# PRELIMINARY STUDIES ON DEVELOPMENT OF A PCR BASED TECHNIQUE FOR THE DIAGNOSIS OF *Trichomonas vaginalis*

PROJECT REPORT PRESENTED BY

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## ABSTRACT

**Preliminary studies on development a PCR based technique for the diagnosis of *Trichomonas vaginalis***

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The aim of this study was to establish a Polymerase Chain Reaction (PCR)-based technique to help future diagnostic studies on the urogenital parasite *Trichomonas vaginalis* in Sri Lanka. *T. vaginalis* causes the most common nonviral sexually transmitted disease (STD) affecting 180 million adults worldwide (World Health Organization, 2001). Whilst the affected women show symptoms such as vaginitis, urethritis and cervicitis, men act as asymptomatic carriers. *T. vaginalis* causes pelvic inflammatory disease, abortions and acts as a risk factor for obstetric complications in pregnant women (Riley *et al.*, 1992; Wendel *et al.*, 2002). The public health significance of the disease has been seriously increased following the findings that *T. vaginalis* infection acts as a risk factor for transmission of human immunodeficiency virus (Wendel *et al.*, 2002) and cervical cancer (Zhang *et al.*,1995).

Although specific diagnosis of trichomoniasis depends on laboratory tests, diagnosis has been hampered due to lack of sensitive detection methods (Riley *et al.*, 1992). Diagnosis of Trichomoniasis is currently based on wet smear microscopy, which is subjective to the parasite viability, parasite number and the experience of the microscopist. Several overseas research groups have recently applied molecular biology based methods to detect extremely low numbers of *T. vaginalis* (Riley *et al.*, 1992; Wendel *et al.*, 2002). Once established, these methods offer rapid, non- subjective and extremely sensitive diagnostic tools (Riley *et al.*, 1992; Madico *et al.*,1998). The present study describes a short term project (Jan –June 2008) in which *T. vaginalis* was maintained under *in vitro* conditions with attempts to establish a genus- specific PCR -based laboratory diagnostic technique to detect *T. vaginalis* in Sri Lanka.

*Trichomonas vaginalis* (originally isolated from a patient) was maintained under *in vitro* conditions at the Department of Parasitology, Faculty of Medicine, University of Peradeniya. Subcultures of this clone was maintained in Robinson's medium (pH 7.2; at 28 °C) with frequent microscopic monitoring. A touch down PCR was carried out with specific primers (BTUB9/2). After completion, 10µl of the reaction mixture was loaded on to a 0.8% agarose gel stained with ethidium bromide for electrophoresis and subsequent visualization by UV transillumination. Known positive and negative controls were used in each run. The reaction mixture was also analysed on 12% SDS-polyacrylamide gel electrophoresis at 120V. The gels were stained with ethidium bromide and visualized by UV transillumination. Known positive control and negative controls were used in each run.

In agarose gels, whilst the positive control showed a distinct band, the parasite material showed a smearing effect. A marked improvement was seen when SDS-PAGE was used. The results showed DNA bands parallel to the molecular marker at 112bp. Ideally this experiment should be run several times to study the reproducibility and the results should be compared with several isolates of *T. vaginalis*. However, the short duration of the time and lack of facilities inhibited the progress of the study.

The results of this study show that the use of a PCR method to amplify the DNA of *T. vaginalis* as a diagnostic tool in Sri Lanka is realistic, achievable and worthwhile. PCR needs extremely low numbers of parasites and hence will offer a method to detect parasites in urine samples and in clinical specimens irrespective of the parasite viability and the number. This noninvasive method will indeed be suitable as a screening method in both men and women. This short term study paves the way to optimize the conditions, to determine the reproducibility and then to apply it as an invaluable diagnostic tool to detect *T. vaginalis* in Sri Lanka. Such a tool will also be useful to study the intra specific variation of *T. vaginalis* in Sri Lanka.