TRICHOMONAS

Comparison of a TaqMan-based real-time polymerase chain reaction with conventional tests for the detection of *Trichomonas vaginalis*

A Pillay, F Radebe, G Fehler, Y Htun, R C Ballard

Sex Transm Infect 2007;**83**:126–129. doi: 10.1136/sti.2006.022376

See end of article for authors' affiliations

Correspondence to: Dr A Pillay, Centers for Disease Control and Prevention, Division of STD Prevention, Laboratory Reference and Research Branch, 1600 Clifton Road, MS-G39, Atlanta, GA 30333, USA; apillay@cdc.gov

Accepted 20 October 2006
Published Online First
7 November 2006

Objective: To compare a TaqMan-based real-time polymerase chain reaction (PCR) with conventional PCR, culture, and wet-mount microscopy for the diagnosis of trichomoniasis in women.

Methods: Vaginal swabs from 119 women were tested for *Trichomonas vaginalis* by wet mount and culture. Paired vaginal lavage and urine specimens were tested by conventional and real-time PCR.

Results: Using an expanded "gold standard", defined as a positive culture result using vaginal swabs and/or a positive PCR test using TVK3/7 primers, the overall prevalence of *T vaginalis* in the study population was 65.5% (78/119). The detection rate of *T vaginalis* was 65.5% (78/119) and 36.9% (44/119) by conventional PCR using vaginal washings and urine specimens, respectively; 68.9% (82/119) by real-time PCR using vaginal washings and 61.3% (73/119) by real-time PCR using urine specimens. The sensitivities of conventional PCR using vaginal washings and urine and real-time PCR using vaginal washings and urine, compared with the gold standard were 100%, 56.4%, 100% and 76.7%, and the specificities of these tests were 100%, 97.6%, 82.9% and 97%, respectively.

Conclusions: The real-time PCR test proved to be significantly more sensitive than culture and wet-mount microscopy, although its specificity was slightly lower than these tests. In addition, it was more sensitive, rapid and less time consuming than conventional PCR for the detection of *T vaginalis*.

It has been estimated that five million new cases of trichomoniasis occur in the USA each year.¹ It is a common cause of sexually acquired vaginal discharge, and infection during pregnancy may result in sequelae such as preterm labour and premature rupture of membranes.² In many settings, particularly in developing countries, *Trichomonas vaginalis* is an important cause of urethritis in men.³

Although wet mount microscopy of vaginal fluid remains the most widely used diagnostic test for vaginal trichomoniasis, detection of *T vaginalis* by culture remains the "gold standard" for the diagnosis of the disease. Both culture and examination of a wet preparation are less sensitive than PCR-based tests for the detection of *T vaginalis*; however, PCR is not routinely used for the diagnosis of *T vaginalis* infections.⁴ The sensitivity of culture compared with PCR ranges from 34.9% to 78%, its specificity is usually 100%.^{5–7} Similarly, the specificity of wet-mount microscopy is generally high, whereas its sensitivity, compared with PCRs is poor, with reported rates ranging from 34.2% to 58.5%.^{4 6 8–10}

The aim of this study was to develop a TaqMan-based real-time PCR and evaluate it against conventional tests for the detection of *T vaginalis* in women.

METHODS

T. vaginalis laboratory strains

T vaginalis strains CDC 1185 and CDC 085 were used as positive controls and for sensitivity testing during PCR amplification. Laboratory strains were grown anaerobically in 12 ml of Diamond's medium at 35°C for 48 h. Trichomonads were counted using a Cellometer (Nexcelom Bioscience, Lawrence, Massachusetts, USA), and genomic DNA was extracted using the QIAamp DNA mini-kit (Qiagen, Valencia, California, USA).

Study population and specimen collection

Paired vaginal washings and urine specimens from 119 consecutive women attending the Esselen Street STI Clinic,

Hillbrow, Johannesburg, South Africa, and the HIV clinic, Hillbrow Hospital, Johannesburg, South Africa, were tested. These specimens were collected as part of a study to investigate the patterns of vaginal and cervical infections in HIV-positive and HIV-negative women, and their possible role as cofactors in genital shedding of HIV.

In each case, a sterile cotton-tipped swab (Medical Wire and Equipment, Corsham, UK) was used to obtain a high vaginal swab for wet mount microscopy. The swab was placed in 1 ml of saline immediately after collection and then agitated. A drop of fluid was then placed on a clean glass slide and examined microscopically for motile trichomonads. An additional high vaginal swab was used to inoculate a tube of Diamond's medium for culture of *T vaginalis*. Inoculated tubes were incubated at 35°C under anaerobic conditions, and a drop of the inoculated medium examined microscopically daily for 7 days for motile trichomonads.

Subsequently, cervicovaginal lavage (CVL) specimens were collected to assess HIV shedding in vaginal secretions. A CVL was performed by flushing out the cervix with 10 ml of sterile normal saline using a sterile 20 ml disposable syringe. The CVL fluid was allowed to collect in the posterior fornix for 2–3 min and then aspirated using a sterile 10 ml disposable pipette. Vaginal swab specimens were not available for molecular detection of *T vaginalis*, therefore, cervicovaginal washings were used instead. A first catch urine specimen was collected after gynaecological examination for detection of *T vaginalis* by PCR. Culture and wet preparations were performed in South Africa, and the molecular testing was performed at the Centers for Disease Control and Prevention, Atlanta.

After collection of specimens, all participants with signs and symptoms of STDs were treated immediately according to

Abbreviations: βTUB, β-tubulin genes; CVL, cervicovaginal lavage; PCR, polymerase chain reaction; RNase, ribonuclease

syndromic management treatment guidelines recommended by the Department of Health of the Republic of South Africa.¹¹ This study was approved by the institutional review board of the Centers for Disease Control and Prevention and the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg.

Polymerase chain reaction

Genomic DNA was extracted from 140 µl aliquots of CVL fluid using the QIAamp DNA mini-kit (Qiagen), and from 500 µl aliquots of urine using a viral RNA kit (Qiagen). Conventional PCR using the primers TVK3 (5'-AT TGT CGA ACA TTG GTC TTA CCC TC-3') and TVK7 (5'-TCT GTG CCG TCT TCA AGT ATG C-3') were used to amplify a 261-bp sequence of a *T vaginalis*-specific repeat DNA fragment.^{12,13} This has previously been shown to be the most sensitive conventional PCR test for *T vaginalis*.⁹

A real-time PCR test was designed to amplify a 92-bp segment of the aforementioned *T vaginalis*-specific repeat.¹³ A 28-mer forward primer TV001 (5'-A AAG ATG GGT GTT TTA AGC TAG ATA AGG-3'), a 22-mer reverse primer TV002 (5'-T CTG TGC CGT CTT CAA GTA TGC-3') and a 26-mer probe TV003P (5'-AG TTC ATG TCC TCT CCA AGC GTA AGT-3') were selected for real-time PCR amplification using Primer Express Software (Applied Biosystems; Foster City, California, USA). The probe was labelled with fluorophor cyanine at the 5' end and black-hole quencher 2 at the 3' end. Real-time PCR was performed in a reaction volume of 25 µl with the following components: 2 µl of deoxynucleoside triphosphate mix (2.5 mM of dATP, dCTP, dGTP, and 5 mM of dUTPs), 3 µl of MgCl₂ (25 mmol/l), 0.3 µM of each primer, 0.3 U of uracil N-glycosylase, 2.5 U of AmpliTaq Gold polymerase, 2.5 µl of 10× PCR buffer (all Applied Biosystems), 0.15 µM of probe and 15 µl of template DNA. Thermocycling was performed in a Rotor-Gene 3000 instrument (Corbett Research, San Francisco, California, USA) as follows: 50°C for 2 min and 95°C for 10 min, followed by 50 cycles of 95°C for 20 s and 60°C for 1 min.

A confirmatory real-time PCR test was designed to amplify a segment of the β-tubulin genes (βTUB; βtub1, βtub2 and βtub3) of *T vaginalis*.⁸ A 26-mer degenerate forward primer TV4C (5'-CW CTT TAC GAT ATY TGC TTC CGT ACA-3'), a 19-mer reverse primer TV5 (5'-TG CCG GAC ATA ACC ATG GA-3') and a 26-mer probe TV7P (5'-AA GCT CAC AAC ACC AAC ATA CGG CGA-3') were selected using Primer Express Software (Applied Biosystems). A forward primer with degeneracy in the second and 14th bases was designed to amplify all three copies of βTUB. Real-time PCR was performed in a reaction volume of 25 µl comprising the following: 2 µl of deoxynucleoside triphosphate mix (2.5 mM of dATP, dCTP, dGTP, and 5 mM of dUTP; Applied Biosystems), 3 µl of MgCl₂ (25 mmol/l; Applied Biosystems), 0.3 µM of forward primer, 0.2 µM of reverse primer, 0.3 U uracil N-glycosylase, 2.5 U of AmpliTaq Gold polymerase, 2.5 µl of 10× PCR buffer (all Applied Biosystems), 0.1 µM of probe and 15 µl of template DNA. The probe was labelled with the fluorophor fluorescein phosphoramidite at the 5' end and black-hole quencher 1 at the 3' end. Thermocycling was performed in a Rotor-Gene 3000 instrument as described above. Negative and positive controls were included in each run. A positive result was defined as a sample having a cycle threshold value between 15.9 and 45 using a manually set baseline.

A real-time PCR targeting the human ribonuclease (RNase) P gene was used to test for PCR inhibition in vaginal washings and urine samples that were negative by the *T vaginalis* repeat real-time assay.¹⁴ The RNase P assay was initially performed identically to the *T vaginalis*-specific repeat real-time PCR, with

the exception that primers and probes were used at a concentration of 0.2 µM. Samples showing PCR inhibition were re-extracted and tested using 10 µl of DNA in a 50 µl reaction volume.

The specificity of the real-time PCR assays was evaluated against microorganisms that are closely associated with or related to *T vaginalis* (table 1). The detection limit of the real-time PCR tests was determined by amplifying duplicate 10-fold serial dilutions of *T vaginalis* genomic DNA. The detection limit was defined as the highest dilution with a positive result in each of the two duplicate samples tested.

Although the analytical sensitivity of the primary real-time PCR was better than the confirmatory assay, vaginal washings or urine specimens that were positive by the primary real-time PCR alone were retested with the βTUB real-time PCR using DNA that was re-extracted from 1 ml of CVL fluid or between 500 µl and 1.5 ml of urine.

RESULTS

Using an expanded gold standard, defined as a positive culture result using vaginal swabs and/or a positive PCR test using TVK3/7 primers, the overall prevalence of trichomoniasis in the study population was 65.5% (78/119). After resolving discordant results, the overall prevalence was 68.9% (82/119). By culture alone, and by wet preparation alone, the prevalence of

Table 1 Microorganisms used for specificity testing of real-time polymerase chain reaction

<i>Bacteroides fragilis</i> ATCC 25285D
<i>Branhamella catarrhalis</i> H1215
<i>Borrelia burgdorferi</i>
<i>Candida albicans</i>
<i>Chlamydia trachomatis</i>
<i>Chlamydia pneumoniae</i>
<i>Dientamoeba fragilis</i>
<i>Escherichia coli</i> ATCC 25922
<i>Enterococcus faecalis</i> SS1273
<i>Enterococcus faecium</i> SS1274
<i>Gardnerella vaginalis</i> ATCC 4104
<i>Giardia intestinalis</i> Portland 1 ATCC 30888D
<i>Giardia lamblia</i> P290801
<i>Haemophilus ducreyi</i>
Herpes simplex virus 1
Herpes simplex virus 2
<i>Leptospira weilii</i> serovar <i>celledoni</i> strain Celledoni
<i>Leptospira noguchii</i> serovar <i>pomona</i> strain Pomona
<i>Mobiluncus curtisii</i> subspecies <i>curtisii</i>
<i>Mobiluncus curtisii</i> subsp. <i>mulieris</i>
<i>Moraxella atlantae</i> H1401
<i>Moraxella nonliquefaciens</i> H2066
<i>Mycoplasma hominis</i>
<i>Mycoplasma genitalium</i>
<i>Mycoplasma penetrans</i>
<i>Neisseria cinerea</i>
<i>Neisseria flavescens</i> KC920
<i>Neisseria meningitidis</i>
<i>Neisseria gonorrhoeae</i>
<i>Pentatrichomonas hominis</i> ATCC 30098
<i>Streptococcus agalactiae</i> SS617
<i>Streptococcus anginosus</i> SS1266
<i>Streptococcus intermedius</i> SS1653
<i>Streptococcus constellatus</i> SS1652
<i>Streptococcus pneumoniae</i> 49619
<i>Treponema denticola</i>
<i>Treponema pallidum</i> subsp. <i>pallidum</i>
<i>Treponema phagedenis</i> Reiter
<i>Treponema refringens</i> Noguchi
<i>Trichomonas vaginalis</i> ATCC 30001
<i>Trichomonas tenax</i>
<i>Trichomonas foetus</i> LIL1
<i>Ureaplasma urealyticum</i>
<i>Mycoplasma pneumoniae</i>

infection was 28.6% (34/119); by conventional PCR using vaginal washings 65.5% (78/119); by conventional urine PCR 36.9% (44/119); by real-time PCR using vaginal washings 65.5% (78/119); and by real-time urine PCR 55.5% (66/119).

Seven vaginal washing specimens gave discordant results with the primary real-time PCR compared with the gold standard. These specimens remained positive after retesting with the primary real-time PCR using existing DNA preparations. However, upon re-extraction and retesting of DNA with the BTUB real-time PCR, two of the seven specimens tested positive, while the remaining five were negative. The cyclic threshold values for the five negative specimens varied between 39.42 and 43 when originally tested with the primary real-time PCR, which indicates a low DNA copy number. In addition, these specimens remained positive after re-extraction and testing with the primary real-time PCR.

Of the 20 urine specimens that were negative for *T vaginalis* with the primary real-time PCR but for which paired vaginal washings tested positive, 16 were available for re-extraction, of which seven were positive after retesting with this assay. The urine samples that corresponded with vaginal washings that initially tested positive by the primary real-time PCR alone remained negative after re-extraction and testing with this same test. One urine specimen was positive by real-time PCR alone and remained positive after re-extraction and testing on a separate occasion.

PCR inhibition was detected in 1 of 34 vaginal washings and in 5 of 37 urine samples using the RNase P real-time assay. On re-extraction and testing, the vaginal washing sample was positive by the primary real-time PCR but negative by the BTUB confirmatory assay. Four of the five urine samples that were available for testing were negative by the primary real-time assay.

Table 2 shows the sensitivities and specificities of culture, wet preparation, conventional urine PCR, and real-time PCR using vaginal washings and urine. The sensitivities of culture, wet preparation, conventional PCR using vaginal washings and urine, and real-time PCR using vaginal washings and urine, compared with the gold standard, were 43.6%, 43.6%, 100%, 56.4%, 100% and 76.7%, and the specificities of these tests were 100%, 97.6%, 100%, 97.6%, 82.9% and 97%, respectively. After retesting the seven vaginal washing specimens that gave discordant results with the β TUB real-time PCR, the resolved sensitivity of the primary real-time PCR was 100% and the specificity 91.9%. The resolved sensitivity and specificity of the primary real-time PCR using urine specimens was 84.9% and 97.0%, respectively. One urine specimen tested positive for *T*

vaginalis by conventional and real-time PCR, whereas the vaginal washings, vaginal swab and wet preparation from this patient yielded negative results. Another urine specimen was positive by the primary real-time PCR alone and one specimen was positive by wet preparation alone.

The detection limits for the primary and BTUB real-time PCR tests were about 1 and 10 copies of target DNA, respectively. Both tests were specific for *T vaginalis*, as negative results were obtained for all closely related microorganisms.

DISCUSSION

This study describes the development and evaluation of a new TaqMan-based real-time PCR test using the Rotor-Gene thermocycler platform. By applying an expanded gold standard, defined as a positive culture result using vaginal swabs and/or a positive PCR test using TVK3/7 primers, the overall prevalence of trichomoniasis in the study population was found to be 68.9% after resolving discrepancies. The sensitivity of the primary real-time PCR using vaginal washings was 100% compared with 43.6% for both culture and wet preparation. The specificity of the real-time PCR was 82.9% compared with 100% and 97.6% for culture and wet preparation, respectively. After resolving discrepancies with a second real-time PCR, the specificity of the primary real-time PCR using vaginal washings improved from 82.9% to 91.9%, while the sensitivity remained the same.

The specificity of the primary real-time PCR using vaginal washings was lower than culture, wet-mount microscopy and conventional urine PCR, as only three of seven specimens that were positive by this test were confirmed by the BTUB real-time PCR. There are several possible reasons for the discordant results. Firstly, the theoretical detection of the BTUB real-time PCR is 10-fold lower than the primary real-time PCR, therefore, the primary assay is expected to be more sensitive than the confirmatory test. Secondly, the three specimens that were positive by the primary real-time PCR alone were re-extracted and tested by this assay and, although they remained positive, the cycle threshold values were high (≥ 39.4), suggesting low copy numbers in these specimens. Thirdly, precautions were taken to avoid cross-contamination of specimens, and positive and negative PCR controls gave the expected results for all runs. Lastly, as some time had elapsed before the samples were retested with the β TUB confirmatory assay, the DNA may have degraded, leading to a false negative result.

In all, 7 of 20 urine specimens that initially gave negative results were positive on re-extraction and testing by the

Table 2 Performance of diagnostic tests for *T vaginalis* (n = 119)*

Assay or test	Number of tests				% Sensitivity (95% CI)	% Specificity (95% CI)
	True positive	False positive	False negative	True negative		
Culture, vaginal swab	34	0	44	41	43.6 (32.4 to 55.3)	100 (91.4 to 100)
Wet preparation, vaginal swab	34	1	44	40	43.6 (32.4 to 55.3)	97.6 (87.1 to 99.9)
Conventional PCR						
Vaginal washing	78†	0	0	41	100 (95.4 to 100)	100 (91.4 to 100)
Urine	44‡	1	34	40	56.4 (44.7 to 67.6)	97.6 (87.1 to 99.9)
Real-time PCR						
Vaginal washing	78†	7	0	34	100 (95.4 to 100)	82.9 (67.9 to 92.8)
Urine	66§	1	20	32	76.7 (66.4 to 85.2)	97.0 (84.2 to 99.9)

PCR, polymerase chain reaction.

*The gold standard was a positive culture result using vaginal swabs and/or a positive PCR test using TVK3/7 primers (n = 78).

†Includes all 34 culture-positive specimens.

‡Six culture-positive specimens were negative by this assay.

§One culture-positive specimen was negative by this assay.

primary real-time PCR. DNA was re-extracted from 1.5 ml of urine using the Qiagen viral RNA kit, which contains a lysis buffer that has been optimised for removal of PCR inhibitors from urine. The cycle threshold values for these specimens were ≥ 35.49 , which theoretically corresponds to <1 microorganism. Taking these two factors into account, the urine specimens initially tested negative probably as a result of low copy numbers of trichomonads and not due to PCR inhibitors. PCR inhibition was observed in one vaginal washing and five urine samples using the RNaseP assay; however, re-extraction and testing of five of the samples with the primary real-time assay yielded only one positive sample.¹⁴

Using a conventional PCR with TVK3/7 primers, the *T vaginalis* detection rate using vaginal washings was 65.5% (78/119) compared with 68.9% (82/119) for the real-time PCR.¹² Although the sensitivity of conventional PCR was similar to real-time PCR, five specimens tested by conventional PCR gave no visible product on agarose gels and would have been missed if amplicons were not subsequently analysed on the ABI Genetic Analyzer.¹²

In this study, cervicovaginal washings were used for real-time PCR because vaginal swabs were unavailable for testing. Although this sampling method is impractical for routine use, it may prove to be superior to vaginal swabs for detection of *T vaginalis* as the entire vagina is sampled compared with a relatively small area using swabs. This hypothesis is supported by a recent study which showed that CVL specimens were better than vaginal swabs for the diagnosis of *T vaginalis* by wet-mount microscopy.¹⁵ CVL may be an appropriate specimen for studying the association between *T vaginalis* and other causes of vaginal discharge, and therefore warrants further investigation.

Wet-mount microscopy is the most widely used test for the clinical diagnosis of trichomoniasis in the USA and many other countries. As has been shown previously, the use of wet preparation and culture for detection of *T vaginalis* will result in a considerable number of infections remaining undetected.^{4 7 12 16} The sensitivity of culture compared with real-time PCR and conventional PCR was 41.5% and 47.5%, respectively, in this study, which is significantly lower than in previous reports and may reflect the increased sensitivity of the PCR methods used here.^{4 7 17} The high prevalence of trichomoniasis, based on real-time PCR, in this study is not surprising, as two previous studies in South Africa involving STD clinic attenders and female sex workers reported prevalence rates of 47% and 41.3% by culture and/or wet-mount microscopy, respectively.^{18 19} As a result of the high prevalence setting, the number of patients free of disease was small in this study, therefore, the measurement of specificity could be relatively imprecise. Further studies undertaken in low prevalence settings are therefore indicated.

Real-time PCR has advantages over conventional PCR as post-PCR processing of amplicons is not required, thereby eliminating the possibility of amplicon contamination, and the assay can be completed within 2.5 h. Given the high sensitivity of real-time PCR versus culture and wet-mount microscopy, its use as a diagnostic test for screening and treating trichomoniasis should be explored. A modification of the real-time platform into a portable format with the ability to detect multiple microorganisms through multiplexing would allow wide-scale application of real-time tests especially in high-risk populations. The primary real-time assay described here, targeting the *T vaginalis*-specific repeat fragment, is recommended for the molecular diagnosis of trichomoniasis; however, in research settings, a confirmatory real-time PCR may be required, in which case both real-time PCR tests described here should be performed.

Authors' affiliations

A Pillay, R C Ballard, Laboratory Reference and Research Branch, Division of Sexually Transmitted Diseases Prevention, NCHSTP, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

F Radebe, G Fehler, Y Htun, Sexually Transmitted Infections Reference Centre, Institute for Communicable Diseases, National Health Laboratory Services, Johannesburg, South Africa

Funding: None.

Competing interests: None declared.

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention.

AP was the principal investigator for the study and lead author for the paper; FR and GF performed wet-mount and culture testing; AP, YH and RCB designed the study.

The authors would like to express their sincere thanks to Evan Secor, David Cox, Patricia Shewmaker, Paul Levett and Maria Tondella from the Centers for Disease Control, Atlanta; Jane Schwebke from the University of Alabama at Birmingham; and Tania Crucitti from the Institute of Tropical Medicine, Belgium for providing microbial isolates or DNA used in this study.

REFERENCES

- 1 Cates W Jr. Estimates of the incidence and prevalence of sexually transmitted diseases in the United States. American Social Health Association Panel. *Sex Transm Dis* 1999;**26**:S2-7.
- 2 Cotch MF, Pastorek JG, Nugent RP, et al. Trichomonas vaginalis associated with low birth weight and preterm delivery. The Vaginal Infections and Prematurity Study Group. *Sex Transm Dis* 1997;**24**:353-60.
- 3 Watson-Jones D, Mugeye K, Mayaud P, et al. High prevalence of trichomoniasis in rural men in Mwanza, Tanzania: results from a population based study. *Sex Transm Infect* 2000;**76**:355-62.
- 4 Lawing LF, Hedges SR, Schwebke JR. Detection of trichomoniasis in vaginal and urine specimens from women by culture and PCR. *J Clin Microbiol* 2000;**38**:3585-8.
- 5 Crucitti T, Van Dyck E, Tehe A, et al. Comparison of culture and different PCR assays for detection of Trichomonas vaginalis in self collected vaginal swab specimens. *Sex Transm Infect* 2003;**79**:393-8.
- 6 Wendel KA, Erbeling EJ, Gaydos CA, et al. Trichomonas vaginalis polymerase chain reaction compared with standard diagnostic and therapeutic protocols for detection and treatment of vaginal trichomoniasis. *Clin Infect Dis* 2002;**35**:576-80.
- 7 Smith KS, Tabrizi SN, Fethers KA, et al. Comparison of conventional testing to polymerase chain reaction in detection of Trichomonas vaginalis in indigenous women living in remote areas. *Int J STD AIDS* 2005;**16**:811-15.
- 8 Madico G, Quinn TC, Rompalo A, et al. Diagnosis of Trichomonas vaginalis infection by PCR using vaginal swab samples. *J Clin Microbiol* 1998;**36**:3205-10.
- 9 Mahmoud MS, Abdel-Aziz SS, El-Sherif EA, et al. Diagnosis of symptomatic and asymptomatic Trichomonas vaginalis infection by applying one tube nested PCR to vaginal discharge. *J Egypt Soc Parasitol* 1999;**29**:1031-46.
- 10 Rompalo AM, Gaydos CA, Shah N, et al. Evaluation of use of a single intravaginal swab to detect multiple sexually transmitted infections in active-duty military women. *Clin Infect Dis* 2001;**33**:1455-61.
- 11 Ballard R, Htun Y, Fehler H, et al. Syndromic management of sexually transmitted infections. In: *The diagnosis and management of sexually transmitted infections in southern Africa*. 3rd edn., JDB Designs, Johannesburg, 2000:53-69.
- 12 Pillay A, Lewis J, Ballard RC. Evaluation of xeno-strip-Tv, a rapid diagnostic test for Trichomonas vaginalis infection. *J Clin Microbiol* 2004;**42**:3853-6.
- 13 Kengne P, Veas F, Vidal N, et al. Trichomonas vaginalis: repeated DNA target for highly sensitive and specific polymerase chain reaction diagnosis. *Cell Mol Biol* 1994;**40**:819-31.
- 14 Emery SL, Erdman DD, Bowen MD, et al. Real-time reverse transcription-polymerase chain reaction assay for SARS-associated coronavirus. *Emerg Infect Dis* 2004;**10**:3111-16.
- 15 Kissinger PJ, Dumestre J, Clark RA, et al. Vaginal swabs versus lavage for detection of Trichomonas vaginalis and bacterial vaginosis among HIV-positive women. *Sex Transm Dis* 2005;**32**:227-30.
- 16 van der Schree C, van Belkum A, Zwiijgers L, et al. Improved diagnosis of Trichomonas vaginalis infection by PCR using vaginal swabs and urine specimens compared to diagnosis by wet mount microscopy, culture, and fluorescent staining. *J Clin Microbiol* 1999;**37**:4127-30.
- 17 Caliendo AM, Jordan JA, Green AM, et al. Real-time PCR improves detection of Trichomonas vaginalis infection compared with culture using self-collected vaginal swabs. *Infect Dis Obstet Gynecol* 2005;**13**:145-50.
- 18 Kharsany AB, Hoosen AA, Moodley J. Bacterial vaginosis and lower genital tract infections in women attending out-patient clinics at a tertiary institution serving a developing community. *J Obstet Gynaecol* 1997;**17**:171-5.
- 19 Ranjee G, Karim SS, Sturm AW. Sexually transmitted infections among sex workers in KwaZulu-Natal, South Africa. *Sex Transm Dis* 1998;**25**:346-9.