

# Urine culture for the detection of *Trichomonas vaginalis* in men

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**SUMMARY** Urine samples were collected from 248 men, 21 of whom were known contacts of women infected with *Trichomonas vaginalis*. This organism was cultured from only three of the 21 specimens from patients in the contact group. The cultural technique was shown to be capable of reliably detecting small numbers of organisms under practical conditions; it appears, therefore, that most male contacts shed relatively few trichomonads and that the infective dose for women must be correspondingly small.

## Introduction

Although most cases of trichomoniasis are believed to be sexually transmitted, demonstration of this organism in men has always been difficult. Here we report a small study designed to assess the value of urine culture in the diagnosis of this infection. We have also attempted to estimate the sensitivity of the method.

## Patients and methods

### SPECIMENS

Samples of urine were collected from men attending James Pringle House, Middlesex Hospital, during a six-week period in the spring of 1978. Samples were taken from all men except known homosexuals and those already undergoing any kind of treatment. During, and for two weeks before, the study contact slips were issued to all women diagnosed as having trichomoniasis in an attempt to increase the number of male contacts in the study. Samples (20 ml) were stored at room temperature for no longer than four hours before being processed. After centrifugation (500×g for 10 min) the sediment was inoculated into 5 ml of modified Lumsden's medium<sup>1</sup> and incubated for 14 days at 37°C. Cultures were examined by wet-film microscopy after any colour change and also on the fourteenth day of incubation before being discarded.

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Received for publication 14 May 1979

### MEDIA

Three media were used: modified Lumsden's medium;<sup>1</sup> Robinson's medium;<sup>2</sup> and a modification of Bushby's medium.<sup>3</sup> The latter contained (in 2 litres) Tryptone (Difco), 60 g; D-glucose, 40 g; liver infusion (Difco), 36 g; and calcium pantothenate (0.5% solution), 2 ml. After filtering and autoclaving, the following additions were made: sterile inactivated horse serum, 500 ml; cysteine hydrochloride (sterile 30% solution), 10 ml; and chloramphenicol (1% solution), 25 ml.

For the enumeration of small numbers of organisms, pour plates were prepared and incubated as described by Hollander.<sup>4</sup>

### DETERMINATION OF MINIMUM INOCULUM

A cryopreserved stock of *T vaginalis* (LUMP 873) was opened and grown in modified Lumsden's medium. Organisms were harvested by centrifugation (at 500×g; for 5 min), washed once in phosphate-buffered saline (pH 7.2; PBS), and resuspended in PBS. After counting in a haemocytometer, dilutions were made in PBS to give 1 to 10<sup>5</sup> organisms in 0.1 ml. Five-millilitre samples of medium were inoculated with 0.1 ml in quintuplicate and incubated for 14 days at 37°C. The number of organisms necessary to infect half of the bottles (ID<sub>50</sub>) was calculated from the number becoming positive by the method of Reed and Muench.<sup>5</sup>

### DETERMINATION OF SENSITIVITY

To test the sensitivity of the urine-culture method, *T vaginalis* organisms prepared as above were added to 20-ml samples of fresh urine from men previously shown to be free from *T vaginalis*. The number of

organisms used ranged from 800 to 13 (doubling dilutions). After the urine samples had been inoculated, they were held at room temperature for four hours and then treated exactly as the specimens from the clinic. Cultured *T vaginalis* organisms were also added to urine from men, kept at room temperature, and examined microscopically at intervals. Motility was not significantly reduced after standing for up to 24 hours.

## Results

Since the number of organisms present was expected to be low, a preliminary experiment was conducted to determine the best medium for growing *T vaginalis* from small inocula. Using organisms grown in modified Lumsden's medium, known numbers were added to 5-ml quantities of different media; modified Lumsden's and modified Bushby's media both yielded 5/5 positive when inoculated with 10 organisms whereas a much larger inoculum was necessary with Robinson's medium (the ID<sub>50</sub> was 10 organisms but 10<sup>4</sup> organisms were necessary to give 100% positive cultures). Modified Lumsden's medium was therefore used for urine culture in the remainder of this work.

### STUDY POPULATION

Two hundred and eighty-four urine samples were obtained during the study period. Thirty-six patients were excluded from the study because their last known contacts were men, their records were incomplete, or treatment had already begun. Of the remaining 248, 21 were contacts of women diagnosed as having trichomoniasis. One hundred contact slips were issued to women with trichomoniasis during the eight weeks before the study and during the study; as a result, nine men attended and are included in the figure of 21 given above.

### CULTURES

*T vaginalis* was grown from three (14%) of the 21 samples from contacts; in 10 samples, however, bacterial growth was very rapid despite the presence of antibiotics, and it is possible that more samples would have grown *T vaginalis* if this had not occurred. None of the samples from men not known to have been in contact yielded a positive culture, giving an overall success rate of three (1.2%) out of 248 samples.

### SENSITIVITY OF TECHNIQUE

In view of these very low results, an attempt was made to assess the sensitivity of the whole technique. Varying numbers of cultured trichomonads were added to 20-ml urine samples and treated exactly as

the study samples. The accumulated results of the three separate experiments (see Table) show that fewer than 50 organisms in 20 ml of urine are unlikely to be detected but that 200 or 400 probably will be; the ID<sub>50</sub> is 70 organisms. To provide a check on the counting and diluting techniques, samples of the four lowest dilutions were added to pour plates of Hollander's medium; the number of colonies produced is also shown in the Table. The numbers confirm the accuracy of the calculated number of organisms being added to the urine.

TABLE Accumulated results of three experiments to determine sensitivity of urine-culture technique

No of <i>T vaginalis</i> organisms added to urine	Cultures becoming positive		Mean No of colonies/plate	
	No	%	No	%
800	2/2	100	ND	
400	2/3	67	ND	
200	6/7	86	ND	
100	7/11	64	68	68
50	8/8	100	30	60
25	1/8	13	14	56
13	2/14	14	10	80

ND = not done

## Discussion

We have shown here that our frequent failures to cultivate *T vaginalis* from male urine is not due to a lack of sensitivity of the method. Lumsden *et al*<sup>6</sup> showed that growth could be initiated from about six organisms, although some media require much larger numbers,<sup>7</sup> and we have confirmed that adding 10 or more trichomonads to 5 ml of medium invariably gives a positive result. More realistically, the results suggest that, with a 20-ml urine sample containing more than 200 *T vaginalis* organisms treated by the procedure described, there is an approximately 90% chance of obtaining a positive culture result. The organisms used in these in-vitro studies were grown in fluid medium and therefore might not represent the true behaviour of organisms taken directly from the patient. On the other hand, they were only on their third subculture since isolation and so any changes were probably minimal. Subject to this proviso, therefore, the fact that only 14% (3 out of 21) of samples from known contacts yielded positive culture results implies that the urine of these men contains few viable organisms. An earlier study<sup>8</sup> using identical methods yielded positive culture results from 6/37 (16%) of urine and 6/41 (15%) of semen samples from known male contacts. If the method is as sensitive with semen as with urine samples, most male contacts produce semen which

contains only a small number of trichomonads; the infective dose for women, therefore, would also appear to be small.

In previous studies of trichomoniasis in male contacts of infected women, Schapira<sup>9</sup> found 28% positivity by culturing semen samples whereas Summers and Ford<sup>10</sup> found a remarkable 71% positivity using Papanicolaou staining of prostatic fluid and urine deposits (although this method has been criticised).<sup>11</sup> Watt and Jennison<sup>12</sup> obtained 55% positivity from urine cultures in a group of men whose wives had trichomoniasis; in this case, however, the women had long-standing and chronic infections. Our results suggest that, possibly because infected women are seen and cured more rapidly now, urine culture (even in the case of known male contacts) is not likely to lead to many positive results and that it is unlikely to be worth applying routinely to all men who attend.

This work was partly supported by a project grant from the Medical Research Council. We are grateful to Dr R D Catterall and all his staff at James Pringle House for their help in making this study possible.

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