

Growth of *Trichomonas vaginalis* in Commercial Culture Media

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There are only two commercially available, ready-to-use culture media which are approved by the Food and Drug Administration for clinical diagnosis of vaginal trichomoniasis: Kupferberg's STS and Diamond's medium (modified). Diamond's medium (Klaas modification), recommended by the Centers for Disease Control for the isolation of *Trichomonas vaginalis*, was compared in vitro to Kupferberg's (STS) medium. Growth studies using six fresh clinical isolates, all from different patients, showed that while generation time was about 6 h in both STS and Diamond's, the period of exponential growth was longer in Diamond's. More important, in STS there was a 4-h lag period during which the population significantly decreased prior to exponential growth. This did not occur in Diamond's medium. Three hundred organisms inoculated into Diamond's reached a population of over 10^5 organisms in 72 h. In STS, the same inoculum could multiply to only 6×10^3 organisms. The fact that there is a lag phase in STS which is not seen in Diamond's could explain why low numbers of *T. vaginalis* do not multiply in STS but do multiply and can be detected in Diamond's. We conclude that because Diamond's medium (modified) allows more prolific growth over a shorter period of time, it is more suitable than Kupferberg's (STS) for detecting *T. vaginalis* in patients with vaginitis.

Trichomonas vaginalis infects an estimated 180 million women worldwide per year with sexually transmitted vaginitis (10). It causes approximately one-third of all vaginal discharge complaints. Despite the fact that the disease was characterized and the protozoan was described in 1836 by Donné (5), its detection remains a problem today (6, 10, 12). Culture, with a sensitivity of 86 to 97%, is considered the best method for detection of trichomonads (12). There are two commercially available, ready-to-use media that have been approved by the Food and Drug Administration for clinical diagnostic use to detect *T. vaginalis*: Kupferberg's (STS) and modified Diamond's (Klaas modification). Kupferberg's medium, developed in 1948, evolved from a medium developed by Johnson and Trussell originally known as cysteine peptone liver maltose medium (9, 11). The main ingredients of Kupferberg's medium are cysteine, tryptone, bovine serum, and maltose. Modified Diamond's evolved from Diamond's (TYM) medium, originally developed in 1957 (2). The main ingredients were trypticase digest, yeast extract, cysteine, maltose, ascorbic acid, and sheep serum. Several modifications of this medium have been made, and Diamond later developed several other forms of media for other protozoans (3, 4). All of these were used for propagation of *T. vaginalis* and were called modified Diamond's. In 1980, Klaas, at the Centers for Disease Control, returned to the original Diamond formulation and made the following changes: horse serum replaced sheep serum; the concentrations of maltose, cysteine, and ascorbic acid were increased; agar was eliminated; and antibiotics were added to suppress the growth of bacteria and fungi (5). It is this medium that was recommended by Lossick (12) and Schmid et al. (15). Although Cox and Nicol (1) and Garcia-de-Lomas et al. (7) studied the growth of *T. vaginalis* in a variety of culture media, they did not use either Kupferberg's or modified Diamond's. Recently, clinical evaluations comparing Kupferberg's with modified Diamond's found

that in a clinical setting Diamond's was statistically superior for the detection of trichomoniasis in symptomatic patients. Trichomonads were detected in 52 of 163 patients (32%) by Diamond's medium (modified) but in only 40 of 163 patients (24.5%) by Kupferberg's ($P < 0.001$). This was confirmed in a later study which compared various formulations of Diamond's and Kupferberg's as well as other media (15). The purpose of this study was to examine the growth characteristics of freshly isolated strains of *T. vaginalis* in the two commercially available, ready-to-use culture media that were approved for diagnostic use and to help determine the reason that modified Diamond's medium was so superior in the clinical identification of trichomonads.

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MATERIALS AND METHODS

Strains and media. Fresh clinical isolates of *T. vaginalis* were obtained from six patients attending the Obstetrics and Gynecology Infectious Disease Clinic at Sinai Samaritan Medical Center. Patient consent was obtained. All isolates were obtained from different patients. Motile trichomonads in wet-mount preparations of vaginal secretions obtained on cotton swabs premoistened with sterile saline were simultaneously inoculated into the two culture media to be tested. Kupferberg's and Diamond's medium modified are commercial products obtained premade from Remel (Lenexa, Kans.). Both media were assigned a shelf life of 26 weeks from date of manufacture when kept refrigerated. Appropriate quality control of the media to verify pH and ensure that the media were able to support organism growth was performed with *T. vaginalis* ATCC 30001 and stock clinical isolates. The media were stored at 4 to 8°C and brought to room temperature before use.

Counting procedure. Samples of freshly inoculated media were placed into a Levy double counting chamber hemacytometer and counted at $\times 400$ magnification. Appropriate dilution was made with the medium to be tested to obtain an

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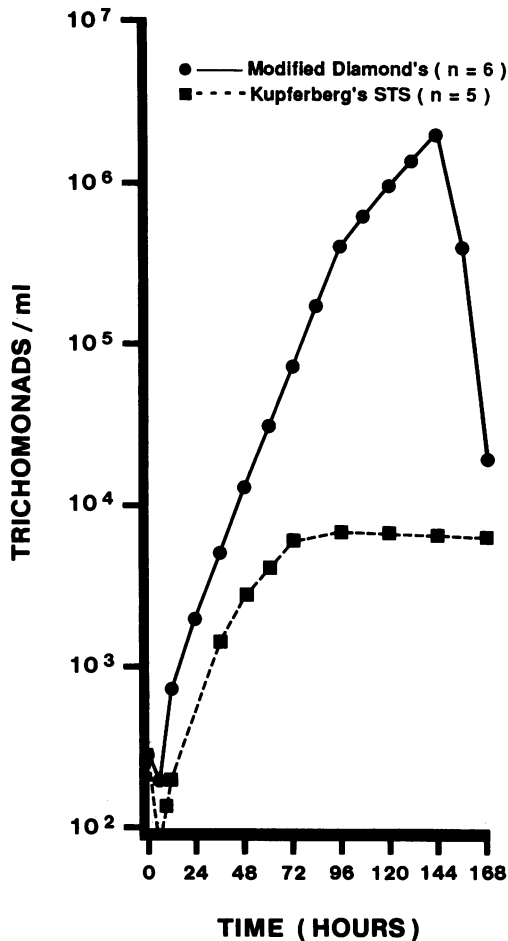


FIG. 1. Growth of *T. vaginalis* in modified Diamond's and Kupferberg's media. Each curve shows the average of the composite counts of the six isolates inoculated into the two media. One strain of *T. vaginalis* could not be recovered from Kupferberg's medium after initial inoculation.

initial concentration of 300 organisms per ml. The starting concentration of 300 protozoans per ml was based on the recommendation of Philip et al., who suggested that the minimal detection limit be on the order of magnitude of 100 CFU (trichomonads) per ml (13). The tubes of media were then incubated at $36 \pm 1^\circ\text{C}$ in a Napco (National Appliance Company, Portland, Oreg.) controlled-environment incubator in 5 to 7% CO_2 at 80 to 85% humidity. Samples were taken for counting after mechanical vortexing for 5 to 10 s, using long-tipped Pasteur pipettes to sample the bottom of the tubes every hour for the first 12 h and then every 12 h thereafter for 7 days. The tubes were kept tightly capped and opened only for sampling. Only motile protozoans were counted. Examination was thorough; the entire ruled area within the hemacytometer grids was counted. Six freshly isolated clinical isolates were studied in each of the media. Each experiment was performed in duplicate, with duplicate counts made for each sample.

RESULTS

The growth curves of the six clinical isolates are presented in Fig. 1. A composite curve for all strains is presented, as

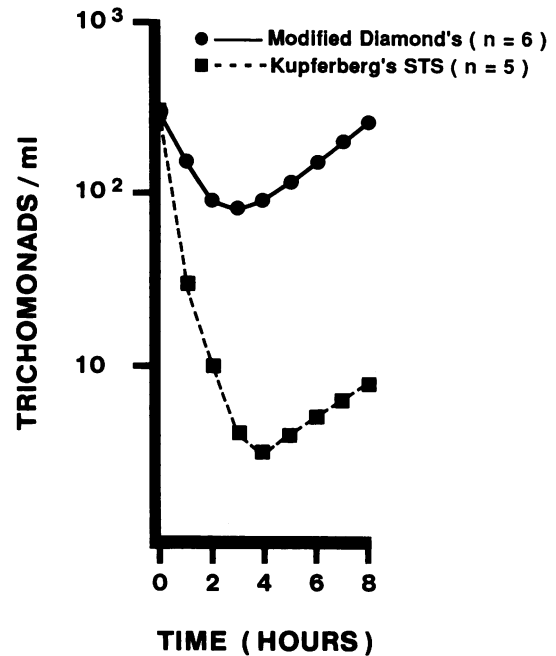


FIG. 2. Lag phase of *T. vaginalis* growth in modified Diamond's and Kupferberg's media. Each curve shows the average of the composite counts of the six isolates inoculated into the two media. One strain of *T. vaginalis* could not be recovered from Kupferberg's medium after initial inoculation.

was done by Cox and Nicol (1) and Garcia-de-Lomas et al. (7). One strain of *T. vaginalis* when inoculated into Kupferberg's medium was lost during the lag phase and could not be recovered in subsequent sampling. All strains were recoverable in modified Diamond's. The lag phase (Fig. 2), which lasted for 3 h in modified Diamond's and 4 h in Kupferberg's, was far more catastrophic in Kupferberg's. Over 90% of the initial inoculum became nonmotile in Kupferberg's. In contrast, there was only a slight loss of motility in modified Diamond's during the lag phase.

Exponential growth occurred well beyond 96 h in modified Diamond's, resulting in a maximum concentration of 2×10^6 organisms per ml. The mean generation time for the six isolates in both media was the same, i.e., 5.6 h (5.0 to 6.2 h). Exponential growth stopped at approximately 48 h in Kupferberg's, with the maximum concentration of organisms equalling only 6×10^3 , representing only a 20-fold increase over the concentration in the initial inoculum. Motility was far more obvious after 72 h of growth in modified Diamond's than after this time in Kupferberg's. However, stationary phase persisted for over 7 days in Kupferberg's. Nearly all organisms were motile. In contrast, after 7 days in modified Diamond's, 99% of the culture were nonmotile and presumed dead.

DISCUSSION

Culture for *T. vaginalis* has been considered the "gold standard" for *T. vaginalis* detection, although this attitude has been debated (12, 16). It was recognized that a relatively large number of organisms must be inoculated for adequate growth (1, 14). Cox and Nicol reported that strain variation caused difficulty in reproducing growth curves (1). This problem was not reported by Garcia-de-Lomas, who used a much larger initial inoculum (7). When fresh clinical isolates that had not been grown in culture medium were used, these

difficulties were not encountered. Apparently, the growth characteristics of the organism are modified by passage in culture medium or by freezing, as was done by Garcia-de-Lomas. Kupferberg's medium did not appear to be as sensitive as modified Diamond's at initially propagating the growth of *T. vaginalis* in a clinical setting (8, 15). This finding could be explained by the significant loss in motility during lag phase that initially followed inoculation into Kupferberg's medium.

Cultures were not incubated under anaerobic conditions. The tubes were tightly capped and opened only for sampling. The incubator used was the one available in the clinic used for culturing gonorrhea. The same type would be used for *T. vaginalis* culturing in a clinical setting. The 5 to 7% CO₂ or 80 to 85% humidity is not necessary. Any incubator can be used. The manufacturers of the media for trichomonad identification do not recommend anaerobic incubation. They specifically recommend ambient air. Nevertheless, *T. vaginalis* is an obligate anaerobe, and the effect of repeated sampling on the anaerobiosis of the medium has to be considered a possible parameter which was not investigated.

Modified Diamond's medium uses 12% horse serum, while Kupferberg's contains only 5% bovine serum. This is a major difference between the two media. The additional serum may be protective, diminishing the catastrophic lag phase that occurs when clinical specimens are inoculated into media. Increasing the serum concentration of Kupferberg's might have a similar effect. Although not explored in the study, future investigation of serum concentration effect may substantiate the hypothesis.

On the other hand, by not being as rich in nutrients, Kupferberg's medium does not permit extensive multiplication, thus considerably delaying the death phase of the protozoan. This fact can be very beneficial to laboratories wishing to maintain axenic cultures for long periods. Modified Diamond's, however, must be examined within 3 to 5 days. There is significant loss of motility after 7 days.

In recent studies for the detection of *T. vaginalis* in clinical specimens, modified Diamond's proved to be superior to Kupferberg's medium (8, 15). At the time of the Centers for Disease Control study (15), modified Diamond's medium was not commercially available. It is now available. Its sensitivity appears to approach 100%. Our study confirms the observations of others (8, 15), and modified Diamond's medium is recommended as the medium of choice for *T. vaginalis* detection. One factor that would diminish the sensitivity of culture medium is the adequacy of the patient sample. However, even with a poorly obtained patient sample with few organisms or a sample obtained from a patient in whom there were only rare trichomonads, modified Diamond's medium could be relied upon to give a more accurate result than Kupferberg's.

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