Detection of *Trichomonas vaginalis* in Vaginal Specimens by Direct Immunofluorescence Assay

RODNEY F. SMITH

Contra Costa County Public Health Laboratory, Martinez, California 94553

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Vaginal discharge specimens from 105 women were examined by wet mount, culture, and a new direct immunofluorescence assay to detect *Trichomonas vaginalis*. The organism was detected by culture in 31 patients, by direct immunofluorescence in 26 patients, and by wet mount in 21 patients.

The isolation of *Trichomonas vaginalis* has been shown to be both more sensitive and specific than direct methods, including the wet mount, Papanicolaou smear, acridine orange, and numerous clinical parameters (2-6, 9-11). With the advent of monoclonal antibodies, highly sensitive and specific immunofluorescence assays are being developed for the direct detection of pathogens in clinical specimens. Chang et al. (1) recently prepared such a conjugate for *T. vaginalis* which is now available as a commercial kit. The purpose of this study was to compare a direct immunofluorescence assay (DFA) kit to wet mounts and culture for the detection of *T. vaginalis*.

A group of 105 women who attended a clinic for sexually transmitted diseases were studied. Each patient received a pelvic examination. Vaginal discharge material from the posterior vaginal fornix was removed with a sterile cotton-tipped swab for three consecutive procedures. First, a smear of the discharge the size of a dime was placed on a glass slide and air dried at room temperature for the DFA. A second droplet of discharge was mixed with saline on another slide for the wet mount. Third, discharge was removed with the same swab and placed in a tube containing 8 ml of modified Hollandier medium.

The wet mount was immediately examined by a 3-min scan using ×100 microscopy with spot checks as needed at ×400 to detect motile trichomonads. The medium was incubated at 35°C for 7 days and examined daily by wet mount for motile trichomonads. The medium was made from basic ingredients (7) and is not commercially available. The smallest inoculum that could produce a positive culture in this medium was between 1 and 5 cells per ml (8). After air drying, the DFA slide was fixed in acetone. Both positive and negative control slides were prepared in the same way. The fixed smear was covered with *Trichomonas* direct specimen test reagent (Integrated Diagnostics, Berkeley, Calif.) and incubated at room temperature in a moist chamber for 20 min. Excess reagent was removed, and the slides were rinsed in deionized water and air dried. Each slide was mounted and examined with an epifluorescence microscope. Trichomonads appeared as apple-green fluorescence-stained cells with visible flagella and nuclei. The sensitivity of the DFA is at least 300 trichomonads per ml.

In this comparative study (Table 1) on women with signs of vaginitis, 31 (29.5%) were positive by culture, 26 (24.7%) were positive by DFA, and 21 (20%) were positive by wet mount. Compared with culture, the DFA had the following: sensitivity, 80.6%; specificity, 98.6%; predictive positive value, 96.1%; and predictive negative value, 92.4%; agreement between the two tests was 93.3%.

It was not possible in the clinical setting of this study to change the sequence in which the assays were prepared, for example by inoculating the medium first or altering the slide preparation sequence. Complete randomizing of the three assays into six sequences was not possible; it would have required a much larger study population. Such a study may have affected the results. However, even with the culture taken last, the ability of the medium to detect 1 to 5 *Trichomonas* cells per ml explains the greater sensitivity of culture over the DFA, which may not detect fewer than 300 cells per ml (Integrated Diagnostics product insert). Fouts and Kraus (2) also found that approximately 1,000 *Trichomonas* cells per ml of discharge were required to produce a positive wet mount.

Although the wet mount is obviously the least sensitive of the three tests performed, it is indispensable in the clinical setting for simplicity and speed in confirming positive cases of trichomoniasis and in initiating treatment. The Papanicolaou smear has been proposed as a cost-effective means of screening women who harbor *Trichomonas* organisms (11) and can provide additional information about the patient. Under some circumstances, this test for *T. vaginalis* may not be available or appropriate for rapid results. The DFA can be performed in a clinic within 30 min if a wet mount is negative. The DFA is a useful alternative to the more time-delayed culture method. In this study, of 10 wet-mount-negative, culture-positive specimens, 8 were positive between 48 and 72 h and 2 cultures were positive at 4 days.

In this laboratory, I (8) previously determined that the cost of a wet mount is $5.40 and the cost of a culture is $6.75. With a 5-min scan of a DFA slide that is then deemed to be negative, the cost of a DFA is about $7.00. Information on the cost of a Papanicolaou smear was not available. The DFA slide does not require immediate testing and may be held up to 6 days before being stained and examined (Integrated Diagnostics product insert).

**TABLE 1. Comparative methods for detection of *T. vaginalis***

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<thead>
<tr>
<th>No. of specimens</th>
<th>DFA</th>
<th>Wet mount</th>
<th>Isolation</th>
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<tr>
<td>20</td>
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Additional studies with the DFA on asymptomatic patients are required to further determine the application of the DFA as a screening test.

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LITERATURE CITED