World Journal of Medical Images, Videos and Cases

ORIGINAL ARTICLE

Alternative methodology for preparing a thermally intact lysate of *Trichomonas vaginalis*.

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Introduction

Trichomonas vaginalis is now the 4th leading sexually transmitted agent in the world according to WHO. Its easily transmitted during sexual intercourse between both sexes. It may also be transmitted by sharing intimate hygiene products. Currently, many cases have an asymptomatic course. *T. vaginalis* is known to produce virulence factors such as cysteine proteinases, which are involved in its cytotoxicity [1].

This pilot study is part of a bigger research project and is aimed at working out the methodology for lysing the cells of *T. vaginalis.*

Materials and Methods

Strains. For this pilot study the following parasite strains were used:

- *Trichomonas vaginalis* ATCC PRA-92 24h and 48h culture.
- *Trichomonas vaginalis* Katedra Mikrobiologii UJCM strain (KAT) 24h and 48h culture (Figure 1).

Materials: Besides of sterile plastic containers, we used solid-glass borosilicate beads 2 mm (Sigma-Aldrich, Poland), Diamonds medium, an Environmental Shaker-Incubator ES-20/60 (Biosan, Latvia), FastPrep FP120 Cell Homogenizer (Thermo Electron, USA), SureSeal Screw Cap Microcentrifuge Tubes 2 ml (MTC Bio, USA) with 180 µm glass beads (Sigma-Aldrich, Poland) and a MPW-212 Centrifuge (MPW Med. Instruments, Poland).

Methodology.
First trial: 2.5 ml of 24-hour Diamonds medium culture of *T. vaginalis* was placed in 4 plastic containers with 2 mm borosilicate beads. *Trichomonas vaginalis* ATCC PRA-92 was placed in two and *Trichomonas vaginalis* Katedra Mikrobiologii UJCM strain in the other two containers. At that time one of each strain was moved to –20°C for 30 minutes and the other two containers were kept at room temperature. The containers were then placed in the mechanical shaker, as above, for 30 minutes at 250 rpm. The two samples that were not frozen were kept at room temperature during the shaking process. The frozen samples were heated from –20°C to 37°C during the shaking.
Second trial: Using standard centrifuge test tubes, 2.5 ml of each of the 24h cultures (PRA-92 and KAT) were centrifuged at 12000 rpm for 5 and 15 minutes.

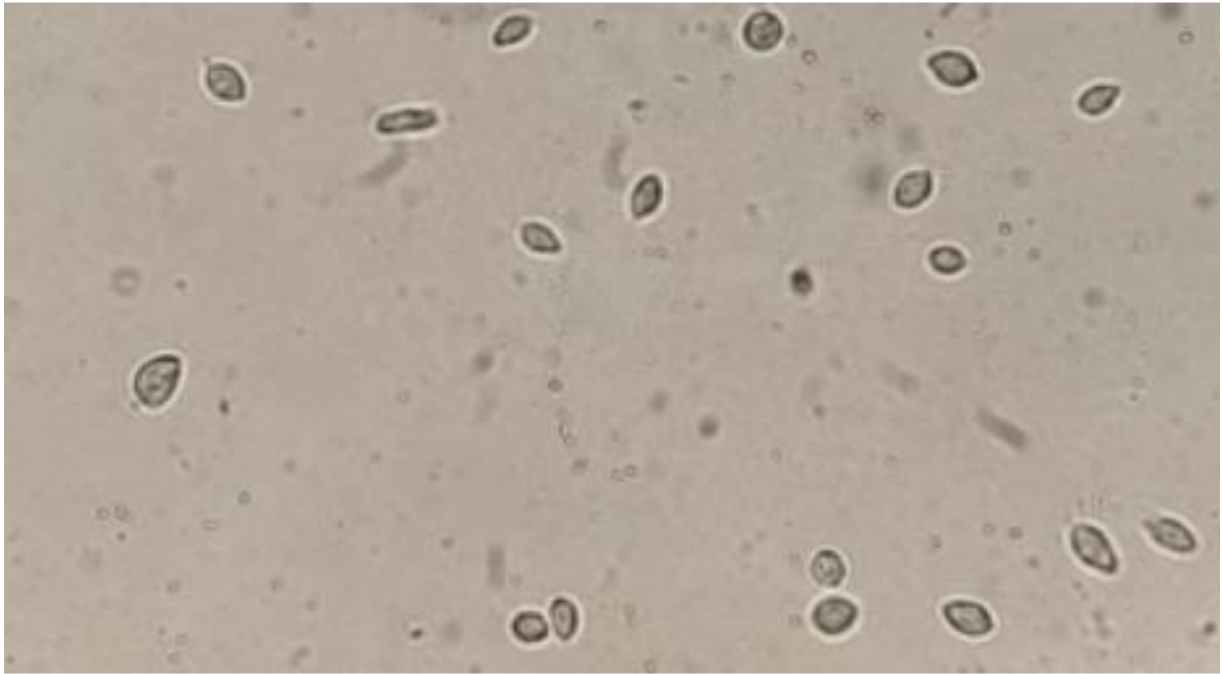

Figure 1. Screenshot of the film showing a viable culture of *Trichomonas vaginalis* in Diamond's medium. Please click on the link to view the film on the official WJOMI YouTube channel: https://youtu.be/fuOwxrNxt0g

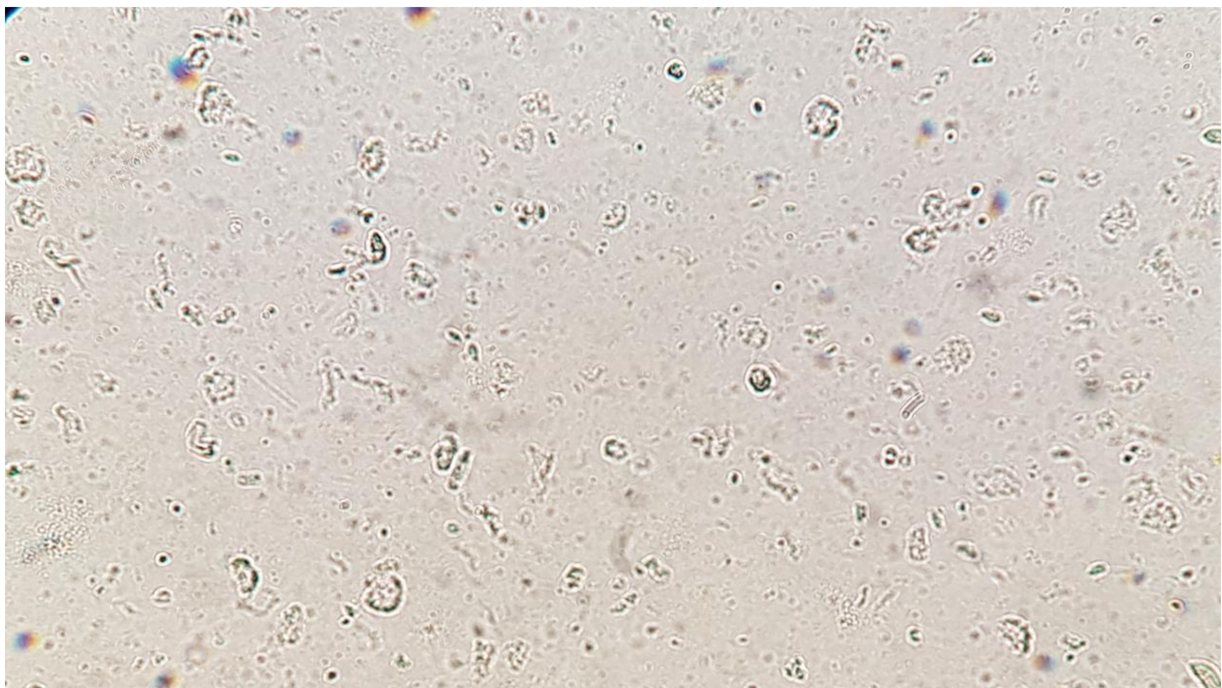

Figure 2. Microscopic photograph of the lysed *Trichomonas vaginalis* trophozoites (using 10× objective and digital camera zoom).

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Third trial: 1 ml of each of the 24h cultures were placed in two different FastPrep test tubes with 180 µm glass beads at 1 cycle of 45 seconds at 6.5 m/s.

Fourth trial: 1 ml of each of the 48h cultures were placed in two different FastPrep tubes with 180 µm glass beads. We then ran 3 cycles of 20 seconds at 6.5 m/s, with 15 minute intervals between each cycle to avoid the samples overheating. The temperature was measured to be approximately 25°C after each cycle, which confirmed that the content was not lysed due to high temperature, but by mechanical force. This is important because it prevents denaturation of the intracellular enzymes.

Results with discussion

The results of trial one, two, and three were all failed, i.e. the cells were not completely lysed. Especially in trial two, the trophozoites appeared to be even more active than the control after the centrifugation.

In trial four, however, we managed to completely lyse the cells with no viable trophozoites (Figure 2). This was achieved without compromising the active substances within the trophozoites. The current approach for making lysate of *T. vaginalis* without denaturating the enzymes has been sonication of the sample [2]. However, sonication ultrasound apparatus is not always readily available in the lab and the FastPrep method provides an easy and cheap alternative to the sonication method.

Conclusions

The FastPrep method allows for a mechanical alternative for making a thermally intact lysate of *T. vaginalis*.

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Conflict of interest: none declared

Acknowledgements: Big thanks to Ms Barbara Papir for help with *Trichomonas* cultures.

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To cite this article: Rønneberg H, Lunde V. Alternative methodology for preparing a thermally intact lysate of *Trichomonas vaginalis*. World J Med Images Videos Cases 2019; 5:e14-16.

Submitted for publication: 18 March 2019
Accepted for publication: 29 March 2019
Published on: 31 March 2019

ISSN: 2450-5773
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