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Comparison of Four Methods To Detect *Trichomonas vaginalis*

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Four methods for the detection of *Trichomonas vaginalis* in vaginal secretions from 88 symptomatic patients were compared: wet-mount examination, Kupferberg liquid medium, Hirsch charcoal agar, and the Papanicolaou smear. Hirsch diphasic slants and Kupferberg medium did not significantly differ in sensitivity from direct examination of wet mounts. The Papanicolaou smear identification of trichomonads was found to be the least sensitive method evaluated.

Although several reports claim that culturing is the best method for detecting *Trichomonas vaginalis*, many scientists feel that the minimally increased yields do not justify the increased cost involved with culturing (1, 3, 7, 8; J. L. Thomason, L. M. Wilcoski, and C. A. McLaughlin, Clin. Microbiol. Newsl. 8:9–12, 1986). Commercial laboratories do not routinely do cultures for trichomonads. We wished to ascertain the degree to which culturing was superior to direct observation of the motile protozoan in a wet-mount preparation or to examination of fixed specimens in a Papanicolaou (Pap) smear from symptomatic patients in a low-risk obstetrical-gynecological population. We wished to test both the medium commercially approved for diagnostic use, Kupferberg simplified Trypticase serum, and a newer medium, Hirsch charcoal agar diphasic slants, to see how they compared with each other, with wet mounts, and with Pap smears. Our goal was to demonstrate the cost-effectiveness of culturing in this setting.

Hirsch medium, which was originally designed for mycobacteria and later used for amoebae, was prepared as described by McQuay (4). Immediately prior to inoculation with specimens, 1,000 U each of penicillin and streptomycin was added per tube (5). We chose Hirsch medium as a possible alternative to Kupferberg for two reasons. First, Hirsch medium was recommended by Melvin and Brooke (5) for cultivation of intestinal protozoans, and we reasoned that trichomonads, being protozoans, should grow well on this medium. Second, although we made our own medium for this trial according to the instructions of Melvin and Brooke, the medium was available commercially in dehydrated form as Hirsch Charcoal Agar Modified (BBL Microbiology Systems, Cockeysville, Md.). Kupferberg medium was obtained premade in tubes from Remel (Lenexa, Kans.) and was used according to the instructions of the manufacturer. Appropriate quality control to show the effectiveness of the media for organism recovery was maintained by using clinical isolates inoculated at a concentration of 10^4 motile *Trichomonas* cells per ml.

A total of 88 sexually active women with complaints about vaginal discharge who were attending a family planning clinic were tested for *T. vaginalis*. Ages ranged from 14 to 40. The mean age was 20.9, and the median age was 17. Although all patients had vaginal discharge complaints, only approximately half (43) actually presented with a discharge at the vaginal introitus upon physical examination. A listing of patient complaints and physician observations is given in Table 1. After insertion of a sterile speculum, specimens from vaginal secretions were obtained on five cotton-tipped applicators, four of which were premoistened with sterile physiological saline. These four swabs were not identified so that when they were subsequently placed in appropriate media or used to make stains there would be randomization of sampling. One dry cotton applicator was used to measure vaginal pH on phenaphthazine paper (E. R. Squibb & Sons, Princeton, N.J.). Another applicator was expressed onto a glass slide, covered, and immediately examined first at ×100 and then at ×400 for motile trichomonads. Examination was thorough, with over 100 fields being examined before a specimen was considered negative. All positive reports were confirmed by a second observer. Bacterial vaginosis was diagnosed if any three of the following four characteristics were present: lactobacillus morphotypes < background bacteria, clue cells, pH of >4.5, or positive amine odor (9). Swab 3 was inserted into a tube of Kupferberg medium. The bottom portion of the swab was broken off into the tube. Similarly, inoculation of swab 4 into Hirsch medium was performed, and the swab was used to streak the agar slant before it also was broken. Cultures were incubated at 37°C and were examined at 2, 4, and 7 days by using exactly the same procedure used for examination of wet mounts of vaginal secretions. A drop of culture from the bottom of the tube was transferred to a slide and covered. Tubes were not centrifuged prior to sampling. These slides were examined first under ×100 and then under ×400. Any slides with motile trichomonads were considered positive. A total of 50 fields were examined (at ×100) before the culture was considered negative. Swab 5 was used to prepare Pap smears.

Trichomonads were identified in 37 patients (42%) either by wet mount or by positive culture, with 31 specimens positive by both methods. Kupferberg medium detected 32, while Hirsch medium detected 30. Four cases detected by Kupferberg medium were not detected by Hirsch medium, while two cases detected by Hirsch medium were missed by Kupferberg medium. Wet mount detected 34 positive specimens, but three of them were not confirmed by either the culture method or the Pap smear method, which detected only 26 cases (34%). Overall, observation of wet mounts by well-trained individuals who thoroughly examined each slide and reported only motile trichomonads confirmed by a second observer was as accurate as culture methods. Only when data from both culture methods were combined in our study was the culture method as accurate as wet-mount examination. However, when the culture and wet-mount methods were combined, the greatest sensitivity was ob-

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TABLE 1. Comparison of diagnoses in 88 patients with symptoms of *T. vaginalis*

Characteristic	No. of patients when *T. vaginalis* was:	
	Absent (n = 51)	Present (n = 37)[a]
Symptoms		
Itching	5	14
Burning	1	9
Other complaints	5	14
Signs		
Introital discharge	14	29
Odor	15	27
Inflamed cervix	18	27
Microscopic analysis		
Leukocytes > epithelial cells	27	34
Clue cells	32	25[b]
Bacterial vaginosis	16	32
pH > 4.5	18	32

[a] $p < 0.005$, except where indicated, by chi-square with Yates correction.
[b] Not significant.

tained. Pap smear examination was far less sensitive than either culture or wet mounts ($P < 0.005$; McNemar test).

We conclude from this study that Hirsch medium is equally as effective as Kupferberg medium ($P > 0.05$; McNemar test), but neither represents a "gold standard" for trichomonad isolation from vaginal secretions. While many investigators consider culture methods a "gold standard" with which all other methods must be compared, virtually all previous studies reveal that some specimens will contain motile trichomonads that will not grow in culture medium (2, 6; J. G. Lossick, Editorial, J. Am. Med. Assoc. **259**:1230, 1988). We agree with Krieger et al. that specimens which are positive by wet mount represent true positives even though the trichomonads fail to grow in culture medium (2). This is probably due to the fact that a large number of organisms must be inoculated to initiate proliferation of the protozoans in the medium (6). In this study, wet mounts and culture methods both performed at 86% sensitivity, which made the cost-effectiveness of the culture method unacceptable. Culture costs far exceed those of a wet-mount examination. In addition to the cost of culture medium, a maximum of three additional wet-mount examinations from the culture medium would be required per specimen. Admittedly, any evaluation of direct microscopic examination of specimens depends on the thoroughness and skill of the practitioner (Lossick, Editorial). Our results may not be reflective of a clinic where painstaking examination of the wet mounts is not possible, nor is wet-mount examination feasible in clinics where microscopes are not available. A review of current concepts concerning *Trichomonas* recovery and identification was recently presented (Lossick, Editorial). Sluggishly motile trichomonads or low numbers of trichomonads can be missed easily when they are in the presence of large numbers of leukocytes. Douching prior to physical examination can also lower the detection of motile trichomonads (1). Currently, Kupferberg simplified Trypticase serum is the only commercially available medium approved for diagnostic use for the isolation of *T. vaginalis*. It appears from our study and preliminary studies by others (G. Schmid, L. Matheny, A. Zaidi, and S. Kraus, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 733, 1987) that this medium is not as sensitive as one would like for the purpose of identifying *T. vaginalis*.

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