

## DIAGNOSTICS

# Comparison of latex agglutination, wet preparation, and culture for the detection of *Trichomonas vaginalis*

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**Objectives:** To compare the performance of three diagnostic methods for *Trichomonas vaginalis* infection—latex agglutination, saline wet mount, and culture.

**Methods:** Vaginal swabs from 3807 women attending antenatal clinics were tested for the presence of *T vaginalis* by latex agglutination. All positives and the following two negatives were tested by wet preparation and culture.

**Results:** The prevalence of infection by latex agglutination was 5.4%. Using an expanded gold standard based on the wet mount and culture results, the sensitivity of the latex agglutination test was 98.8% (95% CI 95.9 to 99.9) and specificity was 92.1 (89.2 to 94.5). The kappa index for test agreement was 0.93 for latex and culture and 0.88 for latex and wet preparation.

**Conclusion:** The latex agglutination test is a highly sensitive test for detecting *T vaginalis* infection. It is a simple rapid test and has the potential for use in screening and diagnostic settings.

**T**richomoniasis is a sexually transmitted infection caused by the protozoan parasite, *Trichomonas vaginalis*. It is the commonest curable sexually transmitted infection; the World Health Organization estimates that 170 million new infections occur each year.<sup>1</sup> The highest prevalence of infection has been reported from resource constrained countries<sup>2–4</sup> and in disadvantaged populations in developed countries.<sup>5</sup> Infection with *T vaginalis* may facilitate the transmission of HIV<sup>6–7</sup> and is associated with adverse pregnancy and perinatal outcomes.<sup>5</sup> Thus, the control of *T vaginalis* infection is important in reproductive health settings and also in the control of HIV/AIDS.

Diagnosis of *T vaginalis* infection in most parts of the world is carried out by the saline wet preparation ("wet prep") method, a technique which has not changed since it was first reported by Donné in 1836.<sup>8</sup> However, this technique has a low sensitivity of 30–80%<sup>9–11</sup> and requires trained and experienced microscopists. The gold standard in *T vaginalis* diagnosis—culture—has a higher sensitivity of 71–100%,<sup>12</sup> but requires an incubator with a constant electricity supply and relatively expensive culture media, in addition to an experienced microscopist. Further, it can take up to 7 days for results to be obtained, and currently is outside the reach of many health settings. In recent times, the sensitivity of *T vaginalis* diagnosis has been much improved by the use of nucleic acid amplification technology.<sup>13–14</sup> Non-invasive diagnostic material (self obtained swabs, tampons, and urine)<sup>15–16</sup> appealing to patients, have also been used. Polymerase chain reaction (PCR) currently, however, is also outside the reach of many diagnostic centres in resource poor settings.

A less technologically demanding technique with good test performance characteristics is needed, especially in resource poor countries. We compared a latex agglutination test with culture and Donné's wet prep technique for the diagnosis of *T vaginalis* infection.

## PATIENTS AND METHODS

A total of 3807 consecutive women attending antenatal clinics in Kumasi, Ghana, were screened for vaginal infections between September 2002 and May 2003. Following

informed consent, self administered vaginal swabs were screened for *T vaginalis* infection using the Kalon TV latex agglutination test (Kalon Biological, Surrey, UK). In this test, vaginal swabs were eluted by agitation in phosphate buffered saline. One drop of this eluate was mixed with a drop of test latex on a reaction zone on a black glass slide, and the slide manually rocked continuously for 2 minutes. Agglutination of the test, but not control latex, indicated presence of *T vaginalis* antigen.

All study subjects testing positive for *T vaginalis* on latex agglutination testing, and the following two consecutive women testing negative had two further vaginal swabs taken. The triplet of tests was not matched according to symptom presentation. The swabs were obtained by a nurse from the posterior fornix, after speculum insertion and were tested for *T vaginalis* by wet prep and culture. In the wet prep technique, the swab was agitated in 0.9% saline and a drop of this was observed under wet mount microscopy at  $\times 100$  for the characteristic morphology and motility of *T vaginalis*. Any observed trichomonad was confirmed at  $\times 400$ . Culture was undertaken using the *T vaginalis* InPouch system (Biomed Diagnostics, San José, CA, USA) as previously described.<sup>17</sup>

Latex agglutination and wet prep microscopy were done on site within 10 minutes of specimen collection. Inoculation of the InPouch was also done on site within 10 minutes of specimen collection and incubated at 37°C within an hour of specimen inoculation. All three tests were read independently and blind to the results of the other tests by different technicians.

Test sensitivity, specificity, and predictive values were compared. Kappa statistic for tests agreement between latex and wet prep, and latex and culture was also determined.

## RESULTS

Of the 3807 pregnant women, 206 (5.4%) were positive for *T vaginalis* on screening with the latex agglutination kit; 618 women (206 latex positives, 412 latex negatives) were selected for wet prep and culture testing. At presentation, 343 women (55.5%) were symptomatic for vaginitis (either vaginal discharge or itch) and 275 (44.5%), asymptomatic.

**Table 1** Diagnostic comparison of latex agglutination, wet prep, and culture for detection of *Trichomonas vaginalis*

<i>T vaginalis</i> infection (n = 618)	Diagnostic test		
	Latex agglutination	Wet prep	Culture
True positive*	171 (27.7%)	141 (22.8%)	170 (27.5%)
False positive	35 (5.7%)	0 (0%)	0 (0%)
False negative	2 (0.3%)	32 (5.2%)	3 (0.5%)
True negative†	410 (66.3%)	445 (72.0%)	445 (72.0%)

\*Wet prep microscopy or InPouch culture positive.

†Both wet prep microscopy and Inpouch culture negative.

Significantly more symptomatic subjects were positive on testing ( $\chi^2 = 10.3$ ,  $p = 0.001$ ); 64.5% of the 206 subjects testing positive with the latex as against 51% of the 412 testing negative, were symptomatic for vaginitis.

All but one of the latex positive samples was positive on culture, and all but one sample positive on latex agglutination testing was positive on wet prep. These two latex negative samples had discrepant results for wet prep and culture testing. Three samples with flagellates having characteristic morphology and motility of *T vaginalis* in the direct smear did not grow in culture.

Using an expanded gold standard for the comparison, patients were considered to have *T vaginalis* infection when either wet prep microscopy or InPouch culture were positive. They were considered negative for infection when both wet prep microscopy and Inpouch culture were negative. Table 1 shows the diagnostic comparison and table 2 test performance.

The kappa index which measures agreement between tests was 0.93 (95% CI 0.91 to 0.94) for latex and culture, and 0.88 (95% CI 0.86 to 0.90) for latex and wet prep.

## DISCUSSION

In this diagnostic comparison, the test performance of the wet prep and culture are in conformity with other published studies.<sup>12</sup> Sensitivity of the latex agglutination test compares favourably with culture and is superior to the wet prep. Moreover, the test is simple to perform, requires no equipment other than a glass slide and mixing stick and gives a result in less than 3 minutes. This study is the second evaluation of the latex agglutination test and the first to be conducted in Africa. Carney *et al*<sup>18</sup> in their evaluation of this kit in the United Kingdom in 1988 reported a sensitivity and specificity of 95.2% and 99.4% respectively using wet prep and culture as reference standards. In spite of these encouraging results, the test has not been widely used.

Recent reports suggest that *T vaginalis* enhances the transmission of HIV,<sup>6</sup> and that symptomatic *T vaginalis* infection increases the amount of HIV shed in semen.<sup>19</sup> Moreover treatment of *T vaginalis* infection significantly lowers the vaginal and seminal HIV viral load in dually infected subjects.<sup>20-21</sup> Given the high prevalence of *T vaginalis* infection, its control could have a significant impact on the

HIV epidemic in Africa, and may reduce the incidence of adverse pregnancy outcome.<sup>5</sup>

Two ingredients important in the control of sexually transmitted infections are accurate diagnosis and prompt treatment. Properly done, this will reduce the reservoir of infection and thus reduce the incidence of infection. In many health settings worldwide, wet mount microscopy is the preferred option for prompt diagnosis of trichomoniasis. This method, though timely and enabling patients to receive same day treatment, has a poor sensitivity<sup>9-11</sup> and patients could remain infected and untreated. Culture, with a much better sensitivity,<sup>12</sup> does not allow same day treatment, and patients, especially if asymptomatic for infection may continue to transmit infection. In many developing countries where the cost of return to the health facility can be substantial, patients may not bother to return for their culture results, thus prolonging infection, leading to further transmission and the possibility of sequelae. Partner notification efforts would also be defeated. This latex agglutination kit allows for prompt laboratory diagnosis of infection and thus treatment. With a sensitivity of 98.8% and a kappa index of 0.93 it compares favourably with culture, and its use could be more cost effective than culture. It provides same day results (test takes 2 minutes), thus saving time and costs due to incubation. At a present cost of £1 (\$1.56) per test it compares with other rapid tests made for reproductive health settings. In resource poor settings where the cost of purchasing microscopes for wet prep and culture examinations may be prohibitive, and where trained and skilled personnel are unavailable, this latex agglutination test can fulfil such need. We have trained secondary school graduates to perform the test correctly. The kit as presently manufactured contains everything that is needed and no further purchases are needed.

In analysing a model of treatment interventions for trichomoniasis, Bowden and Garnett<sup>22</sup> indicate that identifying individuals with both symptomatic and asymptomatic infection and giving appropriate treatment could be the most efficient method of controlling *T vaginalis* infection. This would be greatly augmented by a good screening tool that would detect most infections and allow prompt treatment. Currently available diagnostic tools do not allow this. The *T vaginalis* latex agglutination test could fill that void. In high *T vaginalis* endemic areas, which unfortunately are also often

**Table 2** Performance of the three diagnostic tests and 95% confidence intervals

	Latex agglutination	Wet prep	Culture
Sensitivity (%)	98.8 (95.9 to 99.9)	81.5 (74.9 to 87.0)	98.2 (95.0 to 99.6)
Specificity (%)	92.1 (89.2 to 94.5)	100 (99.2 to 100)	100 (99.2 to 100)
Positive predictive value	83.0 (77.2 to 87.9)	100 (97.4 to 100)	100 (97.9 to 100)
Negative predictive value	99.5 (98.3 to 99.9)	93.3 (90.7 to 95.4)	99.5 (98.1 to 99.9)

### Key messages

- Present diagnostic tests for *T vaginalis* in resource poor settings are either insensitive or too expensive
- The *T vaginalis* latex agglutination test is a 2 minute test and its performance compares favourably with culture, the current gold standard in *T vaginalis* diagnosis
- It is a simple to use kit, requires no equipment, and is suitable for developing country settings

high HIV prevalence settings, this simple and easy to use *T vaginalis* diagnostic would go a long way in the control of HIV/AIDS and possibly reduce preterm, premature low birth weight deliveries.

About 6% (35) of samples were latex positive but negative for the traditional tests. It would be important to establish whether these are false positives by latex agglutination or false negatives by the traditional tests. Recent studies using molecular methods for the diagnosis of *T vaginalis* infection have shown culture to be less than 100% sensitive. Wendel *et al* comparing the polymerase chain reaction (PCR) and culture, reported sensitivities of 84% and 78% respectively<sup>13</sup> using an expanded gold standard of wet prep, culture, and PCR. Also, in the series by Tabirizi *et al*, *T vaginalis* infection prevalences of 9% by traditional tests as against 15% by PCR in the same population was reported.<sup>14</sup> This could also be important in many areas of the world where women are less empowered because of dependence on their spouses. False positive results could lead to considerable adverse social and relationship effects during partner notification.

Owing to the high sensitivity exhibited by nucleic acid amplification tests,<sup>2, 13, 14</sup> the sensitivity of *T vaginalis* PCR could well surpass that of the latex agglutination test, and there could still be missed infections using the latex kit in resource poor centres where PCR is unlikely to be used for diagnosis.

One of the weaknesses of the WHO vaginal discharge syndromic management approach is its poor specificity.<sup>23</sup> This can be greatly improved by the availability of rapid tests for vaginal discharge diagnosis. The WHO has commissioned field trials for rapid tests for the diagnosis of gonorrhoea and chlamydia infection.<sup>24</sup> When the results of these trials are favourable, and the diagnostics affordable, together with a rapid *T vaginalis* diagnostic, vaginal discharge management would greatly improve. Even in very low *T vaginalis* prevalent populations in developing countries, the use of the *T vaginalis* latex could be useful in eliminating infection and for effective partner notification. At a present cost of £1, this could be cheaper than the cost of blind therapy with metronidazole, unwarranted drug adverse effects, and the probable adverse social and marital outcomes with partner notification.

We have in our hands a good screening and diagnostic tool for *T vaginalis*. There is already available a cheap single dose antimicrobial against *T vaginalis* with cure rates over 80%.<sup>25</sup> It is time to evolve routine screening and treatment for *T vaginalis* in reproductive health settings.

### CONTRIBUTORS

YAS, HAW, and DM designed the study; BKO and KAD trained the nurse-midwives and supervised clinical aspects of the study; YAS trained and supervised laboratory aspects of the study; data analysis

was done by YAS and HAW; the manuscript was drafted by YAS, HAW and DM and written by YAS; all authors have reviewed and approved the final version of the paper.

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