

A comparison of current laboratory methods and a new semi-solid culture medium for the detection of *Trichomonas vaginalis*

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SYNOPSIS A comparison of three different laboratory methods currently in use for the detection of *Trichomonas vaginalis* indicated that each method had some disadvantages. Examination of a wet preparation was the simplest method but yielded the lowest proportion of positive results; the cultural method, although more fruitful, was relatively slow and failed to detect non-viable trichomonads; examination of a Leishman-stained film yielded the highest proportion of positive results but probably failed to detect trichomonads when they were present in very small numbers. The best combination of methods was found to be Leishman film and culture: the positive yield was 99%.

Also described is a new semi-solid agar medium which represents an improvement upon the liquid medium in current use.

Differing opinions are held regarding the value of fixed stained smears for the detection of *Trichomonas vaginalis*. Whittington (1957), comparing the laboratory methods used for the detection of this parasite, repeated staining methods advocated by various workers but found none to be wholly reliable and excluded this method of examination from her investigations. Contrary to Whittington's findings, however, the Leishman method for staining smears of secretions (vaginal and urethral exudates) has been successfully employed in this laboratory for many years and has appeared to compare favourably with the wet preparation and the more recent cultural technique. In view of these conflicting findings it was felt that a comparison and reappraisal of the laboratory methods used for the detection of *Trichomonas vaginalis* was indicated.

MATERIALS AND METHODS

The laboratory methods compared in this investigation were the examination of wet preparations of exudates, the examination of smears stained by the Leishman method, and the cultivation of *Trichomonas vaginalis* in the medium of Feinberg and Whittington (1957).

Specimens of vaginal and urethral exudates examined were obtained from patients attending the Venereal Diseases Department and the Gynaecological Out-patient Department of the Royal Gwent Hospital, Newport.

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WET PREPARATION AND CULTURAL METHOD Swabs wound on wooden applicator sticks impregnated with activated charcoal are used in this laboratory for the routine collection of vaginal and urethral discharges for bacteriological examination, the swabs being transmitted to the laboratory in Stuart's transport medium. Samples of secretions required for the wet preparation and cultural method were collected on similar swabs. The swabs were placed into bijoux bottles containing 5 ml. of culture medium which was prepared according to the original formula of Feinberg and Whittington (1957), and were cut or snapped off flush with the rim of the bottle and the cap was screwed down firmly. On arrival at the laboratory a wet preparation of the exudate was made by withdrawing with a Pasteur pipette material from around the swab and the surrounding culture medium; a drop of this fluid was delivered on to a slide, covered with a cover-slip and examined under the 4 mm. objective. The culture medium was then incubated at 37°C. and examined by the same procedure daily for three successive days before discarding.

SMEARS STAINED BY THE LEISHMAN METHOD Duplicate swabs of secretions were taken and smears made, the unfixed films being usually air-dried on arrival at the laboratory.

The slide was flooded with Leishman stain and left for two minutes. Double volume of buffered distilled water pH 6.8 was added, mixed by gentle rocking, and left for 15 minutes. The stain was washed off with buffered distilled water and then dried by blotting. The stained film was examined under the 2 mm. oil immersion lens.

TABLE I

COMPARISON OF LEISHMAN-STAINED SMEARS, WET PREPARATIONS AND CULTURAL METHOD FOR THE DETECTION OF *TRICHOMONAS VAGINALIS*

No. of Specimens Examined	Total No. of Positive Samples	Total No. of Positive Results		
880	100 (11%)	Leishman + 86 Leishman or culture + 99	Wet preparation + 74 Leishman or wet preparation + 90	Culture + 83 Culture or wet preparation + 91
		Leishman + Wet preparation - } 9 Culture -	Leishman - Wet preparation + } 1 Culture -	Leishman - Wet preparation - } 10 Culture -

RESULTS

The results are summarized in Table I; 880 patients were investigated and of these 100 (11%) were found to be harbouring *Trichomonas vaginalis*. Of the three methods investigated, the Leishman-stained smear gave the highest number of positive results, 86, thus confirming observations made in this laboratory. The cultural method gave a similar number of positives, 83. The number of positive results obtained with the wet preparation method was only 74. A larger number of positive results was obtained by using combinations of methods; the Leishman method coupled with culture gave 99 positives, whereas the Leishman film with wet preparation and culture with wet preparation gave fewer positive results, 90 and 91 positives respectively. In nine instances the Leishman film gave the only positive result; this was probably due to the detection of non-viable trichomonads. The presence of trichomonads in small numbers probably accounted for the 10 instances where the culture was the only method found to be positive.

It is of interest to note that the failure of culture in the Feinberg and Whittington medium in nine of the total positive specimens would not have been revealed if the Leishman film had been excluded from this series. The majority of the patients investigated were attending the clinics for the first time and loss of trichomonad viability could not be due to treatment, although self treatment or cleansing by the patient before examination could devitalize or kill the parasites. Unfavourable conditions developing in the medium could also have an adverse effect. It was found that in many instances the bacterial content of the medium was considerable after 24 hours' incubation and it was apparent that the penicillin and streptomycin included in the medium was ineffective against many strains of bacteria found in vaginal and urethral exudates. *Candida (Monilia)* were also found to be a troublesome contaminant. The usefulness of this medium for the diagnosis of yeast infections is doubtful as no distinction can be made between the commensal, contaminant, and infective conditions; diagnosis by

examination of stained films and direct culture on to suitable solid culture media is to be preferred.

SEMI-SOLID AGAR MEDIUM

Experiments were carried out in order to devise a medium which would eliminate the unfavourable conditions described. The following medium was found to meet these requirements and was technically easier to prepare since it obviated Seitz filtration.

CULTURE MEDIUM The formula of this medium is as follows:

- Liver extract (Panmede)¹ 12.5 g.
- Sodium chloride 2.5 g.
- Maltose 0.5 g.
- Distilled or deionised water 500 ml.

Dissolve the solids by heat, adjust the pH to 6.2, then add 1.25 g. of agar powder. Steam at 100°C. for one and a half hours, mixing well at intervals. Cool to 54°C. and add aseptically the following:

- Sterile inactivated horse serum (Burroughs & Wellcome No. 3) 50 ml.
- Fildes extract (Oxoid) 0.5 ml.
- 1% sterile aqueous solution of chloramphenicol 5 ml.
- 1% sterile aqueous solution of streptomycin 5 ml.
- 1% sterile aqueous solution of neomycin 5 ml.
- 0.5% sterile aqueous solution of nystatin (freshly prepared) 10 ml.

Mix well and transfer aseptically into a sterile distributing flask, distribute into sterile bijoux bottles filling up to the shoulder, and incubate overnight for sterility check. The gelling action of agar varies from batch to batch. The gel of the medium should remain intact when the bottle is inverted but should break up readily on shaking. The concentration of agar should be adjusted accordingly to give these conditions.

Stock culture of *Trichomonas vaginalis* and parasites contained in selected samples of exudates were found to grow abundantly in this medium. The antibiotics included did not appear to have any adverse effect on growth. Nystatin, which was incorporated at the strength recommended by Thomas (1964), was found to prevent multiplication of stock cultures of *Candida*.

¹The Panmede brand of liver extract is obtainable from Payne and Byrne, Ltd., Greenford, London.

TABLE II

COMPARISON OF FEINBERG-WHITTINGTON AND SEMI-SOLID AGAR MEDIA FOR THE CULTURE OF *TRICHOMONAS VAGINALIS*

Total No. of Positives	Feinberg-Whittington Medium		Semi-solid Agar Medium		Leishman Smear +
	Wet Preparation +	Culture +	Wet Preparation +	Culture +	
37	24	30	25	33	32

A comparative trial to test the efficiency of the Feinberg-Whittington medium and the medium described above was carried out using the same collection and examination procedures as previously described. The results are shown in Table II. Due to the comparatively small numbers of positive results obtained in this trial the differences in the results appear to be small. However, the semi-solid agar medium gave the highest number of positive results both in the wet preparation and culture; the Leishman film once again gave a high proportion of positive results. Considerable technical improvements were obtained with the semi-solid medium. The material on the swabs was contained and did not readily diffuse into the medium. Wet preparations made from material around the swab were composed almost entirely of exudate. In the majority of instances no bacterial growth occurred in the medium and *Candida* were successfully suppressed. Colonization of the parasite readily took place around the swab, the trichomonads slowly migrating to all parts of the medium. It was found that growth in the Feinberg-Whittington medium usually ceased after three days' incubation; in the semi-solid agar medium motile forms were observed in the majority of isolations after seven days' incubation.

Experiments have also shown that *Trichomonas vaginalis* remains viable from four to 10 days when stored at room temperature (22°C.). Variable results were obtained with refrigerated cultures; some strains were viable after exposure for 48 hours at 4°C.; other strains were dead after 18 hours' exposure at this temperature. A number of positive swabs in the semi-solid agar medium were transmitted to the laboratory by post, being 24 to 48 hours in transit; on arrival all gave positive wet preparation and cultural results.

DISCUSSION

Each of the methods compared here has shown certain disadvantages. The wet preparation was found to be the simplest method but unfortunately gave the lowest number of positive results; the cultural method, although detecting larger numbers

of positives, was relatively slow and failed to detect non-viable trichomonads; the Leishman-stained film also gave a high proportion of positive results but probably failed to detect trichomonads when present in very small numbers. Also the identification of stained trichomonads, although not difficult, should be made by suitably trained personnel. It is of interest to note that *Trichomonas vaginalis* in films made from semi-solid agar cultures stain extremely well by the Leishman method, the flagella, axostyle, and internal structure being clearly visible. Such preparations are suitable for demonstration and instructional purposes. Parasites growing in the Feinberg-Whittington medium fail to stain satisfactorily.

The following routine incorporating the advantages of each method is suggested for the detection of *Trichomonas vaginalis*.

A charcoal swab of the exudate should be snapped off into a bijou bottle containing the semi-solid agar medium described above and a smear should also be made of the exudate. On arrival at the laboratory a wet preparation from the culture medium should be made as previously described. If motile trichomonads are present the culture and the smear can be discarded. If no motile forms are detected the smear is stained by the Leishman method and examined, and the culture incubated at 37°C. and examined on three successive days before discarding. The specimens can be transmitted through the post and should not deteriorate if left at room temperature for 24 hours. It is not advisable to refrigerate specimens. For the examination of urine specimens a wet preparation and culture of the centrifuged deposit are suggested.

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