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# Cell Culture Compared with Broth for Detection of *Trichomonas vaginalis*

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***Trichomonas vaginalis* can be grown in cell culture. We studied the growth kinetics of *T. vaginalis* in McCoy cell culture compared with that in a conventional broth medium (Diamond TYI-S-33 medium supplemented with 10% heat-inactivated bovine serum [TYI]). In the presence of McCoy cells and two parts cell culture medium to one part TYI, a peak concentration of $2 \times 10^6$ to $6 \times 10^6$ *T. vaginalis* per ml was consistently achieved with inocula as low as three *T. vaginalis* cells per ml. Without cells, this medium did not support growth of *T. vaginalis*. *T. vaginalis* in TYI in 1-ml vials with or without McCoy cells demonstrated poor growth. In tubes containing 10 ml of TYI, inocula grew to $2 \times 10^6$ to $6 \times 10^6$ *T. vaginalis* per ml, but at least $3 \times 10^3$ *T. vaginalis* per tube was required to initiate growth. Thus, in vitro, cell culture was more sensitive than TYI broth in detecting low numbers of *T. vaginalis*. In a subsequent clinical comparison of broth and cell culture for isolation of *T. vaginalis* from 188 vaginal specimens and 21 urethral specimens from men, the results were in agreement for 206 specimens (98.6%). There were no situations in which culture was negative and a saline preparation showed motile trichomonads. For women, using a positive culture as the indicator of true positivity, the sensitivity of detection of *T. vaginalis* was 83% with the Pappenheim stain and 77% with saline preparations. In tests with a limited number of men, the Pappenheim stain was neither sensitive nor specific for detection of *T. vaginalis* in comparison with cultures or saline preparations. These studies show that cell culture can be used for isolation of *T. vaginalis* from clinical specimens; it gave results comparable to those of broth culture for the group of mainly symptomatic women. Further studies should be performed to determine its utility in clinical populations such as asymptomatic women and men with and without symptoms, in which *T. vaginalis* is more likely to be present in low numbers.**

*Trichomonas vaginalis* is one of the most frequently encountered sexually transmitted pathogens. It has been estimated that 3 million women in the United States and 180 million women worldwide are infected annually (2, 3). Despite this, clinical and laboratory diagnosis of trichomoniasis, especially in men, may sometimes be difficult (12). *T. vaginalis* will grow in cell culture (1, 4, 9, 10, 14) but has infrequently been grown in cell culture from clinical specimens that had not been frozen before processing (9, 14). As shown in this report, in vitro cell culture was more sensitive than broth in detecting low numbers of inoculated *T. vaginalis*. The objective of this study was to evaluate the cell culture system for detection of *T. vaginalis* infection under clinical conditions.

## MATERIALS AND METHODS

**Broth culture.** Isolates were cultured in Diamond TYI-S-33 medium (pH 6.2) (5) supplemented with 10% heat-inactivated bovine serum (TYI) (8). TYI broth (13 ml) was added to disposable borosilicate glass screw-cap tubes (16 by 125 mm) with Teflon (Du Pont Co., Wilmington, Del.)-lined caps (Kimble Co., Toledo, Ohio) and incubated at an angle of 45° at 37°C. At the time of initial isolation from patients, antibiotics (penicillin, 100 U/ml; streptomycin, 100 μg/ml; and gentamicin, 50 μg/ml) were added to remove bacterial contamination. Axenic cultures were subcultured in TYI without antibiotics. For storage, additional serum (final concentration, 15%) and 10% dimethyl sulfoxide were added and the isolates were frozen in liquid nitrogen.

**Cell culture.** McCoy cells were grown in Eagle minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal bovine serum (GIBCO/BRL-Life Technologies Inc., Burlington, Ontario, Canada), 2 mM glutamine, 7 mM sodium bicarbonate, 24 mM glucose, 8 μg of gentamicin per ml, and 25 U of nystatin per ml (pH 7.5) (CMGA). Culturing for *T. vaginalis* was performed in vials. A CMGA suspension (1 ml) containing $1.5 \times 10^5$ McCoy cells per ml was inoculated into borosilicate glass screw-cap 1-dram (1.177-g) MLB polyshell snap cap vials (15 by 45 mm; John's Scientific, Toronto, Ontario, Canada). After 48 h of incubation at 37°C, monolayers were confluent and contained $3 \times 10^5$ to $5 \times 10^5$ cells per vial. The CMGA was aspirated immediately before inoculation with clinical specimens or media containing *T. vaginalis*.

**In vitro comparison of growth in broth and cell culture.** *T. vaginalis* isolates removed from liquid nitrogen were quickly warmed to room temperature and inoculated into TYI medium. Cultures were incubated for 48 to 72 h. The *T. vaginalis* cells were centrifuged at $500 \times g$ for 5 min. The pellet was washed twice by suspension and centrifugation in 0.01 M phosphate-buffered saline (pH 7.2). The *T. vaginalis* cells were then suspended in TYI, CMGA, or CMGA-TYI at a ratio of 2:1. The initial suspensions were counted with a hemacytometer and then standardized to a ratio of 1 *T. vaginalis* cell to 10 McCoy cells, which corresponded to $3 \times 10^4$ to $5 \times 10^4$ *T. vaginalis* per ml. Serial 1:10 dilutions of *T. vaginalis* were prepared in the three media, and 1-ml portions of the *T. vaginalis* suspensions containing from $3 \times 10^4$

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to $5 \times 10^4$ down to a calculated inoculum of $3 \times 10^{-1}$ to $5 \times 10^{-1}$ *T. vaginalis* were added to vials containing confluent McCoy cell monolayers, control vials without McCoy cells, or 10 ml of standard TYI broth in culture tubes. The cultures were incubated aerobically at 37°C.

Representative vials or tubes were sampled daily. Only motile *T. vaginalis* cells were counted. For vials with McCoy cells, the supernatants were separated, the monolayers were washed twice with 1 ml of a balanced salt solution containing 137 mM sodium chloride, 5.4 mM potassium chloride, and 5.6 mM glucose, and the washings were pooled with the supernatants. The McCoy cell monolayers were then trypsinized with 0.2 ml of 0.2% trypsin, and motile *T. vaginalis* cells were counted. The count from the monolayer was added to the count from the pooled supernatant and washings to give the total motile *T. vaginalis* count per vial. In addition, McCoy cell monolayers were examined visually for confluence, and the viability of dispersed McCoy cells was determined by trypan blue exclusion after trypsinization of the monolayer.

**Clinical comparison.** Both men and women were seen at the Sexually Transmitted Disease Clinic (STD Clinic) in the Provincial Health Laboratories Building, and women were seen at the Downtown Community Health Centre (DCHC). At initial visits, women at both locations had symptoms suggestive of vaginitis (vaginal discharge, itch, odor, external dysuria, or introital dyspareunia), but usually only women at the STD Clinic had saline preparations examined to detect motile trichomonads. Most of the women at the STD Clinic were known to have had positive saline preparations at the time initial specimens were obtained. The DCHC information on direct preparations was not available when specimens were obtained for culture. Men were either contacts of women with proven or possible trichomoniasis or were screened because of urethritis. Patients shown to have *T. vaginalis* (usually by a positive saline preparation) at the time of a visit were treated with metronidazole (2 g orally as a single dose).

Women underwent standard genital examinations. For detection of *T. vaginalis* by direct examination, most women at the STD Clinic had a saline preparation to detect motile *T. vaginalis* and almost all women had a Pappenheim smear performed on vaginal secretions fixed onto a slide. For cultures, separate vaginal swabs of secretions pooled around the cervix were obtained. One swab was placed into TYI broth containing antimicrobial agents and broken off into the broth, and the broth was transported to the laboratory for processing. It was then observed for the presence of motile trichomonads, and a 1:10 dilution in fresh TYI medium with antimicrobial agents was prepared. Both preparations were incubated for at least 7 days and observed daily or every other day for motile trichomonads. The other swab was broken off into CMGA-TYI medium containing supplemental antimicrobial agents (final concentrations: penicillin, 200 U/ml; streptomycin 200 μg/ml; gentamicin, 50 μg/ml; and amphotericin B, 2.5 μg/ml) without cells and transported to the laboratory. In the laboratory, the medium was removed from the transport vial and used to inoculate vials containing CMGA-TYI and McCoy cells. A 1:10 dilution into a fresh vial with McCoy cells and CMGA-TYI was made from this vial. Both vials were observed daily or every other day for at least 7 days.

For men, after urethral swabs were taken for cultures for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, or both, as indicated, calcium alginate endourethral swabs (Spectrum Diagnostics Inc., Glenwood, Ill.) were inserted 5 cm into the urethra to obtain specimens for analysis by the two methods described above. The first 10 ml of voided urine was then obtained in sterile polystyrene centrifugation tubes (Evergreen Scientific, Los Angeles, Calif.). It was centrifuged for 5 min at $500 \times g$, and all but 0.5 ml containing the pellet was poured off. The pellet was then immediately resuspended in the residual urine, and 1 to 2 drops were inoculated into both the TYI broth and CMGA-TYI transport medium for subsequent handling as described above.

For specimens obtained in the STD Clinic, transport to the laboratory and initial processing in the laboratory were performed within 1 to 6 h. For specimens from the DCHC Clinic, a maximum of 9 h, but usually no more than 5 h, elapsed before they were processed in the laboratory. Specimens which were not processed until the next day were excluded from analysis.

**Pappenheim smear.** A modification of the Pappenheim stain developed by the British Columbia Provincial Health Laboratories and based on previous formulations of the stain (11) was used. The stain was prepared by sequentially dissolving 8.0 g of methyl green (Color Index, 42585; BDH, Poole, England) and 4.0 g of pyronin Y (Color Index, 45005; certified 70% dye content; Eastman Kodak Co., Rochester, N.Y.) in 200 ml of absolute methanol. Glycerol (99%; 400 ml) was added, and the mixture was allowed to stand overnight. Two liters of 2% (vol/vol) aqueous phenol solution was added, and the final stain mixture was passed through Whatman no. 4 filter paper (Whatman, Inc., Clifton, N.J.) into a brown bottle for storage.

Air-dried smears prepared from vaginal or urethral swabs were fixed by being passed through a flame, cooled, and then flooded with Pappenheim stain for a minimum of 30 s. The slides were rinsed thoroughly with tap water, blotted dry, and examined at a magnification of ×500 with an oil immersion scanning objective. The slides were examined for a minimum of 3 min before being considered negative. Slides positive for *T. vaginalis* showed round, oval, or irregular amoeboid cells 15 to 20 μm in diameter. The nuclei of the trichomonads were ellipsoid and stained pink, with a deeper shade of pink at the periphery. The cytoplasm stained different shades of pink and was granular with moderate-size vacuoles. In contrast, the nuclei of epithelial cells and polymorphonuclear leukocytes stained blue and were round or lobed, respectively. The cytoplasm of polymorphonuclear leukocytes did not take up the stain, and the cytoplasm of epithelial cells stained pink.

## RESULTS

**In vitro evaluation of growth.** The results of initial experiments evaluating potential combinations of media and cells in vials are shown in Table 1 for a single recent isolate from a woman with vaginitis. The results shown are the ranges for six replicate experiments with the isolate. Results of experiments with three other recent clinical isolates, each performed in triplicate, were essentially identical (data not shown). In comparing these culture systems, we evaluated the presence or absence of growth (>50% increase in the number of *T. vaginalis*), the minimum inoculum from which growth was detected, and the peak concentration of *T. vaginalis* attained. For comparison, results in the standard TYI broth system in tubes are also shown. *T. vaginalis* did not grow in CMGA with or without McCoy cells or in CMGA-TYI without McCoy cells, regardless of the inoculum size. With TYI in vials, the presence or absence of McCoy cells had no effect on the growth of *T. vaginalis*.

TABLE 1. Growth of *T. vaginalis* in CMGA, TYI, and CMGA-TYI media in the presence and absence of McCoy cells in vials and in TYI medium in tubes

| Culture system[a] | McCoy cells[b] | Minimum inoculum to detect growth (*T. vaginalis*/ml) | Peak concn attained (*T. vaginalis*/ml) | |
|---|---|---|---|---|
| | | | At minimum inoculum with growth | With inoculum of $4 \times 10^4$/ml |
| CMGA | + | NG[c] | | |
| | − | NG | | |
| CMGA-TYI | + | 3–5 | $2 \times 10^6$–$6 \times 10^6$ | $2 \times 10^6$–$6 \times 10^6$ |
| | − | NG | | |
| TYI | + | $3 \times 10^3$–$5 \times 10^3$ | $10^4$ | $2 \times 10^6$–$6 \times 10^6$ |
| | − | $3 \times 10^3$–$5 \times 10^3$ | $10^4$ | $2 \times 10^6$–$6 \times 10^6$ |
| TYI tube (10 ml) | − | $3 \times 10^2$–$5 \times 10^2$ | $2 \times 10^6$–$6 \times 10^6$ | $2 \times 10^6$–$6 \times 10^6$ |

[a] All systems used 1-ml vials except where otherwise indicated.
[b] +, Cells present; − cells absent.
[c] NG, no growth detected with an inoculum of $4 \times 10^4$ *T. vaginalis* per ml.

With an inoculum of $4 \times 10^4$ *T. vaginalis* per ml, a peak concentration of $3 \times 10^6$ *T. vaginalis* per ml was attained. However, with an inoculum of $3 \times 10^3$ *T. vaginalis* per ml, the lowest inoculum for which growth was detected in this culture system, the peak concentration measured was only $10^4$ *T. vaginalis* per ml. In contrast, in tubes containing 10 ml of TYI, a peak concentration of $2 \times 10^6$ to $6 \times 10^6$ *T. vaginalis* per ml was achieved whenever growth was detected. In this system, the minimum inoculum for which *T. vaginalis* growth was consistently detected was $3 \times 10^2$ to $5 \times 10^2$ *T. vaginalis* per ml ($3 \times 10^3$ to $5 \times 10^3$ *T. vaginalis* per 10-ml tube).

When *T. vaginalis* was grown in CMGA-TYI in the presence of McCoy cells, growth was detected with all inocula tested and the peak concentration was $2 \times 10^6$ to $6 \times 10^6$ *T. vaginalis* per ml. As few as 3 to 5 *T. vaginalis* cells per ml consistently grew to this peak. With a further 1:10 dilution to a calculated inoculum equivalent to 0.3 to 0.5 *T. vaginalis* cells per ml, growth was seen in 10 of 38 vials. When growth was present, the same peak of $2 \times 10^6$ to $6 \times 10^6$ *T. vaginalis* per ml was achieved. In hundreds of subsequent experiments with the cell culture system and CMGA-TYI in vials, with more than 50 different isolates of *T. vaginalis*, the peak concentration of *T. vaginalis* attained was consistently $2 \times 10^6$ to $6 \times 10^6$ per ml. The larger the initial inoculum, the earlier this peak growth was attained. With each 10-fold decrease in the inoculum, the time to peak growth increased by 12 to 24 h.

In the absence of *T. vaginalis*, McCoy cell monolayers remained intact (>75% confluent) and viable for at least 10 days in CMGA and CMGA-TYI. In TYI, the monolayers disrupted in only 18 h. With *T. vaginalis* in CMGA-TYI, the monolayers disrupted when the concentration exceeded approximately $5 \times 10^5$ *T. vaginalis* per ml, and the McCoy cells were almost all nonviable 24 h later.

**Clinical comparison.** There were 209 occasions on which specimens from patients were inoculated into both broth and cell culture (Table 2). The 90 pairs from initial visits by women consisted of 31 from the STD Clinic and 59 from the DCHC Clinic. Among the 209 paired specimens, there were three discordant results, with broth alone being positive for 2 and cell culture alone being positive for 1. Among women, results were concordant for 29 of 31 initial visits and all 66 follow-up visits at the STD Clinic and for 58 of 59 initial visits and all 32 follow-up visits at the DCHC Clinic. Follow-up cultures were positive for 12 of 98 women. Eight women had not been treated by the time of the second visit, and four were positive posttreatment. No woman who was initially culture negative became culture positive at follow-up.

If a woman was considered to have trichomoniasis if she had a positive culture by one or both tests or a positive saline preparation, then in this population of symptomatic women, the sensitivity of the TYI broth was 97% and that of cell culture was 96%. By definition, for both culture systems, the specificity and predictive value of a positive culture was 100%. The predictive value of a negative culture was 98% for both systems. The time to a positive culture was 2 or more days longer in cell culture than in broth on one occasion and 2 or more days longer in broth than in cell culture on one occasion.

Including additional evaluations in which broth cultures, but not necessarily cell cultures, were performed, Pappenheim smear results were available in 227 evaluations of women, and saline preparations were examined in 134 of these (Table 3). The Pappenheim smear was positive in two situations in which the saline preparation and both cultures were negative. Based on the culture results, the sensitivity of the Pappenheim smear was 83% and the sensitivity of the saline preparation was 77%. For the Pappenheim stain, the specificity was 99%, the predictive value of a positive smear was 97%, and the predictive value of a negative smear was 89%. For the saline preparation, the specificity and predictive value of a positive test were 100% and the predictive value of a negative test was 87%.

For men, no *T. vaginalis* detection method resulted in a

TABLE 2. Comparison of broth and cell culture for detection of *T. vaginalis*

| Sex | Clinical situation | No. of culture results[a] | | | |
|---|---|---|---|---|---|
| | | Broth +, cell + | Broth +, Cell − | Broth −, cell + | Broth−, cell − |
| Female | Initial visit | 51 | 2 | 1 | 36 |
| | Follow-up | 12 | | | 86 |
| Male | Initial visit | 1 | | | 9 |
| | Follow-up | | | | 11 |

[a] +, Positive culture; −, negative culture.

TABLE 3. Results of culture, Pappenheim stain, and saline preparations for detection of *T. vaginalis* in women

| Pappenheim smear[a] | Saline prepn[a] | No. of cultures | |
|---|---|---|---|
| | | Positive | Negative |
| + | + | 36 | 0 |
| | − | 9 | 2 |
| | ND | 32 | 0 |
| − | + | 5 | 0 |
| | − | 3 | 79 |
| | ND | 8 | 53 |

[a] +, Positive; −, negative; ND, not done.

high yield, and results with the Pappenheim smear did not predict culture results. The only man who was culture positive in the comparison of the broth and cell culture techniques was positive by both and had motile *T. vaginalis* in the sediment of the first-voided urine but a negative Pappenheim smear. One other man who had a positive broth culture and motile *T. vaginalis* had a negative Pappenheim smear. Cell culture was not performed. For men making an initial visit, when either a urethral swab or first-voided urine sediment or both were cultured at least in broth, Pappenheim smears were positive for 3 and negative for 19. Cultures were negative for all three men with positive smears, as was the evaluation of the first-voided urine sediment for motile trichomonads.

## DISCUSSION

Previous investigations using large inocula have shown that *T. vaginalis* will disrupt a cell culture monolayer (1, 4, 9, 10, 14). However, by comparing the growth of *T. vaginalis* in McCoy cell culture to growth in the same system without McCoy cells, the present study demonstrated that the presence of eucaryotic cells appears to enhance the growth of *T. vaginalis*. Furthermore, by comparing growth in cell culture to that in TYI medium, it was apparent that the cell culture system could support the growth of very low *T. vaginalis* inocula (<3 to 5 *T. vaginalis* cells per ml). A previous report has indicated the ability to detect low *T. vaginalis* inocula in broth culture systems (7). However, the cell culture system was far more reliable and consistent in our experiments and thus appeared more sensitive than standard culture in tubes.

The cell culture system was compared with broth culture in a clinical comparison to assess the utility of the cell culture system. The cell culture technique was shown to be feasible, and the use of antimicrobial agents and a 1:10 dilution circumvented the problem of contamination of cell lines. The results were highly concordant. The group studied consisted primarily of women who were symptomatic at their initial visit. Too few men were evaluated in the comparative study to assess the cell culture system. To assess the relative sensitivity of cell culture in situations in which there are likely to be fewer *T. vaginalis* cells present, studies will need to be performed with populations of asymptomatic women and with larger numbers of men.

We could not readily quantitate the relative ease of using the two systems. Both systems were easy to use to screen for motile *T. vaginalis*. The cell culture system was less easy to use in that it required cell culture and the overlaying medium had to be aspirated before use. There was no apparent need for cells of any specific age, however, and the results seemed comparable when monolayers of any age up to 7 to 10 days were used. The 1:10 dilution was performed to circumvent possible loss of cultures due to contamination. In 15 of 62 (24%) situations, contamination of the undiluted cell culture resulted in a negative undiluted culture with a positive 1:10 dilution. In three situations, the undiluted culture was positive and the 1:10 dilution was negative. Most cultures were positive at 24 h, but two cell cultures first became positive at 6 days. One of these was the specimen that was negative in TYI broth.

Nonculture techniques were also assessed since the culture techniques, especially cell culture, might not be available under some circumstances. As expected, these techniques were less sensitive than culture (7, 13, 16). The saline preparation was evaluated because it is simple and rapid, although it requires reading soon after preparation. In our study, there were two occasions when false-negative saline preparations were grouped. The first was terminated by switching to a fresh batch of saline. The second grouping occurred when the research nurse was not able to read the slides as quickly as is desirable. Thus, the sensitivity may have been underestimated in our study. However, for asymptomatic women, it is likely that the sensitivity would be less than we found for symptomatic women. The specificity, however, was high. Our results with the sensitivity of wet mounts were similar to or better than those previously reported (6, 7, 15, 16).

The Pappenheim smear was evaluated because in our Provincial Health Laboratory it is the routine stain used for detection of gonococci in submitted slides and because it does not require stringent transport or storage conditions. For women, the results were comparable to those with the saline preparation, and the use of this technique to evaluate specimens from high-prevalence populations of women is likely an acceptable alternative to culture. We do not have data on low-prevalence populations of women, but for men, the Pappenheim smear results were negative when cultures were positive and positive for a small proportion of individuals for which there was no other documentation of trichomoniasis. These preliminary results caution against acceptance of this technique for men, pending further evaluation.

In conclusion, *T. vaginalis* growth in vitro is enhanced by the presence of eucaryotic cells. We are not advocating that cell culture become the standard for cultivating clinical specimens of *T. vaginalis*, but it could provide an advantage over broth culture in clinical situations in which low numbers of *T. vaginalis* may be present, such as in male urethritis or in studies of asymptomatic females. At least for high-prevalence populations of women, the saline preparation and Pappenheim smear are alternative nonculture techniques with acceptable, although not ideal, sensitivity and specificity.

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