Evaluation of Acridine Orange Stain for Detection of 
Trichomonas vaginalis in Vaginal Specimens

J. R. GREENWOOD1,2* AND KATHLEEN KIRK-HILLAIRE1

Public Health Laboratory, County of Orange, Santa Ana, California 92702,1 and Division of Epidemiology, 
School of Public Health, University of California, Los Angeles, California 900242

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Vaginal exudates from 105 patients were examined by direct wet-mount microscopy and acridine orange stain for the presence of Trichomonas vaginalis. Both procedures demonstrated greater than 90% agreement in both sensitivity and specificity. When specimens can be examined immediately after collection, it appears that wet-mount microscopy is almost as sensitive as acridine orange staining for detection of T. vaginalis.

Although numerous methods are presently available to detect Trichomonas vaginalis in vaginal exudates (1–3, 5), direct microscopic examination of clinical material continues to be one of the most rapidly performed and least expensive techniques in use. However, in a recent comparison of T. vaginalis detection methods (4), acridine orange (AO) staining was shown to be significantly more sensitive than wet-mount examination. Because of the striking increase in positive specimens reported with AO staining (8.5% versus 2.4% for wet mount), we chose to evaluate this procedure for possible use in our laboratory.

Vaginal specimens were collected on a swab (Culturette, Marion Scientific) and then submitted to the laboratory within 20 min of collection. Each swab collected was used to prepare a wet mount and also a thin smear on a 22-by-40-mm cover slip. Wet mounts were examined immediately for T. vaginalis exhibiting typical motility. Thin smears were air dried and then stained with AO by the method of Fripp et al. (3). Immediately after staining, smears were mounted on clean slides with the smear side down and then observed with a Zeiss fluorescence microscope equipped with a BG 12 exciter filter and a combination of number 44 and 53 barrier filters.

When observed under ultraviolet light, T. vaginalis trophozoites stained with AO are brick red with a yellowish-green, elongated or round nucleus. Yeast cells also stain red, but can be differentiated from T. vaginalis on the basis of morphology. Epithelial cells fluoresce light yellow-green with a bright yellow-green nucleus, and leukocytes only show slight yellow-green nuclear fluorescence.

Of 105 vaginal specimens examined, 15 (14.3%) were found by wet mount to be positive for T. vaginalis. AO stain also showed all of these same 15 to be positive, plus one additional specimen (16 or 15.2%). Thus, there was 93.8% agreement with positive specimens and 98.9% agreement with negative specimens.

Our results indicate that vaginal exudates, when examined by wet mount immediately after collection, can yield results comparable to AO staining. In addition, wet-mount examination continues to be a faster procedure and doesn't require use of a fluorescence microscope, which is frequently unavailable to smaller laboratories or clinics. AO stain would be a useful procedure, however, for those clinics that have no on-site laboratory services and must transport specimens to a central laboratory.

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LITERATURE CITED


