

COMPARISON OF CULTURE MEDIA FOR THE GROWTH OF *Trichomonas vaginalis**

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A number of different media are now in use for the culture of *Trichomonas vaginalis*. The medium described by Feinberg and Whittington (1957) has been used routinely in this laboratory since 1957. Whittington found that it was a more sensitive method of detecting the flagellate in secretions from the female genital tract than direct microscopical examination of fresh smears. This superiority of cultures over fresh smears was not, however, apparent in the examination of urethral secretions from men with non-gonococcal urethritis. This disparity may be due to the number of *T. vaginalis* in the inoculum, as the organisms are usually more plentiful in material from the female than from the male genital tract. Whittington (1966) has stated that an inoculum of the order of 5×10^4 trichomonads is generally needed to initiate growth in the Feinberg-Whittington medium.

The minimal inoculum of trichomonads to initiate growth in the Feinberg-Whittington medium under aerobic and anaerobic conditions was therefore determined and compared with that for the medium described by Squires and McFadzean (1962) and the cysteine-peptone-liver-maltose (C.P.L.M.) medium described by Johnson and Trussell (1943), and quoted by Trussell (1947). Parallel cultures of secretions from male and female patients were also made with media, and the results were compared with those of direct microscopy of fresh material.

Material

(1) The Feinberg-Whittington (F-W) medium was prepared as described by the authors (1957). For the minimal inoculum experiments it was tubed in 5 ml. volumes and for the examinations of material from patients, in bijou bottles. When ready for use the medium has a pH of about 6.2. If cysteine hydrochloride is added to the medium in the same proportion as it is present in the

C.P.L.M. medium, the pH is reduced to about 5.7.

(2) The medium of Squires and McFadzean (S-M) was prepared as described by the authors (1962), except that in some batches Oxoid tryptone soya broth was substituted for ox heart broth. The pH value was adjusted to 6.0 before autoclaving.

(3) The C.P.L.M. medium was prepared as described by Johnson and Trussell (1943) and bottled in 80 ml. vols. 1.5 ml. of stock antibiotic solution containing 50,000 international units of penicillin/ml., and 50 mg. streptomycin/ml., was added to each bottle. The medium was tubed in $5 \times \frac{1}{2}$ " stoppered tubes in 8 ml. volumes for the examination of material from patients and 5 ml. volumes for the minimum inoculum experiments.

The methylene blue in the medium gives a green colouration at the surface of the medium. As the conditions become increasingly aerobic because of oxidation, this colouration moves downwards. When the green layer reaches more than half-way down the tube, the medium should be discarded as unfit for use. If at least 8 ml. of medium are placed in the tubes, the green layer remains above half-way for several weeks. The medium is inoculated below this layer. The pH is 5.6-5.7.

Minimum Inoculum Experiments

These experiments were performed with both stock strains of the organism and freshly-isolated strains which had not been subcultured more than once. The cultures were incubated, usually in the F-W medium for 24 hours, and checked for the presence of actively growing trichomonads. They were then centrifuged at low speed, and the organisms were washed free from medium with nutrient broth and re-suspended in about 1.0 ml. broth, and the number present was counted with a haemocytometer. Serial 10-fold dilutions were made in broth and inoculated into the media under investigation. The cultures were incubated at 36°C.

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and a small volume was removed daily with a Pasteur pipette for 5 to 8 days and examined for the presence of trichomonads.

Results of Minimum Inoculum Experiments

Table I shows clearly that the C.P.L.M. medium established growth with a very much smaller inoculum than the F-W or S-M media, with both stock and freshly isolated strains. The C.P.L.M. medium contains enough agar to make it semi-solid and cysteine hydrochloride as a reducing agent, so that it affords at least partial anaerobiosis. The other two media both contain glucose but no other reducing agent. Whittington (1966) found that 5×10^4 organisms were necessary to initiate growth in 10 ml. of F-W medium although occasionally fewer were sufficient. She stated that growth was established in 24 to 48 hours if it was to occur at all and this is in agreement with the present findings.

The effects were examined of incubating the F-W medium under anaerobic conditions (95 per

cent. nitrogen and 5 per cent. carbon dioxide) and of adding to it a comparable amount of cysteine hydrochloride to that present in the C.P.L.M. medium. The results of these experiments (Tables II and III) demonstrate that the F-W medium will initiate growth from a smaller inoculum if the medium is incubated under partially or completely anaerobic conditions, but the results are still not as

TABLE III
EFFECT OF ADDITION OF CYSTEINE HYDROCHLORIDE ON MINIMUM INOCULUM TO INITIATE GROWTH OF TRICHOMONADS IN F-W MEDIUM

Strain	Routine		With Cysteine Hydrochloride	
	Minimal Inoculum	Time for Growth (hrs)	Minimal Inoculum	Time for Growth (hrs)
Stock ..	8.5×10^4	24	8.5×10^3	24
Stock ..	2.2×10^5	24	2.2×10^3	24
Fresh ..	2.5×10^5	24	2.5×10^4	24

TABLE I
MINIMUM INOCULUM OF TRICHOMONADS TO INITIATE GROWTH

Strain	Medium					
	F-W		S-M		C.P.L.M.	
	Minimal Inoculum	Time for Growth (hrs)	Minimal Inoculum	Time for Growth (hrs)	Minimal Inoculum	Time for Growth (hrs)
Stock	$>4.4 \times 10^4$	N.G.	4.4×10^3	48	4.4×10^3	96
	3.0×10^5	48	3.0×10^3	48	N.D.	
Fresh Isolate .. .	2.3×10^4	24	2.3×10^4	24	N.D.	
	2.5×10^5	24	2.5×10^4	24	N.D.	
Stock	$>8.0 \times 10^3$	N.G.	N.D.		8.0	96
	$>8.8 \times 10^3$	N.G.	N.D.		8.8	96
Fresh Isolate .. .	2.5×10^4	24	N.D.		1	48
	1.4×10^5	24	N.D.		1.4	48

N.D. — Not Done. N.G. — No Growth.

TABLE II
COMPARISON OF THE EFFECT OF AEROBIC AND ANAEROBIC CONDITIONS ON MINIMUM INOCULUM OF TRICHOMONADS TO INITIATE GROWTH IN F-W MEDIUM

Strain	Medium					
	F-W				C.P.L.M.	
	Aerobic Conditions		Anaerobic Conditions		Minimal Inoculum	Time for Growth (hrs)
Minimal Inoculum	Time for Growth (hrs)	Minimal Inoculum	Time for Growth (hrs)			
Fresh Isolate .. .	1.2×10^4	24	1.2×10^3	72	N.D.	
Stock	5.0×10^4	24	5.0×10^3	48	N.D.	
Fresh Isolate .. .	N.D.		3.6×10^3	72	1	72

good as with the C.P.L.M. medium. Johnson (1942) showed that the organism is a facultative aerobe and that an increased oxygen concentration depresses its growth because the ability to utilize carbohydrates decreases. The C.P.L.M. medium has been shown to initiate growth with an inoculum of between one and forty organisms, although incubation for as long as 96 hours is often necessary to demonstrate growth from these very small inocula. Growth curves in fluid medium show that, if the inoculum is small, the maximum growth is not reached for several days. Since the C.P.L.M. medium will initiate growth from a very small inoculum, the cultures should never be discarded as negative until at least 72 hours after inoculation.

Material from Patients

Although the experiments shown in Table I favoured the C.P.L.M. medium, it was temporarily abandoned in favour of the S-M medium as the latter is less troublesome to make and to store.

A comparison was then run between the S-M and the F-W media in conjunction with the Whitechapel clinic. Vaginal secretion was collected on swabs from 236 female patients at their first attendance at the clinic, and the swabs were dropped into the two media. The investigation was abandoned after 10 weeks as the F-W medium in use was detecting a significantly greater number of positive cases of trichomoniasis than the S-M medium.

A comparison was then made between the C.P.L.M. and F-W media using 203 female patients and 160 male patients. Vaginal secretion was collected from female patients at their first attendance at the clinic, and urethral scrapings from male patients who had non-gonococcal urethritis or were contacts of female patients with trichomoniasis. In some of the later cases centrifuged urine deposits were examined and inoculated into the media. Fresh smears of the material from both male and female patients were examined in most cases by the clinic staff when the cultures were made. The cultures were incubated at 36°C. and examined at 24 hours for growth and again at 72 hours before being discarded as negative.

Results

Comparison of F-W and S-M Medium

Trichomonads were grown from 49 of the 236 cultures in the S-M medium and from 66 of those in the F-W medium.

Comparison of F-W and C.P.L.M. Medium (Table IV)

FEMALES 69 positive cultures (34 per cent.) were obtained in the C.P.L.M. medium but only 34 (16·7 per cent.) in the F-W medium. The difference between these two isolation rates is statistically significant $P=0\cdot01$.

TABLE IV
COMPARISON OF CULTURES IN C.P.L.M. AND F-W MEDIA WITH MICROSCOPIC EXAMINATION OF FRESH MATERIAL

Sex	Female	Male	
		Urethral Scrape	Centrifuged deposit of Urine
No. of Cases ..	203	63	97
Culture Smear			
- -	131	52	82
+ +	51	9	10
+ -	14	1	3
- +	3	1	2
+ Not recorded	4	.	.
+ C.P.L.M.	69 (34%)	10 (15·9%)	13 (13·4%)
+ F-W	34 (16·7%)	0	2
Positive by Any Method (smear and/or culture)	72 (35·5%)	11 (17·5%)	15 (15·5%)

MALES The first samples of material were obtained by scraping the urethral mucosa gently with a platinum loop and inoculating the two media with the scraping. 63 patients who had non-gonococcal urethritis or were contacts of females with trichomoniasis were examined in this way. Ten positive cultures (15·9 per cent.) were obtained in the C.P.L.M. medium but none in the F-W medium. Since these specimens were taken after those of the urethral secretion for the preparation of smears and cultures for *N. gonorrhoeae*, the amount of material available for *Trichomonas vaginalis* cultures was often very scanty.

It was thought that urination might wash out any remaining secretion, so the centrifuged deposit from the patient's urine was used for the inoculation of the two culture media in a further 97 cases. Thirteen (13·4 per cent.) positive cultures were obtained in the C.P.L.M. medium but only two in the F-W medium.

Discussion

The results of both the minimum inoculum experiments and the parallel testing of secretions from patients showed that the C.P.L.M. medium was a more sensitive means of detecting *T. vaginalis* than either the S-M or the F-W. Whittington (1957), examining vaginal discharge specimens, found 21 per cent. positive cases in cultures on the F-W medium from 747 specimens from 563 women. In the present series the comparable figure was 16·7 per cent. from 203 women with F-W medium, but 34 per cent. in the C.P.L.M. medium.

Whittington used an inoculum of "two to three loopfuls" of secretion taken from a mustard spoon used to remove the specimen of vaginal secretion, whereas a swab was used to collect the material for the present investigation. Results with material from males show a more marked difference between Whittington's series and the present one. Whittington found that 20.7 per cent. of 354 urethral scrapings from 200 men and 27.7 per cent. of 378 specimens of urine from 207 men gave positive cultures in the F-W medium. In the present series 15.9 per cent. urethral scrapings and 13.4 per cent. urine deposits grew *T. vaginalis* in the C.P.L.M. medium but the F-W medium gave positive results on only two of the urine deposits and on none of the urethral scrapings. In the 1957 work, all female and male patients attending the clinic were examined for trichomonads, whereas in the present work the numbers were limited to female patients at their first attendance and male patients who were suffering from non-specific urethritis or were contacts of women with trichomoniasis. In the 1957 work all the material obtained was placed into the F-W medium, whereas in the present work approximately the same volume of secretion was divided between two media. In both cases swabs were first taken for the culture of gonococci. Thus, by the time the last sample was obtained, the material present for culture would be scanty. This is particularly significant in the case of the urethral scrapings from male patients where the volume of material available is small and the likelihood of establishing the growth of trichomonads much reduced. The ability of the C.P.L.M. medium to initiate growth from a very small inoculum seems to account for its superiority over the F-W medium in the specimens from male patients. The method of preparation of the F-W medium has remained unchanged since Whittington first introduced it into the laboratory and its poor performance with material from male patients, while giving fairly comparable results to Whittington's with female patients, remains otherwise unexplained.

Since the C.P.L.M. medium can initiate growth from a very small inoculum, it may be several days before growth can be detected. As a routine procedure cultures should be incubated for at least 72 hours before being discarded as negative. In the material from females 15.3 per cent. and from males 50 per cent. of the positive cultures showed growth only after 72 hours incubation.

Both F-W and C.P.L.M. media support the growth of yeasts and *Candida* species, but their growth seems to be less in the C.P.L.M. medium, especially in the case of *Candida* sp., mycelium

being produced less frequently than in the F-W medium. If detection of these organisms is required, reliance should not be placed on the C.P.L.M. medium alone, but selective media for yeasts and *Candida* sp., should be inoculated.

Summary

- (1) The minimum number of *Trichomonas vaginalis* to initiate growth in the Feinberg-Whittington, Squires and MacFadzean, and C.P.L.M. media has been determined. For the C.P.L.M. medium, this was found to be from one to forty organisms, while inocula of the order 10^4 - 10^5 were needed to initiate growth in the F-W medium.
- (2) Vaginal secretions from 203 women and urethral scrapings or centrifuged urine deposits from 160 men have been cultured in parallel in C.P.L.M. and F-W medium. The C.P.L.M. medium was found to be a more sensitive means of detecting the flagellate than the F-W medium.

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Une comparaison entre les milieux de culture utilisés pour la culture des *Trichomonas vaginalis*

RÉSUMÉ

- (1) Le nombre minimum des *Trichomonas vaginalis* nécessaire pour commencer la culture dans les milieux suivants: Feinberg-Whittington, Squires et MacFadzean, et C.P.L.M. a été déterminé. Pour le C.P.L.M. on a trouvé qu'il fallait un à quarante protozoaires tandis qu'un ensemencement de l'ordre de 10^4 à 10^5 avait été nécessaire pour commencer la culture dans le milieu F-W.
- (2) Des sécrétions vaginales obtenues de 203 femmes et des raclures urétrales ou des dépôts d'urine centrifugée provenant de 160 hommes avaient été cultivés parallèlement dans les milieux C.P.L.M. et F-W. Il a été trouvé que le milieu C.P.L.M. était un moyen plus sensible que le milieu F-W pour déceler les flagelles.