from at least eight patients with positive cultures.
[b] $P \geq 0.05$.

The antimicrobial and antifungal agents in the media showed various efficiencies in suppressing contamination, yet they appeared to have little effect on the recovery of *T. vaginalis*. Modified Diamond medium allowed the greatest growth of bacteria. Like Diamond medium, it contains 1,000 U of penicillin per ml, but it contains only 150 μg of streptomycin per ml instead of the 1,000 μg/ml in Diamond medium. Kupferberg medium contains chloramphenicol. There was no difference in yeast suppression among the media, although modified Diamond medium contains an antifungal agent (2 μg of amphotericin B per ml) and the other media do not.

In our project we also sought to study the optimal days on which to read culture media. The greatest yield of positive isolates from wet-mount-negative specimens occurred on day 7. Observing cultures daily for prolonged periods is, however, time-consuming. Growth in Kupferberg medium formulations was slower than in Diamond medium formulations, and all isolates that grew in Kupferberg media were detected on day 7. For Diamond or modified Diamond medium, observing on day 7 alone detected 94 and 89% of the total positive cultures, respectively. For these media, one or two readings at days 1 to 5 and again at day 7 would be highly effective yet labor saving. Smith has also shown, using Hollander medium (not commercially available), that reading tubes at days 3 and 7 would lead to high sensitivity, since he detected 100% of isolates by reading on these two days (5). It is possible that greater yields would have been detected if we had continued observing media for longer than 7 days. Experience with the wet-mount-positive specimens, however, shows that the heavier inocula from these women caused cultures to become positive sooner and that the media were unable to support growth in some tubes by day 7.

The optimal days on which to read cultures, however, were in part medium dependent and in part volume dependent. The less sensitive media benefited from longer periods of incubation. In addition, it is possible that the use of smaller amounts of media than we used may lead to more rapid detection of culture positivity without sacrificing sensitivity. Similarly, we may have found that cultures became positive sooner had we used a larger inoculum per tube.

This study shows that the sensitivity of wet-mount examination for *T. vaginalis* varies with the culture media used, with the less efficacious media having a better wet-mount sensitivity because they can support growth only if large numbers of organisms, as found in wet-mount-positive inocula, are used. Some workers using the culture media that we found to be less sensitive (Kupferberg media) have con-

cluded that culture offers no advantage over wet-mount examination (7). Others using the culture media that we found to be more sensitive might not agree (2). We suspect, however, that the technician performing the wet-mount examination is also important in determining wet-mount sensitivity. Although we did not find a statistically significant difference among the results obtained by technicians reading wet mounts in our study, the differences observed suggest that some technicians are better observers than others (4). Such differences could be due to training, experience, and/or time spent examining the specimens.

Our wet-mount sensitivity, which was 64% of the 102 positive cultures, was also potentially influenced by technical factors. First, we greatly diluted our vaginal swab specimen with 1.5 ml of saline. Second, although we examined vaginal wet-mount slides for at least 15 s, longer periods of observation may be more efficacious. We found, however, that the wet mount of vaginal secretions was 100% specific, confirming that culture of wet-mount-positive vaginal specimens is unnecessary if well-trained technicians perform the examination.

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