JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1988, p. 1684–1686 Vol. 26, No. 9
0095-1137/88/091684-03$02.00/0
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Monoclonal-Antibody-Based Enzyme-Linked Immunosorbent Assay for *Trichomonas vaginalis*

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Received 13 January 1988/Accepted 23 May 1988

Trichomonas vaginalis **is estimated to infect 4 million women per year in the United States. The diagnosis of trichomoniasis is predominantly achieved by direct microscopic examination of vaginal exudates. This subjective diagnostic procedure is reported to be 75% sensitive under ideal circumstances. We have developed an enzyme-linked immunosorbent assay (ELISA) for the detection of *T. vaginalis* directly from vaginal exudates. The ELISA employs a monoclonal antibody specific for a 65-kilodalton surface polypeptide of *T. vaginalis* as the capture antibody in a sandwich format. A polyclonal rabbit anti-*T. vaginalis* antibody labeled with horseradish peroxidase serves as the probe. An evaluation of vaginal specimens from women attending clinics revealed a sensitivity and specificity of the ELISA of 89 and 97%, respectively, versus the culture technique. These results indicate the usefulness of this ELISA as an alternative to microscopic and culture methods for the detection of *T. vaginalis* in vaginal exudates.**

Trichomoniasis is among the most common sexually transmitted diseases known to afflict men and women worldwide. The causative organism, *Trichomonas vaginalis*, is a flagellated protozoan which infects the urogenital tract. The disease, which often results in vaginitis (6), is the cause of significant morbidity among women. In symptomatic women, trichomonal vaginitis is characterized by inflammation of vaginal epithelium, foul-smelling discharge, severe discomfort, and tissue cytopathology (7).

Several methods have been reported for the detection of *T. vaginalis* in vaginal exudates. All, except the culture method, suffer from a lack of sensitivity. The most commonly used method, direct microscopic examination (wet mount), has been reported to be only 50 to 75% as sensitive as the culture method (5, 9, 10, 12). The culture method, which takes days to complete, is performed by relatively few laboratories. This is presumably due to the laborious nature of the technique and the cost of the essential reagents. Clearly, a need exists for a rapid, specific, and sensitive method for the detection of *T. vaginalis*.

The generation of a monoclonal antibody specific for a 65-kilodalton surface polypeptide of *T. vaginalis* has led us to the development of the enzyme-linked immunosorbent assay (ELISA) for the detection of *T. vaginalis* directly from vaginal exudates. The utilization of this monoclonal antibody as the capture antibody in a sandwich ELISA format provided the required broad specificity necessary for the detection of *T. vaginalis* in clinical specimens. In this report, we describe a rapid, sensitive, and specific microtiter ELISA which is comparable to the laborious and time-consuming culture method for the diagnosis of trichomoniasis in women.

MATERIALS AND METHODS

Patient samples. Thirty-six unselected women attending the affiliated clinics of the Hahnemann University Hospital Department of Obstetrics and Gynecology and the Sexually Transmitted Diseases Clinic of the Charlottesville Albemarle Health Department were enrolled in the study. Specimens of vaginal exudates for wet-mount preparation, culture, and ELISA for *T. vaginalis* were obtained during the pelvic examination. The specimens were collected on cotton swabs from independent quadrants of the vaginal fornices.

Microscopy. For the wet-mount examination, the swab was agitated in 1 to 2 ml of saline and 1 drop of the resultant suspension was transferred to a microscope slide. A cover slip was applied, and the slide was evaluated by phase-contrast microscopy at ×100 magnification. At least five fields were examined for the presence of motile trichomonads.

Culture. The swabs for culture were processed by either of the following methods. In one method, the swab was briefly agitated in 2 ml of Feinberg Wittington medium supplemented with 8% heat-inactivated horse serum–penicillin G (1,000 U/ml)–gentamicin (100 μg/ml)–nystatin (25 μg/ml) (4). The tubes were subsequently held at room temperature for up to 3 h until they were incubated anaerobically in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.) for 48 h. Initially, negative cultures were incubated for an additional 24 h and reexamined. In the other method, the swab for culture was briefly placed in a capped, sterile plastic tube (13 by 75 nm) containing 5 ml of sterile BI-S-33 medium containing streptomycin, penicillin, and kanamycin adjusted to pH 6.0 (2). The swabs were removed, and the caps were tightly applied. The tubes were incubated at 37°C. Specimens were examined for the presence of motile trichomonads at 24-h intervals over a 1-week period. For the ELISA, the swab was immersed in 1 ml of 0.01 M sodium phosphate buffer containing 0.15 M NaCl (pH 7.0), frozen at −70°C, and transported on dry ice to the laboratory for testing. The samples were thawed at room temperature, the fluid was expressed from the swab, and a sample was used in the ELISA procedure.

Monoclonal antibody. A mouse monoclonal antibody, des-

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TABLE 1. Sensitivity of ELISA for *T. vaginalis*

Trichomonads[a]/well	OD_{490} (nm)[b]
0	0.052
85 ± 9	0.084
212 ± 21	0.231
850 ± 85	0.475
2,125 ± 213	0.870

[a] Isolate RU375.
[b] Mean of duplicate wells run in three separate assays. OD_{490}, Optical density at 490 nm.

ignated DM116, was produced and characterized by standard procedures as described previously (1). Briefly, this immunoglobulin G1 (IgGl) monoclonal antibody has been shown to bind specifically to a 65-kilodalton surface polypeptide of *T. vaginalis* isolates.

Polyclonal antibody. Polyclonal antibodies to *T. vaginalis* were elicited in rabbits by multiple-site intramuscular immunization with 10^7 whole trichomonads emulsified in Freund complete adjuvant (Difco Laboratories, Detroit, Mich.). Booster injections were given 2 weeks later with equal numbers of organisms in Freund incomplete adjuvant. Rabbits were bled 14 days after the booster immunization, and the sera were stored at −20°C until use.

Polyclonal antibody purification. Purified IgG was obtained from the rabbit antiserum by sequential ammonium sulfate precipitation and ion exchange chromatography. An equal volume of saturated ammonium sulfate was added dropwise to the serum with continuous stirring at 4°C. The precipitate was formed by further stirring at 4°C for 2 h. The suspension was centrifuged at 1,000 × *g* for 30 min at 4°C, and the precipitate was dissolved and dialyzed against 0.02 M sodium phosphate buffer (pH 6.3). Any insoluble material was removed by centrifugation at 1,000 × *g* for 30 min at 4°C. The supernatant was applied to a column of DE-52 cellulose (Whatman, Inc., Clifton, N.J.) previously equilibrated with 0.02 M sodium phosphate buffer (pH 6.3). The purified IgG was eluted in the void volume, and it was concentrated and dialyzed against 0.01 M sodium phosphate buffer (pH 7.0). The concentration of IgG was determined by the optical density at 280 nm (extinction coefficient [1%] = 13.5). The purified anti-*T. vaginalis* IgG was conjugated to horseradish peroxidase essentially as described by Duncan et al. (3).

Solid-phase antibody preparation. Polystyrene microtiter plates (Immulon II; Dynatech Laboratories, Inc., Alexandria, Va.) were coated with the monoclonal antibody (DM116) by adding 0.100 ml of mouse ascites fluid, diluted 1: 1,000 in 0.01 M sodium phosphate buffer (pH 7.0), to individual wells followed by an overnight incubation at room temperature. The wells were washed three times by the addition of 0.250 ml sodium–0.01 M phosphate buffer (pH 7.0) containing 0.15 M sodium chloride and 0.02% Triton X-100. The individual wells were subsequently blocked by incubation with 1% bovine serum albumin in 0.01 M sodium phosphate buffer (pH 7.0) for 2 h at room temperature. The wells were then washed three times as described above.

ELISA procedure. Assay of clinical specimens and cultured *T. vaginalis* isolates involved the addition of 0.050 ml of sample to the antibody-horseradish peroxidase conjugate appropriately diluted in 0.01 M sodium phosphate buffer (pH 7.0) containing 0.015 M sodium chloride and 1.5% bovine serum albumin. After a 30-min incubation at room temperature, the wells were washed three times with 0.250 ml of phosphate buffer containing 0.02% thimerosal and 0.02% Triton X-100. Then, 100 μl of 1.25 M 3,3′,5,5′-tetramethylbenzidine solution in 50% methanol–50% 0.1 M citrate buffer (pH 5.0) containing 0.01% hydrogen peroxide was added to each well. After 10 min at room temperature, the resulting blue color was read in a microtiter plate reader at 650 nm. Specimens were run in duplicate. Samples with an absorbance greater than or equal to the mean plus 2 standard deviations of the culture-negative samples were considered positive. The intra- and interassay variabilities were 10 and 15%, respectively.

TABLE 2. Detection of *T. vaginalis* in vaginal exudates

Method	No. positive	% Sensitivity[a]	% Specificity[b]
Clinical diagnosis	36	100	100
Culture	35	97.2	100
Wet mount	22	61.1	100
ELISA	32	88.9	96.7

[a] (Observed positives/clinical positives) × 100.
[b] (Observed negatives/clinical negatives) × 100.

RESULTS

Sensitivity and specificity of ELISA. The sensitivity of the assay was determined by measuring the response of known concentrations of a cultured *T. vaginalis* isolate. The data in Table 1 indicates that the assay was capable of detecting 85 trichomonads in a 0.050-ml sample. This result indicates that the ELISA is less sensitive than the theoretical sensitivity of the culture method yet more sensitive than the reported sensitivity of the wet mount (14). The assay was demonstrated to be specific for *T. vaginalis* in that no cross-reactivity was observed when *Candida albicans* or *Gardnerella vaginalis* were assayed at concentrations of 50,000 organisms per ml.

Clinical samples. The results of the clinical samples assayed by ELISA are summarized in Table 2. The distribution of the results is depicted in Fig. 1. A positive clinical diagnosis for trichomoniasis was assigned to samples which were either wet-mount positive or culture positive. Of the 36 clinical samples tested from 36 untreated patients, 35 were culture positive and 22 were wet-mount positive for *T. vaginalis*. By comparison, 32 samples were positive for *T. vaginalis* by the ELISA. Of the 30 clinically diagnosed

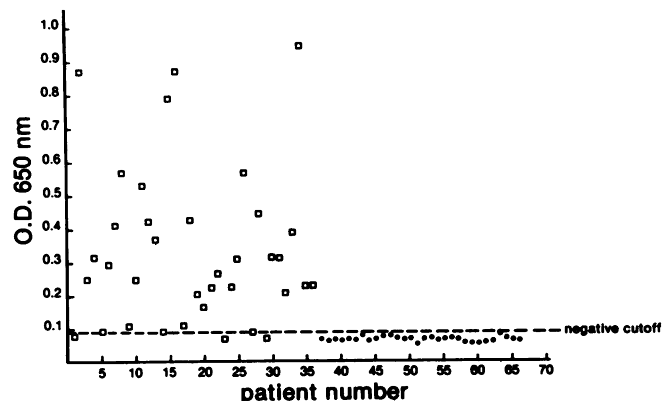

FIG. 1. Distribution of the absorbance values of the clinical samples assayed in the ELISA. Both culture-positive (□) and culture-negative (●) samples were assayed. The negative cutoff value was defined as the mean plus 2 standard deviations of the value of the culture-negative samples.

negative samples (i.e., neither wet-mount nor culture positive), 29 were negative by the ELISA. Thus, the sensitivity and specificity of the ELISA compared with clinical diagnosis was 88.9 and 96.7%, respectively. This exceeds the performance of the wet-mount procedure and compares favorably with the culture method.

DISCUSSION

A rapid microtiter plate ELISA for the detection of *T. vaginalis* in vaginal exudates has been developed. The assay utilizes a mouse monoclonal antibody specific for a 65-kilodalton polypeptide as the capture antibody and a rabbit polyclonal antibody conjugated to horseradish peroxidase as the probe. The sensitivity of this assay far exceeded that achievable with the extensively used wet-mount method. In addition, we have demonstrated comparable sensitivity and specificity of the ELISA to the laboratory standard culture method. The ability to assay vaginal exudates for the presence of *T. vaginalis* in less than 1 h makes the ELISA an attractive alternative to the time-consuming culture methods.

Several laboratories have generated monoclonal antibodies to *T. vaginalis* (8, 13). Each report indicated that because of the apparent heterogeneity of *T. vaginalis* isolates no one monoclonal antibody has, under the conditions described, been capable of binding all clinical isolates tested. In a limited study, we have demonstrated the capability of detecting nearly 90% of the culture-positive clinical isolates tested. Collection of the vaginal exudates on a cotton swab and elution of this organism into a phosphate-buffered saline solution apparently either exposes or releases the common epitope necessary for binding with the antibody. Some previously ascribed heterogeneity of *T. vaginalis* may be defined in terms of the inaccessibility of epitopes to antibody binding rather than the absence of antigen from the trichomonal surface. The specimen collection process and assay conditions apparently permit the binding of the monoclonal antibody to a common antigen. Further experimentation is necessary to elucidate the precise mechanism.

Two of three culture-positive samples which were negative by the ELISA were also negative by wet mount. This finding suggests that the concentration of trichomonads in the sample is critical to the performance of the ELISA. For this study, the order of collection of vaginal swabs was not stipulated and this may be reflected in these results. This study also identified one sample which was wet-mount positive and culture negative yet was positive in the ELISA. The ELISA will detect nonviable as well as viable trichomonads. In some clinical settings in which, for example, delivery of the specimen to the laboratory may be delayed, nonviability of trichomonads may be an important factor in choosing assay methods.

Alternatives to the widely used wet-mount procedure for the diagnosis of trichomoniasis have recently been reported. Direct (11) and indirect (8) immunofluorescence assays using monoclonal antibodies have been described. While reportedly more sensitive than the wet mount, these assays require the use of a fluorescence microscope and a trained microscopist to process each specimen individually. Others have reported the use of an ELISA for the detection of *T. vaginalis* antigens in clinical specimens. One report (14) described an assay as an alternative to the wet-mount procedure yet fell short of providing an assay to replace the laborious culture method. A second report (15) described an assay which had comparable sensitivity to the culture method yet needed four sequential incubation periods totaling 4 h before a result could be reported. Both previously described ELISAs utilized affinity-purified polyclonal antibody to achieve the sensitivity and specificity reported. The assay described herein represents the first report of the use of a monoclonal antibody in an ELISA for the direct detection of *T. vaginalis*. The results presented here suggest the use of this ELISA as a rapid assay alternative for the diagnosis of trichomoniasis.

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