

Evaluation of an enzyme-linked immunosorbent assay for the detection of antibody to *Trichomonas vaginalis* in sera and vaginal secretions

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SUMMARY Using a whole-cell antigen antibody to *Trichomonas vaginalis* was measured by an enzyme-linked immunosorbent assay (ELISA). IgG antibody was found in sera from only three of 99 children under 12 years of age. In contrast, serum IgG or IgM antibody or both were detected in 80·4% of women who had vaginal trichomoniasis and in 13·7% of uninfected women. Although antibody was found in cervical and vaginal secretions, the correlation between current infection and the presence of antibody was poorer than found between circulating antibody and infection. IgG or IgA antibody or both was detected in the secretions of 73·2% and 41% of infected and uninfected women respectively. This may be accounted for, at least partly, by previous infection since antibody, circulating or local, was found most often in women who had a history of trichomoniasis. There was no indication that some other vaginal micro-organism stimulated antibody directed against *T vaginalis*.

Introduction

Although none has advantages over existing diagnostic microscopy and cultural methods many serological techniques (for example, haemagglutination, complement-fixation, immunofluorescence, and radioimmunoassay) have been used to measure antibody to *Trichomonas vaginalis*.¹ The enzyme-linked immunosorbent assay (ELISA) has been used to study and diagnose many parasitic diseases²⁻⁵ and has proved to be a rapid and sensitive technique. We have developed an ELISA for detecting antibodies to *T vaginalis* and have found IgG and IgM antibodies in the sera of 63% and 18·5% respectively of women with trichomoniasis.⁶ In addition, IgG and IgA antibodies were detected in vaginal secretions. The aim of the present study was to evaluate further this method of detecting antibodies and to compare the serological results with those obtained by testing vaginal and cervical secretions.

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Patients and methods

Seventy-three women who attended the special clinic at St Mary's Hospital, London, were examined. Material for culture for *T vaginalis* was obtained from the posterior fornix of the vagina of women in whom the organism had been detected by microscopic examination of a saline mount of secretion. The swabs on which vaginal material was collected were inoculated into 4 ml of medium (Oxoid No 2) which was incubated at 37°C for up to 14 days. Cultures of *T vaginalis* were mixed with dimethyl sulphoxide (final concentration 5%), distributed in 0·2 ml aliquots, and cooled immediately at 1°C/min to -100°C before being stored in liquid nitrogen.

For local antibody studies high vaginal and cervical secretions were collected from 31 of the 73 women. Surgical sponges (Weck-cel, Edward Weck Co Ltd, North Carolina, USA), measuring 0·5 × 0·5 cm, were soaked in secretion and then stored for convenience at -196°C. Blood for the estimation of serum antibodies was collected from all 73 of the women and the sera stored at -70°C.

Cervical secretions were collected from 110 women who attended the Department of Genitourinary

Medicine, Royal Infirmary, Edinburgh. Secretion was aspirated gently through a sterile polyethylene capillary tube attached to a 5-ml syringe containing 1 ml sterile physiological saline, then ejected into a sterile polyethylene container.⁷ Blood was collected from a different group of 136 women. The diagnosis of trichomoniasis in both these groups of women was based on microscopical examination of a wet preparation of vaginal secretion. The secretions and sera were kept at -20°C until tested for antibody.

Sera were also obtained from 99 children under 12 years of age who had various non-infectious conditions and attended Northwick Park Hospital, Harrow.

After completion of the antibody assays the case records of 31 women from whom secretions had been obtained were examined and the following information recorded: duration of symptoms, degree of inflammation, presence of additional venereal infections, and past history of *T vaginalis* infection.

ANTIGEN

A frozen culture of *T vaginalis* was thawed rapidly and the trichomonads inoculated into 10 ml Diamond's medium⁸ without agar. After 48 hours at 37°C the cells were washed twice in phosphate-buffered saline (PBS, pH 7.2) and then twice in carbonate buffer (pH 9.6) before being resuspended in carbonate buffer at a concentration of 10^7 cells/ml. The suspension was either sonicated briefly on three occasions, after which whole cells could not be detected, or kept as whole cells. The sonicate and whole-cell suspension were stored at -70°C .

CONJUGATED ANTISERUM

Antihuman globulin, prepared in goats and conjugated with alkaline phosphatase, was obtained from Sigma (London). A preliminary titration of the conjugated antiserum was performed to determine the optimum concentration for the ELISA. The dilution chosen was that which provided the best resolution between antibody-positive and antibody-negative sera.

ELISA TECHNIQUE

Antigen, diluted 1/100 in carbonate buffer, was placed in $50\ \mu\text{l}$ amounts in each well of a micro-ELISA plate (Dynatech) and adsorption allowed to occur overnight at 4°C . The plate(s) was then subjected to three five-minute washes in PBS containing 0.1% Tween 20.

Sera were diluted serially fourfold in PBS-Tween. Secretions were expressed from the sponges into $50\ \mu\text{l}$ of PBS-Tween containing 4% bovine serum albumin (BSA) to give an approximate twofold dilution and then further diluted fourfold to produce final

dilutions of 1/8 to 1/512. The diluted sera or secretions, in $50\text{-}\mu\text{l}$ volumes, were added to the wells and the plates kept for two hours at 4°C , after which they were washed again three times in PBS-Tween. A $50\text{-}\mu\text{l}$ volume of conjugated antiserum, diluted 1/1000 in PBS-Tween containing 4% BSA, was then added to each well and the plates kept for three hours at 4°C , after which they were washed as before.

The solution of phosphatase substrate was freshly prepared before each test by dissolving one substrate tablet (Sigma 104) in 5 ml of substrate buffer (Don Whitley) to give a solution containing 1 mg/ml of p-nitrophenyl phosphate. A $50\text{-}\mu\text{l}$ volume of this solution was added to each well and after 30-40 minutes at 22°C the reaction was stopped by adding $20\ \mu\text{l}$ of 1 mol/l NaOH. The absorbance value of the yellow product was read at 405 nm on a Titertek Multiskan (Flow Laboratories).

The antibody titre was taken as the dilution of the serum or secretion at which the optical density value for the sample was greater than that of the mean of 10 antibody-negative samples $+2.5\ \text{SD}$. on this basis, we considered a titre of ≥ 32 as indicating antibody.

Results

TESTS WITH HOMOLOGOUS AND HETEROLOGOUS ANTIGENS

T vaginalis was isolated from 20 of the 73 women examined at St Mary's Hospital. Antigen was prepared from each of these cultures. Serum from each culture-positive woman was diluted serially in twofold steps and tested against the homologous antigen and against seven of the heterologous antigens. The results are given in table I.

The antibody titres were greater with some antigens than with others, not necessarily the homologous ones. Antigen prepared from three isolates (case Nos 76835, 77961, and 87616) against which the highest antibody titres were demonstrated were combined in equal amounts and used for tests on the remaining samples. Antibody titres were approximately twofold higher with the antigen stored as whole cells than with the sonicated antigen, so the former was used for all further tests.

TESTS ON SERA

Sera from children

IgG antibody titres of ≥ 32 were found in only three (3%) of 99 children's sera; IgM antibody titres ≥ 8 were not found.

Sera from women

Forty-one of the 209 women from whom blood samples were obtained had *T vaginalis* infection.

TABLE I Titres of IgG antibody to *T vaginalis* obtained by testing eight isolates against homologous and heterologous human sera

Antigens from case Nos:	Reciprocal titres of sera from cases Nos:							
	57886	68471	75187	76835	77961	83515	87553	87616
57886	64	8	32	1024	128	64	64	256
68471	128	<8	32	512	128	64	64	64
75187	128	<8	32	512	256	64	64	256
76835	512	8	128	4096	1024	512	256	512
77961	128	8	64	2048	1024	512	128	64
83515	128	8	32	2048	512	128	128	32
87553	128	<8	64	512	256	128	128	512
87616	512	8	128	4096	1024	256	256	512

Serum IgG antibody at a titre of ≥ 32 was detected in 28 (68.3%) of the 41 infected women and in 20 (11.9%) of the 168 women who had no evidence of vaginal trichomoniasis. IgM antibody at a titre of ≥ 8 was found in the sera of nine (21.9%) infected women and five (3%) of the apparently uninfected women. Thus, 33 (80.5%) of the infected women and 23 (13.7%) of the uninfected women had either IgG or IgM antibody to *T vaginalis* in their sera.

TESTS ON SECRETIONS

Antibody of IgG and IgA classes was found in cervical and vaginal secretions (table II). The proportion of women who had these antibodies in cervical secretions was similar to the proportion who had them in vaginal secretions. About half of the women who were infected had IgG antibody in their secretions and less than half had IgA antibody. Of the 41 women who had trichomoniasis, 30 had either IgG or IgA or both classes of antibody at a titre of ≥ 32 in cervical or vaginal secretions or both. Nonetheless, material from 41 of 100 women with no evidence of infection contained one or both of these classes of antibody at a titre of ≥ 32 .

ASSOCIATION BETWEEN ANTIBODY AND CLINICAL DATA

The mean duration of symptoms of nine infected women before collection of specimens was 40 days.

TABLE II Presence of antibody to *T vaginalis* in cervical and vaginal secretions

Secretion	Antibody class	Proportion (%) of women with antitrichomonal antibody:	
		Infected	Apparently uninfected
Cervical	IgG	5/9 (56)	3/22 (14)
	IgA	3/9 (33)	2/22 (9)
Vaginal	IgG	17/41 (41)	29/100 (29)
	IgA	14/41 (34)	16/100 (16)
Cervical or vaginal	IgG or IgA or both	30/41 (73)	41/100 (41)

Eight of these women had serum IgG antibody but one, known to have had symptoms for 60 days, had no detectable serum IgG or IgM antibodies although she did have local IgA antibody. One other woman who had had symptoms for seven days had no local antibody despite copious discharge. Indeed, there was no association between local or serum antibody and a copious discharge. Thus, 11 (69%) of 16 women who had such antibodies had a copious discharge as did nine (60%) of 15 women without antibodies. The presence of antibody seemed to be associated with a history of trichomoniasis. Thus, eight (50%) of 16 women who possessed antibodies had a history of infection, whereas only three (20%) of 15 women without antibodies had a history. Finally, all 16 women with antibodies were infected with one or more other sexually transmitted micro-organisms (gonococci, chlamydiae, ureaplasmas, mycoplasmas), but so also were 12 (80%) of 15 women without antibodies. This does not suggest that antibody reacting against *T vaginalis* was stimulated by these other micro-organisms.

Discussion

The existence of serotypes of *T vaginalis* and the possibility that some might be more useful as antigens than others for detecting specific antibody⁹ prompted us to test several isolates and to combine three selected strains for the antigen in the ELISA.

The results of subsequently testing a large number of sera and cervical and vaginal secretions showed that the technique could be used to measure antibodies to *T vaginalis* in these samples and that it was at least as sensitive as other methods. Nevertheless, antibodies of the IgM and IgG classes were not detected in the sera of 19.5% of women who were infected; conversely, antibodies were found in 13.7% of apparently uninfected women. An explanation for the former observation could be that the assay was insufficiently sensitive or that in early infections antibodies are not produced. The existence of antibody in a proportion of apparently uninfected

women is not surprising. Although the duration of circulating antibody is unknown it is likely that antibody in some women persisted after the infection had resolved either spontaneously or due to specific treatment. Furthermore, as we did not routinely culture vaginal exudate for *T vaginalis*, a procedure which can be¹⁰ more sensitive than microscopy of a wet or stained smear, the occurrence of antibody in some women may have been a reflection of undiagnosed infection. Trichomoniasis in prepubescent children, usually neonates, has been recorded.¹¹ In this study IgG antitrichomonal antibody at a titre of ≥ 32 was found in only three of the 99 children's sera examined. Two of these children had juvenile chronic arthritis, and it is possible that heterophile antibodies which develop in this condition may have reacted with *T vaginalis* antigen. The third child was in the neonatal period and possibly the antibody was of maternal origin.

Using a direct immunofluorescence method Chipperfield and Evans¹² found an increase in the number of plasma cells containing IgA, IgM, and IgG in the endocervix of women with trichomoniasis. Antibodies were not detectable, however, by direct agglutination¹³ or by indirect immunofluorescence¹⁴ in the local secretions of women harbouring the parasite. Clearly, these procedures were insufficiently sensitive, since Ackers and his colleagues¹⁵ found IgA antibody to *T vaginalis* in vaginal secretions by a radioimmune assay in 76% of 29 women who were infected and in 42% of 19 apparently uninfected women; the mean number of counts for the former group were twice that for the latter.

Our results from the examination of secretions were similar, antibody of the IgG or IgA classes or both being found in 73% of 41 infected women and in 41% of 100 apparently uninfected women. Thus local antibodies appear to be of no diagnostic value. Indeed the correlation between the detection of local antibodies and the presence of active infection was poorer than that between serum antibodies and infection. There was no correlation between the presence of antibody and the degree of vaginal inflammation. Antibody was, however, found more frequently in patients with a history of trichomoniasis than in those without, although it is not known to what extent previous infection with a possible anamnestic response accounts for antibody in current disease. Certainly, infection with the other microorganisms which were found does not seem to have been responsible for antibody against *T vaginalis*.

Infection of the male urethra with *T vaginalis* occurs far less frequently than infection in women or

is much more difficult to detect. It would be valuable to examine sera from men known to be infected and from male contacts of infected women to determine the usefulness of the ELISA in detecting previous or current male infection. In clinical practice the onset of the infection is uncertain. Similarly, it is difficult to determine whether the episode is an initial or a subsequent infection. These uncertainties cause difficulty in determining the relationship between antibody production and the duration of infection in both women and men. Further insight into this aspect, particularly the speed of antibody development and the persistence after successful treatment, may be gained best by studies in animal models, preferably non-human primates.

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