



GROWTH OF *Trichomonas vaginalis* IN MODIFIED FEINBERG-WHITTINGTON MEDIUM

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ABSTRACT

Trichomonas vaginalis is a protozoan pathogen of the human urogenital tract. A number of different media are now in use for the culture of *T. vaginalis*, but they are too complex and expensive for ordinary routine work, to that the modification and simplification with available resources in culture medium has a paramount importance. To facilitate using of *Trichomonas* culture media in routine diagnostic work by modification and simplification of Feinberg-Whittington medium (F.W) with available resources. Sheep liver powder prepared and 8% horse Serum replaced by 5% human serum. Urine samples containing *T.vaginales* obtained from Bashaier teaching Hospital. The sediment containing parasite was inoculated in prepared medium and incubated at 37⁰C for 24 to 48 hours. The growth was evaluated. *T. vaginales* and *Candida* species were growth in modified (F.W) medium. Other flagellates detected in urine samples were converted to cyst after overnight incubation. Conclusion for availability and low cost of modified (F.W) medium, this new medium might be used as an alternative choice for *Trichomonas* culture method and avoider misdiagnosis with other intestinal flagellates.

INTRODUCTION

Trichomonas vaginalis is a protozoan pathogen of the human urogenital tract. The World Health Organization (WHO) estimates that trichomoniasis accounts for more than half of all curable sexually transmitted infections (STIs) worldwide [1]. *T. vaginalis* infects an estimated 180 million women worldwide per year with sexually transmitted vaginitis [2]. 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 Donn  [3], its detection remains a problem today [4,5]. Trichomoniasis is of worldwide importance especially because in recent years, it has been implicated in amplifying human immunodeficiency virus (HIV) transmission [6].

In addition, *T. vaginalis* acts as a potential catalyst in the acquisition of secondary infections including human papillomavirus (HPV), the organism responsible for pathogenesis of cervical cancer [7]. Furthermore, a relationship between trichomoniasis and prostate cancer has been reported [8].

The laboratory methods usually employed for detecting *T. vaginalis* in secretions are the microscopic examination of wet films or fixed, stained smears, and the culture of the parasites in artificial media [9]. The microscopic examination of vaginal fluid has a sensitivity of only about 60% compared to that of culture. [10] Culture, with a sensitivity of 86 to 97%, is considered the best method for detection of trichomonads.

There are many attempts have been made to grow *T. vaginalis* in a variety of media. More of these media had the disadvantage of favouring the growth of the accompanying bacteria as well as that of the trichomonads. Johnson and Trussell and Sprince and Kupferberg used elaborate media for maintaining bacteria-free cultures of *T. vaginalis*. McEntegart simplified Johnson and Trussell's

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formula for use in his studies on *T. vaginalis*, and Feinberg substantially modified McEntegart's medium and succeeded in growing the parasites in large numbers for experimental work.

Feinberg and Whittington medium has been used routinely in London Hospital Research 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.

However, in vitro cultivation of the organisms is invaluable for diagnosis and considers the right hand of axenic cultivation in studying the biochemistry, physiology, metabolism, immunology and ultra-structure of the organisms as well as for screening drugs so that advances in chemotherapy can be achieved.

Now, there are more types of culture media for *T. vaginalis*, but they are too complex and expensive for ordinary routine work, for these the aim of this study to modify and simplify of Feinberg-Whittington (F-W) medium to suggest using in routine diagnostic work, by prepared sheep liver powder and replace Horse Serum by human serum.

MATERIAL AND METHODS

Study type and approach

This study is experimental study, tried to find out potency of modified culture medium in proliferation of *T. vaginalis*.

Study area

This study was carried out in Bashaier teaching Hospital, Khartoum state.

Study population

The study was conducted on reviewers attended to Bashaier teaching hospitals with trophozoite of *Trichomonas vaginalis* in their urine sample.

Sample collection:

Urine samples were collected from patients in clean and dry wide mouth containers.

Materials

Supplies

Disposable sterile Pasteur and serological pipettes.

- Microscope slides.
- Cover slips.
- Volumetric flasks (100 ml)
- Graduated cylinders (1,000 ml).
- Flasks or beakers.
- Screw –cap tubes.
- Funnel.
- Culture tubes, racks.
- PH paper.

Equipment

- Binocular microscope with 10x, 40x, 100x objectives.
- Centrifuge.

- Water bath.
- Sensitive balance.
- Incubator.
- Autoclave.
- Refrigerator.
- Oven.
- Neubauer chamber (haemocytometer).

Reagents

Agar	1.0g.
Sodium chloride	6.5g.
Dextrose	5.0g.
Liver powder	25.0g.
Distilled water	1,000 ml.
Penicillin	1,000,000 units.
Streptomycin	5,00,000 units.
Inactivated human serum	50.0 ml.

Culture medium

Preparation of sheep liver powder

Liver powder was prepared by cute up sheep liver into small pieces , put it in room temperature , leaved to dry (far from sun's rays), crushed with miller electric , filtered to separate the large particles from small one and stored in cool , dry place until use .

Preparation of inactivated human serum

Human sera were collected from blood group O positive healthy volunteers, supplementary takers (Multi Vitamins, Iron, folic acid).

Blood sample was placed 30 minutes to clotted, centrifugation was done, separated the serum into plan tube.

Serum inactivated by maintained in water bath at a temperature of 56⁰C for 30 minutes, stored in freezing at -20⁰C until use.

Preparation of culture medium

Solid components were suspended in 1 liter of distilled water and bring to the boil to dissolved, then autoclaving at 121⁰C for 15 minutes to sterilized. After cooled to approximately 50⁰C, added serum and antibiotic and measured pH (pH 6.0). Culture medium distributed in test tubes, 3 ml in each test tube, covered and stored in the refrigerator at 4⁰C for 2 months.

Procedure

Urine sample was centrifuged at 1,500 rpm for 10 min at room temperature, discharged supernatant and transferred one drop of urine sediment in to clean glass slide, and immediately examined microscopically using x10 and x40. Sediment containing *T. vaginalis* was inoculated into the warmed medium. The tubes Incubated in slanted position (45⁰ angle), at 37⁰C aerobically. Culture media examined for 2 days. Parasite number counted by Neubauer chamber (haemocytometer).



RESULTS

We were prepared sheep liver powder and replaced 8% horse Serum by 5% human serum.

T. vaginalis was grown up to 250 parasite /ml in 3 ml in culture medium after 24 h incubated in 37°C, aerobically. After 48h Candida was detected.

We found some flagellates in female urine samples. Cyst was found after cultured there parasites in 37°C overnight.

DISCUSSION AND CONCLUSION

Culture for *T. vaginalis* has been considered the "gold standard" for *T. vaginalis* detection. Different culture media have been employed for this purpose with varying degrees of success. The need for a medium to be used for routine work of cultures for detected *T. vaginalis* of compounds made a cheaper medium desirable.

Whittington (1966) found that was a more sensitive method of detecting the flagellate in secretions from the female genital tract than direct microscopically examination of fresh smears.

Liver digest and horse serum are very important supplement in parasite culture. They provide culture by nitrogen, amino acids, vitamins and carbon. The highly

cost of them, is the main disadvantages'. From this problem, needed for existence of resources of cheap and available is the reason for the establishment of this modification?

Our results describes a medium which seems particularly suitable, because it is cheap, easy to prepare and use, and keeps well.

The growth was established in 24 to 48 hours, yeasts and Candida species were growth .This result agrees with similar studies done by Whittington et al 1966.

Some flagellates were detected in some females' urine samples, they caused misdiagnosis with *T.vaginalis*, after cultured cysts were appear, unsuitable environmental condition cause of this convert. Contamination of urine samples with feces cause of intestinal flagellates detected in there samples. From this point we can use this modified medium for differentiation between *T.vaginalis* and other intestinal flagellates to avoid misdiagnosis.

Therefore, for costless and viability, we recommended that the modified (F.W) medium an alternative culture medium, especially in laboratories with limited material resource and also laboratories present in small cities and health centers.

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