

Review of current methods for the detection of *Trichomonas* in clinical material

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SYNOPSIS Methods of 'wet preparation', stained films, and culture were used to detect trichomonads in vaginal exudate and the results of these methods are compared. A method using a wet preparation and Oxoid no. 2 *Trichomonas* medium is reliable for routine use.

The correct diagnosis of trichomoniasis has become more important at Sefton General Hospital since the start of a cytology unit for the exfoliative cytology of cervical smears, but the interpretation of smears is rendered more difficult by the presence of trichomonads.

It was therefore decided to review the current methods of detecting trichomonads and to assess their reliability for routine use.

Specimens of vaginal discharge were received on sterile cotton wool swabs from patients with vaginal infections (or suspected infections). Two smears were prepared from the vaginal material on microscope slides, and fixed by immersing in ethyl alcohol for 15 minutes. One of the smears was stained by Leishman's stain and the other by Papanicolaou's method. The remaining material was suspended in approximately 1 ml of sterile quarter-strength Ringer's solution, and was used for the wet preparation and for inoculating *Trichomonas* culture media. Ringer's solution was used as it was thought that physiological saline might be slightly antagonistic to trichomonads (Stenton, 1957).

METHODS AND MATERIALS

WET PREPARATION The suspension of the vaginal material in sterile quarter-strength Ringer's solution was warmed slightly to activate any trichomonads present. A few drops of this suspension were examined microscopically for trichomonads.

LEISHMAN-STAINED FILMS Two volumes of buffered distilled water (pH 6.8) were mixed with one volume of Leishman stain. This solution was added to an alcohol-fixed film and left for 15 minutes. The stain was washed off, and the slide dried by blotting. The film was examined microscopically for trichomonads using a 2 mm oil immersion lens.

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PAPANICOLAOU-STAINED FILMS The second alcohol-fixed smear was stained using a Shandon-Elliott staining machine. After processing the smears were left immersed in xylol until they were mounted. The films were examined microscopically for the presence of trichomonads and cellular changes indicative of trichomoniasis.

TABLE I

RESULTS USING SHANDON-ELLIOTT MACHINE

Reagent	Time (min)
1 95% alcohol	2.0
2 Distilled water	2.0
3 Harris haematoxylin ¹	3.0
4 Distilled water	2.0
5 70% alcohol	2.0
6 1% acid alcohol	(quickest change)
7 70% alcohol	2.0
8 Ammonia alcohol ¹	1.5
9 70% alcohol	2.5
10 95% alcohol	1.5
11 95% alcohol	2.0
12 O G 6 ¹	1.5
13 95% alcohol	1.5
14 95% alcohol	1.5
15 E A 50 ¹	2.0
16 95% alcohol	1.5
17 Absolute alcohol	1.5
18 Absolute alcohol	1.5
19 Xylol	2.0
20 Xylol	3.0
21 Mounting xylol	

¹The recommended stains are supplied by Ortho Pharmaceuticals.

²The ammonia alcohol was a 3% solution of 0.880 ammonia in 70% alcohol.

CULTURAL METHODS FOR TRICHOMONAS In order to select a medium suitable for routine use, five different types of *Trichomonas* media were employed:

Thomas's medium is a modification of the medium of Feinberg and Whittington (1957), described by Thomas (1964).

Cysteine-peptone-liver-maltose (CPLM) medium This is described by Johnson and Trussell (1943), and the modification of Smith (1965) was used.

Semi-solid medium Described by Lowe (1965).

Oxoid Trichomonas medium (CM 161) An Oxoid

modification of the medium of Feinberg and Whittington (1957), and the basal medium was obtained in powder form.

Oxoid Trichomonas medium no. 2 (R27) The medium was obtained ready to use, and is a modification of the medium of Bushby and Copp (1955) and Squires and McFadzean (1962).

The culture media were stored at 4°C. The inoculum was the suspension of vaginal exudate in quarter-strength Ringer's solution. This was divided equally among the five media, using a sterile Pasteur pipette. Cultures were incubated at 34°C and examined daily for five days. The sediment was removed from the bottom of the culture with a sterile pipette and examined microscopically for trichomonads.

CULTURE MEDIA The materials required and methods for making up the media are as follows:

Thomas's medium

Proteolysed liver (Oxoid)	25 g
Sodium chloride	6.5 g
Glucose	5.0 g
Inactivated horse serum (Burroughs Wellcome)	80 ml
Water	1,000 ml
Penicillin G	1,000,000 units
Streptomycin	0.5 g
Nystatin	0.1 g

The pH was adjusted to 6.4 and the medium sterilized by Seitz filtration. The medium was distributed aseptically into sterile ¼ oz. bijou bottles, and the bottles were filled with a minimal air space between the lid and the medium.

Basal CPLM medium

Peptone (Oxoid)	32 g
Maltose	1.6 g
Liver digest (Oxoid)	20 g
Cysteine hydrochloride	2.4 g
½ strength Ringer's soln	1,000 ml
N-sodium hydroxide soln	approx. 9 ml

The ingredients were dissolved by shaking, and the pH adjusted to 6.0. After steaming for 30 minutes at 100°C the medium was filtered through a Whatman's no. 1 filter paper, bottled in 90 ml amounts, and autoclaved at 115°C for 10 minutes. This base was stable for several weeks.

The penicillin/streptomycin solution is made up of:

Penicillin	1 × 10 ⁶ units
Streptomycin	0.1 g
Sterile water	10 ml

The nystatin suspension contains:

Nystatin	5 × 10 ⁴ units
Sterile water	10 ml

The antibiotic solutions were stable for up to 10 days when stored at 4°C.

The complete medium contains:

Basal medium	90 ml
Sterile inactivated horse serum (Burroughs Wellcome)	10 ml
Penicillin/streptomycin soln	1 ml
Nystatin suspension	1 ml

The medium was mixed and distributed in sterile ¼ oz bijou bottles.

Semi-solid medium

Liver digest (Oxoid)	12.5 g
Sodium chloride	2.5 g
Maltose	0.5 g
Distilled water	500 ml

The solids were dissolved by heat and the pH was adjusted to 6.2.

Of agar powder, 12.5 g was added and the mixture steamed at 100°C for one-and-a-half hours with frequent mixing. The medium was cooled to 54°C and the following added aseptically:

Sterile inactivated horse serum (Burroughs Wellcome)	50 ml
Fildes extract (Oxoid)	0.5 ml
1% sterile streptomycin solution	5 ml
1% sterile chloramphenicol solution	5 ml
1% sterile neomycin solution	5 ml
0.5% sterile (fresh) nystatin suspension	10 ml

The medium was mixed and distributed aseptically into sterile ¼ oz bijou bottles.

Oxoid Trichomonas medium (CM 161)

Liver digest	25.0 g/l
Sodium chloride	6.5 g/l
Dextrose	5.0 g/l
Ionagar no. 2	1.0 g/l

Of the base powder, 37.5 g was added to 1 litre distilled water and soaked for 15 minutes. The medium was sterilized by autoclaving at 121°C for 15 minutes. It was cooled to 54°C and the following added:

Sterile inactivated horse serum (Burroughs Wellcome)	80 ml
Penicillin	1,000,000 units
Streptomycin	500,000 units

The medium was mixed and distributed aseptically into sterile ¼ oz bijou bottles.

Oxoid Trichomonas medium no. 2 (R27)

Liver infusion	18.0 g
Hartley digest broth	1,000 ml
Horse serum	250 ml
Calcium pantothenate, 0.5% w/v	1 ml
Dextrose	20.0 g
Chloramphenicol	0.125 g

pH = 6.2 (approx.)

This culture medium was supplied by the manufacturers ready to use.

RESULTS

Eight methods were employed in the detection of trichomonads. One method involved the use of wet preparation, two made use of stained films and five of culture media. The results of the survey are shown in Table IIa.

One hundred and thirty-two swabs were examined and in addition eight urine deposits containing trichomonads (Table IIb).

During the survey cultures were examined daily for five days, and the Oxoid no. 2 medium gave majority of positive results (96%) after three days incubation at 34°C (Table III).

TABLE IIa

RESULTS OF SURVEY USING SWABS OF VAGINAL EXUDATE

Method	No. of Positives
Wet preparation	3
Leishman film	1
Papanicolaou film	3
Thomas's medium	5
Oxoid medium (CM 161)	6
Oxoid no. 2 medium (R27)	14
CPLM medium	7
Semi-solid medium	8

TABLE IIb

RESULTS OF SURVEY USING POSITIVE URINE DEPOSITS

Method	No. of Positives
Wet preparation	8
Leishman film	4
Papanicolaou film	3
Thomas's medium	3
Oxoid medium (CM 161)	2
Oxoid no. 2 medium (R27)	8
CPLM medium	3
Semi-solid medium	5

TABLE III

RESULTS WITH OXOID NO. 2 MEDIUM

Incubation Time (days)	No. Positive
1	6
2	1
3	14
4	—
5	1

Total detected = 22

DISCUSSION AND CONCLUSION

The microscopical detection of trichomonads by either wet preparation or direct smear alone was not satisfactory. The successful use of a wet preparation depends mainly on the correct collection of the specimen, the amount of material on the swab, the alertness of the observer, and the presence and number of motile parasites in the specimen. Thus this method by itself is not to be recommended as a routine procedure.

The staining procedures used during this survey did not yield reliable enough results for routine use. The Papanicolaou film may show cellular changes suggesting infestation of the vagina with trichomonads when the parasites are not easily seen. Such changes are not readily recognized by untrained observers and may, in any case, be obscured by cellular deterioration if there is a delay between collection and examination of the specimen.

The methods involving culture media in the detection of trichomonads in this survey were the most successful. Of the five types used, the Oxoid no. 2 medium gave the highest number of positive results. This medium has the added advantage of being supplied by the manufacturers ready to use. The training of laboratory staff to detect the parasite by culture is relatively easy.

The following procedure is a successful routine method for detecting trichomonads:

1 The specimens of vaginal exudate are collected on sterile cotton wool swabs.

2 A suspension of the vaginal material prepared in approximately 1 ml of sterile quarter-strength Ringer's solution is examined microscopically for the parasites.

3 If the wet preparation is negative then the material should be cultured using Oxoid *Trichomonas* medium no. 2 (R27). A heavy inoculum should be used in the broth, *ie*, the swab stick end and remaining suspension of exudate.

4 Cultures should be incubated at 34°C, and examined daily for three days (as described previously).

This technique is simple to use and is not time consuming. Heavy infestations may be detected more quickly by the wet preparation, and lighter infestations detected within three days by culture. It is easy to train technicians how to use this method, and it is not necessary to prepare large quantities of culture media.

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ADDENDUM

The recommended method for the detection of *Trichomonas* has been adopted at Sefton General Hospital, and the following data have become avail-

TABLE IV
RESULTS WITH RECOMMENDED METHOD

<i>Swab</i>	<i>Positive by Wet Preparation Only</i>	<i>Positive by Culture Only</i>	<i>Total No. Positive</i>
306 examined by wet preparation only	19	—	19
510 examined by recommended routine	19	38	57

able since the completion of this project (Table IV). It can be seen that using a method of wet preparation only, positive specimens were an average of 4.7% of the total received. Using the recommended method of wet preparation and culture, the number of positive specimens detected has increased to 11.2% of the total received.