***Trichomonas vaginalis*: clinical relevance, pathogenicity and diagnosis**

**Abstract**

*Trichomonas vaginalis* is the etiological agent of trichomoniasis, the most prevalent non-viral sexually transmitted disease worldwide. Trichomoniasis is a widespread, global health concern, and occurring at an increasing rate. Infections of the female genital tract can cause a range of symptoms, including vaginitis and cervicitis, whilst infections in males are generally asymptomatic. The relatively mild symptoms, and lack of evidence for any serious sequelae, have historically led to this disease being under diagnosed, and under researched. However, growing evidence that *T. vaginalis* infection is associated with disease states with high morbidity in both men and women has increased the efforts to diagnose and treat patients harbouring this parasite. The pathology of trichomoniasis results from damage to the host epithelia, caused by a variety of processes during infection, and recent work has highlighted the complex interactions between the parasite and host, commensal microbiome, and accompanying symbionts. The commercial release of a number of nucleic acid amplification tests (NAATs) has added to the available diagnostic options. Immunoassay based Point of Care testing is currently available, and a recent initial evaluation of a NAAT Point of Care system has given promising results, which would enable testing and treatment in a single visit.

## Introduction

*Trichomonas vaginalis* is a flagellated protozoan parasite of the human genital tract and the cause of the most prevalent curable sexually transmitted disease globally, with an estimated 276.4 million cases per year, worldwide (World Health Organisation, 2012). Infections of the female genital tract can cause a range of symptoms, including vaginitis and cervicitis (Heine and McGregor, 1993). Infections in males are generally asymptomatic, although mild urethritis or prostatitis can occur (Guenthner et al., 2005). During the last decade, the discovery that *T. vaginalis* infection is associated with a range of more serious conditions, such as prostate cancer, cervical cancer, adverse pregnancy outcomes, and an increased likelihood of HIV infection, has increased the efforts to diagnose and treat patients harbouring this parasite (Bachmann et al., 2011).

## Morphology

*T. vaginalis* has no cystic stage in its life cycle, existing only as a tear drop shaped trophozoite, with an average length and width of 10µm and 7µm, respectively (Petrin et al., 1998). A total of four anterior flagella provide the parasite with its characteristic twitching motility, whilst a single posterior flagellum, which forms the outer edge of the undulating membrane, runs along the length of one side of the cell, assisting in motility and the movement of extracellular nutrients towards the cytosome of the cell. The cell also possesses an axostyl, a bundle of microtubules passing through the cell along its anterior-posterior axis into the extracellular environment (Lee et al., 2012), which has a function in cell attachment and mitosis (Ribeiro et al., 2000). The cytoplasm contains a single defined nucleus, and several hydrogenosomes; primitive redox organelles, evolved from mitochondria, which produce molecular hydrogen and ATP (Schneider et al., 2011).

## Pathogenesis

Transmission occurs almost exclusively via sexual contact, although transmission via fomites has been documented, but is rarely encountered and controversial (Schwebke and Burgess, 2004). Documented suspected non-sexual transmission routes include shared bathing water (Crucitti et al., 2011; Burch et al., 1959), and bathing implements (Adu-sarkodie, 1995); routes that are theoretically possible due to the ability of the parasite to survive for up to three hours in a moist environment (Krieger and Kimmig, 1995).

During sexual intercourse, *T. vaginalis* cells in the genital tract of the infected partner are transferred to the uninfected partner, and come in to contact with the genital epithelia. When in contact with epithelial cells, the typically ovoid *T. vaginalis* cell morphologically adjusts, assuming an amoeboid conformation (Gould et al., 2013). The cells attach to the epithelial surface, with the amoeboid

morphology enabling the parasite to increase the surface area contact, and interaction, with the epithelial cell. *T. vaginalis* adhesion is largely mediated by a range of iron-dependant surface adhesins (Munoz et al., 2012). There are five primary surface adhesins responsible for the attachment of the parasite to the host epithelia; AP120, AP65, AP51, AP33, AP23 (Garcia and Alderete, 2007). With the exception of AP51, the genes encoding these proteins are all transcriptionally upregulated by the presence of iron, which is an essential mediator of *T. vaginalis* growth, and a key factor in virulence (Ryu et al., 2001). Of these surface proteins, AP65 has been hypothesised as the most important; anti AP65 serum IgG antibodies inhibit *T. vaginalis* cytoadherance, which does not occur when the same is carried out on other adhesins (Garcia et al., 2003). Interestingly, it has been shown that AP65 does not have a covalent anchor motif, and is released extracellularly, where it binds to both the *T. vaginalis* and epithelial cell surface (Garcia and Alderete, 2007). The synthesis and transport of these adhesins to the outer membrane occurs in response to the contact of the parasite with vaginal epithelial cells, in tandem with a morphological shift to the amoeboid form. After adherence, the *T. vaginalis* cells recruit further parasites to the location, forming sizeable aggregates of ameboid cells on the epithelial surface (Arroyo et al., 1992). The other primary mediator of cytoadherance to the host epithelia is surface lipophosphoglycan, the most highly expressed protein on the *T. vaginalis* surface membrane ($2 \times 10^6$ to $3 \times 10^6$ copies per parasite) (Fichorova et al., 2006), which binds to the galectin-1 protein located on the surface of human epithelial cells (Ryan et al., 2011). Site directed mutagenesis studies have shown that *T. vaginalis* cells expressing a lipophosphoglycan molecule with altered surface residues have a greatly reduced adherence and cytotoxicity to human vaginal epithelial cells, underlining the importance of this molecule for parasite attachment and virulence (Bastida-Corcuera et al., 2005). The glyceraldehyde-3-phosphate dehydrogenase, GAPDH, is another protein expressed on the surface of *T. vaginalis* that has been determined to be involved in cytoadherance (Lama et al., 2009).

The adherence of *T. vaginalis* to the epithelial cell surface is a crucial factor in pathogenesis; adherence of the parasite is cytotoxic, and typically results in the lysis of the host cell, and erosion of the epithelial monolayer. This process also instigates the inflammatory response, involving the release of chemokines such as IL-8 and the recruitment of neutrophils to infected tissues (Fichorova et al., 2006). Damage to the vaginal epithelial monolayer during infection is known to occur via a variety of mechanisms, and this contact dependent killing does not involve phagocytosis (Krieger et al., 1985). Adherance of *T. vaginalis* to epithelial cells causes a weakening of the junctional complex between individual cells in the epithelial monolayer. This weakening is a result of a decrease in trans-epithelial electrical resistance, an increase in the gap between neighbouring cells, and also

modification of the distribution of junction complex proteins, all resulting from the interaction with the parasite (Guenthner et al., 2005; da Costa et al., 2005).

Damage to the host epithelia is also caused by parasite mediated apoptosis of epithelial cells, which is dependent on the release of CP30 cysteine proteases (Kummer et al., 2008). This group of 4 cysteine proteases are also linked to adhesion and the passage of the parasite through the mucosal barrier, making them important factors in *T. vaginalis* pathogenesis. *T. vaginalis* is also capable of the *in vitro* phagocytosis of vaginal epithelial cells, leukocytes and erythroyctes, along with commensal bacteria (Rendon-Maldonado et al., 1998) and yeasts (Pereira-Neves and Benchimol, 2007) of the genital tract. Two distinct mechanisms of phagocytosis have been observed during *in vitro* studies with yeasts; a classic form of phagocytosis involving extension of pseudopodia, which then engulf the target cell, and also a more passive form, where the target cell sinks into the *T. vaginalis* membrane (Pereira-Neves and Benchimol, 2007). Phagocytosis is followed by intracellular killing in lysosomes, and provides the *T. vaginalis* cell with a source of nutrients (Francioli et al., 1983). Phagoyctosis is also thought to be the primary route of horizontal gene transfer between bacteria and *T. vaginalis*, providing the parasite with an important mechanism of genetic diversification and adaptation (de Koning et al., 2000). The precise mechanisms by which the *T. vaginalis* cells recognise target cells appropriate for phagocytosis is poorly understood, although non-specific mannose receptors on the *T. vaginalis* outer membrane have been implicated in the internalisation of yeast cells (Pereira-Neves and Benchimol, 2007). Mannose binding lectins have been shown to bind Gram positive and Gram negative bacteria, as well as yeasts, protozoa and even some viruses (Klein and Kilpatrick, 2004), showing the wide range of organisms identifiable by the presence of this ligand. Mannose is also present on the surface of epithelial cells (Hanada et al., 2014), leukocytes (Rodriguez-Ortega et al., 1987), and erythroyctes (Lodish, 2000), so *T. vaginalis* mannose receptors may play a part in the recognition of these cell types during phagocytosis or lysis. *T. vaginalis* is known to be able to recognise erythrocytes, and is able to lyse erythrocytes both in vitro and in vivo, with haemolysis depending on adherence of the parasite (Fiori et al., 1993). This is thought to provide the parasite with a source of iron, an essential nutrient for *T. vaginalis* growth (Ryu et al., 2001).

**Epidemiology and clinical presentation**

The collection of meaningful epidemiological data on *T.vaginalis* infection is hampered by the fact that trichomoniasis is not currently a reportable infection, in developed or developing countries (Poole and McClelland, 2013). This means that global and local prevalence and infection rates have to be estimated from localised studies, due to the lack of case reporting data available. The most

recent World Health Organisation (WHO) estimates from data collected in 2008 (World Health Organisation, 2012) indicate 276.4 million new cases per year, or 187 million adults infected at any one time. This represents a larger number of new infections than those of the next two most prevalent STI's combined, with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections estimated to number 105.7 million and 106.1 million, respectively (World Health Organisation, 2012). The prevalence and incidence of *T. vaginalis* infection is geographically variable (Table.1), with the highest prevalence found in Africa (20.2% of females, 2% of males), and the Americas (22% of females, 2.2% of males). The prevalence in Europe is estimated as 5.8% of females and 0.6% of Males.

The estimated incidence of *T. vaginalis* is fractionally higher in men than in women, with a male to female ratio of total cases globally of 1.14. There is an obvious discrepancy between this fairly equal relationship, and the sizeable difference in estimated prevalence rates between the sexes. The prevalence of *T. vaginalis* infection in women is around 10 times higher than in men, irrespective of geographic location. This is indicative of the self clearing nature of the infection in males, with the majority of persistent infections occurring in females (Van Der Pol, 2007). The higher likelihood of persistence in the female genital tract has been linked to the availability of iron, which increases during the menstrual cycle, providing the parasite with increased exposure to a major growth requirement (Beltrán et al., 2013). The expression of various adhesion proteins promoting cytoadherance is enhanced in response to increased iron concentration (Sehgal et al., 2012), and this is associated with increased virulence (Ryu et al., 2001). The prevalence of *T. vaginalis* infection has been seen to differ between ethnic populations in the same geographical area, with black females in the US having a higher prevalence (10.5%; 95% CI, 8.3-13.3%) than white females (1.1%; 95% CI, 0.8-1.6%) (Miller et al., 2005).

*T. vaginais* infection in females is symptomatic in around 50% of cases, and around 30% of asymptomatic cases develop some symptoms in the 6 month period post infection (Rein, 1990). Common symptoms include itching and pain during intercourse, a frothy discharge, and vaginitis, which can range from mild to severe (Petrin et al., 1998). In acute cases, punctate hemorrhagic spots may be present on the vaginal and cervical mucosa, a pathology referred to as colpitis macularis, or "strawberry cervix" (Swygard et al., 2004). *T. vaginalis* infection has also been associated with cervicitis, urethritis and also more serious complications such as pelvic inflammatory disease (PID) (Heine and McGregor, 1993), cervical cancer (Zhang et al., 1995) and infertility (El-Shazly et al., 2001). The effects of *T. vaginalis* infection during pregnancy, and on pregnancy outcomes, are well documented. Infections with *T. vaginalis* at the mid gestation point of pregnancy increases the

likelihood of a preterm delivery and low birth weight (Cotch et al., 1997). Interestingly, the successful treatment of *T. vaginalis* infection in pregnant women at the mid gestation point does not prevent this subsequent preterm delivery (Klebanoff et al., 2001). Neonatal genital and nasopharyngeal infections have been reported, with transmission thought to occur during birth (Smith et al., 2002).

Infection of the male genitourinary tract is generally asymptomatic, although mild urethritis, epididymitis (Fisher and Morton, 1969), and prostatitis can occur (Guenthner et al., 2005). *T. vaginalis* colonisation of the prostate can lead to chronic infection, and is thought to be the cause of most persistent infections in males. Trichomonads have been detected in the prostatic urethra, and in the surrounding tissues of the prostate, including the glandula lumina, sub mucosa and stroma (Gardner et al., 1986). Until recently, *T. vaginalis* infection in males was considered a "nuisance infection", without serious consequences (Van Der Pol, 2007). This view has been changed somewhat, with the realisation that colonisation of the prostate by *T. vaginalis* is a risk factor in the development of prostate cancer (Sutcliffe et al., 2006). A study involving 673 subjects with prostate cancer, and an equal number of control subjects, found a statistically significant association between those seropositive for *T. vaginalis* antibodies, and those with prostate malignancy (Stark et al., 2009). The increase in risk in developing prostate cancer has been estimated to be between 23% and 40% (Stark et al., 2009; Sutcliffe et al., 2006). One potential mechanism responsible for the increased likelihood of carcinogenesis seen during *T. vaginalis* infection is the host inflammatory response (Ryu et al., 2004), which involves the increase production of pro-inflammatory cytokines that have been implicated in prostate malignancy (Azevedo et al., 2011). *T. vaginalis* infection of has also been shown to increase the expression of the proto-oncogene *PIM1* in cultured prostate epithelial cells (Sutcliffe et al., 2012). PIM1 is also known to induce the expression of another proto-oncogene, HMGA1, via the PIM1/c-MYC/HMG1 signalling cascade. Both c-MYC and HMG1 are frequently over expressed in malignant prostate cells, and their expression has been linked to increased proliferation and metastasis (Sutcliffe et al., 2012). Recent work has shown that *T. vaginalis* produces a novel protein, *T. vaginalis* macrophage migration inhibitory factor (TvMIF), which has 47% sequence agreement with the pro-inflammatory cytokine Human macrophage migration inhibitory factor (HuMIF) (Twu et al., 2014). TvMIF inhibits macrophage migration, causes inflammation, and activates ERK, Atk, and Bcl-2-associated death promoter phosphorylation, inhibiting with apoptosis and causing cellular proliferation. The same study demonstrates that exposure of benign and malignant prostate cells to TvMIF *in vitro* instigates growth and invasion (Twu et al., 2014).

**Relationship with other microorganisms**

The presence of *T. vaginalis* in female patients can cause extensive changes in the vaginal microbiome, and trichomoniasis often occurs in tandem with bacterial vaginosis (BV) (Fichorova et al., 2013), a condition involving an imbalance in the bacterial flora of the vagina causing vaginal inflammation. This imbalance commonly manifests as a reduction in overall numbers of *Lactobacillus sp*., thought to be crucial for the maintenance of vaginal health, in combination with an increase in other commensal bacteria that are usually only present in lower levels, such as *Gardnerella vaginalis*, *Mobiluncus curtisii*, *Megasphaera sp*., *Atopobium vaginae*, and *Leptotrichia sp*. (Fredricks et al., 2007). The lactobacilii, predominant in women with a typical vaginal microbiota, contribute to vaginal health by releasing lactic acid, which maintains optimal vaginal pH. *T. vaginalis* grows optimally at a pH of 6 to 6.3 (Petrin et al., 1998), whilst the vaginal pH in women with a lactobacilli dominated vaginal environment ranges between 2.8 and 4.2 (O'Hanlon et al., 2011). The disruption of the lactobacilli community seen during trichomoniasis reduces the lactic acid released into the vaginal environment, increasing the pH, and creating more favourable conditions for *T. vaginalis* (Cudmore et al., 2004). Lactobacilli also act against pathogenic organisms in the vaginal environment, by out-competing them for nutrients, and also via the release of hydrogen peroxide, which is toxic to a number of potentially BV causing organisms such as *Gardnerella vaginalis* (Klebanoff et al., 1991). BV can have serious health implications, many of which overlap with trichomoniasis; increased risk of HIV transmission (Mirmonsef et al., 2012), ascending inflammatory infections and preterm birth/low birth weight pregnancy (Taylor et al., 2013). The combination of T. *vaginalis* and BV associated bacteria have been shown to amplify the host immune response, including an up-regulation of chemokines such as IL-8, and a down regulation of SLPI, an enzyme which protects epithelial cells from serine (Fichorova et al., 2013). SLPI has virucidal effects, and the down-regulation of this enzyme, in combination with the damage caused to the epithelial monolayer by *T. vaginalis*, could increased the likelihood of infection with sexually transmitted viral pathogens such as HIV and HPV.

There is a growing body of evidence that suggests *T. vaginalis* infection increases the chance of the acquisition, and transmission of HIV (Mavedzenge et al., 2010).This is of particular concern as *T. vaginalis* is especially prevalent in regions where HIV is considered to be endemic, such as Sub-Saharan Africa, where 32 million *T. vaginalis* infections occur each year (McClelland et al., 2007). Epidemiological studies have recorded an increase in the risk of HIV-1 acquisition of between 1.52 and 2.74 fold in *T. vaginalis* positive women in Sub-Saharan African countries (McClelland et al., 2007; Mavedzenge et al., 2010; Van Der Pol et al., 2008). A similarly sized increase in the risk of

transmitting HIV to a serodiscordant partner has also been recorded in *T. vaginalis* positive women in these regions (Sorvillo et al., 2001). The fairly recent realisation of the impact that *T. vaginalis* prevalence has on HIV rates has hastened a greater public health response (McClelland et al., 2007), and it is now recognised that the control of *T. vaginalis* could have a sizeable impact on the reduction of HIV transmission in these populations.

The exact mechanism by which *T. vaginalis* infection acts to increase the risk of contracting HIV is currently undetermined (Thurman and Doncel, 2011), although a number of theories have been suggested and tested. *T. vaginalis* infection instigates a robust mucosal immune response, involving localised inflammation and the recruitment of lymphocyctes and macrophages (Fichorova et al., 2013). This increases the number of potential cells for the virus to invade and proliferate in, and would make transmission more likely in a HIV-negative individual. Additionally, in a HIV-positive individual, the increase in cells infected with the virus localised in the genital tract would aid HIV shedding during sexual contact, exposing any partners to a higher level of viral particles, facilitating transmission (Shafir et al., 2009). HIV positive men with symptomatic urethritis caused by *T. vaginalis* have been shown to have a higher seminal viral load than those with either *T. vaginalis* negative, or with an asymptomatic (Hobbs et al., 1999). Those with symptoms of urethritis will necessarily have the greatest level of inflammation, and the greatest levels of CD4 lymphocytes and macrophages, increasing the targets for HIV invasion. Additionally, *T. vaginalis* causes damage to the urogenital epithelia, facilitating passage of HIV to deeper layers of the epithelium, and enhancing infection (Guenthner et al., 2005).

One of the more novel intermicrobial relationships of *T. vaginalis* is the association of the parasite with *Mycoplasma hominis*. This symbiotic relationship, first reported by Nielsen (Nielsen, 1975) was the first described association of two obligate human parasites. Co-infection of *T. vaginalis* with a variety of bacterial strains is common, due to the change in vaginal milieu caused by *T. vaginalis* infection, which leads to a favourable growth environment for a number of bacterial species not normally associated with the vaginal microbiota. In the case of *M. hominis* however, the mycoplasma has been found to actually enter *T. vaginalis* cells, surviving and even proliferating internally (Vancini and Benchimol, 2008). The presence of *M. hominis* alongside T. *vaginalis* has been shown to lead to an upregulation of inflammatory cytokines expression in host macrophages *in vitro*, which would potentially increase localised inflammation *in vivo* (Fiori et al., 2013). Initial concerns that infection with *M. hominis* could be related to metronidazole resistance in *T. vaginalis* appear unfounded (Butler et al., 2010).

The relationship with *M. hominis* is not the only symbiotic relationship that occurs involving *T. vaginalis*. The majority of *T. vaginalis* strains encountered in human infections are infected with one or more of a family of four double stranded RNA viruses, from the genus Trichomonasvirus (TVV), family totiviridae (Parent et al., 2013). The presence of this virus has been shown to increase virulence of *T. vaginalis* (Parent et al., 2013), in a process hypothesised to involve the modulation of parasite gene expression (Goodman et al., 2011). *T. vaginalis* borne TVV is also recognised by the host immune system via its interaction with human toll-like receptor 3 (TLR3), which instigates a pro-inflammatory cytokine cascade; a process previously linked to increased susceptibility to epithelial invasion by HIV (Fichorova et al., 2012). The presence of TVV during trichomoniasis can cause up to 30 fold amplification of the immune response, increasing the severity of the infection, and risk of more serious complications such as PID (Fichorova et al., 2013). It has also been demonstrated that this effect is particularly pronounced during simultaneous bacterial vaginosis (Fichorova et al., 2013), highlighting that the interaction between the vaginal microbiome, protozoan parasite, associated endosimbiant TVV and human epithelial cells has a large bearing on the immune response and infection severity.

**Diagnosis**

**Female patients**

Trichomoniasis is the most common non-viral sexually transmitted infection (STI) worldwide, with a higher prevalence than *C. trachomatis* and *N. gonorrhoeae* infections combined (World Health Organisation, 2012). Despite this, there is no routine screening programme in place in the UK or US, apart from during pregnancy, as there is for *C. trachomatis* (Workowski and Berman, 2010; Ross, 2006). This is due in part to the higher incidence of PID and tubal infertility caused by *C. trachomatis*, and also the lower frequency of asymptomatic infection in female patients with trichomoniasis. Symptomatic patients in the UK are tested for *T. vaginalis*, with testing available in the majority of GUM clinics.

The symptoms of *T. vaginalis* infection overlap significantly with those caused by a number of other sexually transmitted pathogens, such as *N. gonorrhoea* and *Mycoplasma genitalium,* and a diagnosis from clinical presentation alone is rarely possible. The specific symptoms of trichomoniasis, such as a the typical inflamed and speckled “strawberry cervix” , and frothy discharge, only occur in a minority of around 2% of cases (Fouts and Kraus, 1980), and so cannot be relied upon as a sole indicator of the infection. Accurate diagnosis of trichomoniasis is important for the subsequent treatment of

infection, as antibiotics given for general urethritis treatment such as azithromycin or doxycycline are not effective treatments for trichomoniasis (Abdolrasouli et al., 2007).

Diagnosis of trichomoniasis in female patients is frequently carried out microscopically, by the examination of a “wet mount” of vaginal or cervical exudates for motile parasites. This method is very simple to carry out, fast, and cost effective, when compared with alternative diagnostic options, including culture or molecular methods (Bachmann et al., 2011). Despite these advantages microscopic evaluation is not considered the optimal detection method, due to the low sensitivity afforded by this technique. Microscopy has been shown to have a sensitivity of around 60%, when compared with PCR based methods (Patil et al., 2012). Microscopy is unlikely to detect low level infections, in which the organism load in the sample may be under $10^4$ cells/ml, and therefore will potentially not be included in the fields examined on the slide (Garber, 2005). The sensitivity of this method decreases rapidly if delays are present between sample acquisition and examination, with a reduction in sensitivity to 20% caused by a as little as a 10 minute delay reported (Kingston et al., 2003). Due to this it has been suggested that any diagnostic service which cannot guarantee the ability to test samples within an hour of acquisition should use alternative methods (Stoner et al., 2013). This is of particular concern for clinics that do not operate an on-site microscopy service, and rely on the transport of samples to remote centralised laboratories for examination. The reduction in sensitivity occurs due to the reduction in parasite motility, making the trichomonads difficult to identify. Due to their similar size and shape, *T. vaginalis* cells can be hard to differentiate between lymphocytes when non motile (Garber, 2005).

The culture of *T. vaginalis* from clinical samples has long been regarded as the gold standard for the diagnosis of this organism (Bachmann et al., 2011). Cultures are typically maintained in a broth medium, and inoculated with swab samples taken from the vaginal canal or cervix of female patients, or urethral discharge from male patients. Cultures can also be inoculated from urine samples, although this is not the optimal sampling method, and reduced sensitivity. Growth can be apparent in as little as 48 hours, but cultures should be incubated for at least 7 days to enable the detection from low inocula (Garber, 2005).

The most common media used are variants of Diamonds (TYM) medium (Diamond, 1957), which originally contained trypticase digest, yeast extract, maltose, cysteine, ascorbic acid and sheep serum. Possibly the most frequently used variant is Diamonds TYI-S-33 medium, which also contains a source of iron, foetal bovine serum in place of sheep serum, and a vitamin 107-Tween 80 mixture (Diamond et al., 1978). *T. vaginalis* is incapable of synthesising a range of macromolecules necessary for survival and growth, including purines, pyrimidines and some lipids, and relies on nutrients

acquired from secretions or phagocytosed human or bacterial cells in the host genital tract. This necessitates the presence of these molecules in any culture media, and serum especially is a key component to support axenic growth of this organism (Petrin et al., 1998).

The use of broth culture to diagnose *T. vaginalis* infection has a higher diagnostic sensitivity than wet mount microscopy. One study of 337 samples, including 97 positive samples, found microscopy and culture had sensitivities of 52% and 78% respectively (Wendel et al., 2002). However, the use of culture to diagnose trichomonas infection does have some significant disadvantages. The week long incubation required means that culture is the diagnostic option with the longest time between sample acquisition and result. Additionally, culture is less sensitive than molecular methods, such as PCR. The use of solid media to culture *T. vaginalis* has been reported in the literature (Stary et al., 2002), although is rarely used in a clinical diagnostic setting. One study found a solid modified Columbia agar medium to be more sensitive (98.4%) than a commercially available Trichomonas medium (92.1%) (Stary et al., 2002).

One commercially available culture based diagnostic test is the InPouch culture system (Biomed Diagnostics, USA). This system combines both culture and microscopy to provide a diagnostic solution that offers the advantages of both methods (Sood et al., 2007). It consists of a clear plastic pounch containing two conjoined chambers full of media, one of which is inoculated via a swab. The other chamber is thinner, and has a thin viewing window enabling examination for any trichomonads using microscopy. This method has been shown to be more sensitive than wet-mount microscopy alone (Draper et al., 1993; Sood et al., 2007), and also removes the need for a pre culture transport medium, as the pouch is inoculated directly from the patient, which improves the likelihood of a successful culture (Schwebke et al., 1999). The fact that microscopic evaluation of the culture can be carried out without any fluid manipulation removes the possibility of contamination and reduces the time taken during the examination. This method is more expensive than standard culture or microscopy based methods however (Draper et al., 1993).

Serological methods for diagnosing *T. vaginalis* have been developed, but are rarely used clinically. A monoclonal antibody based enzyme-linked immunosorbant assay (ELISA) test specific to *T. vaginalis* surface peptides has been shown to offer sensitivities and specificities of 89% and 98% compared with broth culture (Lisi et al., 1988). A detection limit of 100 trichomonads per ml, and a greater sensitivity than wet mount microscopy, has been demonstrated using another ELISA assay (Watt et al., 1986). The Trichomonas Direct Enzyme Immunassay (California Integrated Diagnostics, US), was a commercially released ELISA test, which is no longer on the market. It relied on a mix of peroxidase labelled monoclonal antibodies to an assortment of *T. vaginalis* proteins, and was as sensitive as

broth culture (Petrin et al., 1998). One immunoassay currently available commercially, and the only *T. vaginalis* immunoassay currently awarded FDA approval in the US, is the OSOM Trichomonas Rapid Test (Sekisui Diagnostics, US). The test is an immunochromatographic capillary flow dipstick test, and provides a result within 10 minutes, enabling it's use at point of care (POC). The OSOM test has a sensitivity and specificity of 82% and 97% respectively (Huppert et al., 2007), making it a more sensitive diagnostic test than wet-mount microscopy, culture, and standard ELISA methods, whilst being far quicker and simpler to carry out.

Both commercial, and "in house" PCR based assays for *T. vaginalis* are available, and provide a more sensitive form of testing than the traditional methods of wet-mount microscopy and culture. Although PCR requires more highly trained staff and more expensive equipment and reagents, than alternative methods, the sizeable increase in sensitivity, coupled with a relatively short turnaround time, makes PCR based assays the optimum diagnostic method in developed countries. A range of genes have been exploited as targets for *T. vaginalis* specific PCR tests. A standard PCR assay specific to a sequence of the beta-tubulin gene was found to have a sensitivity and specificity of 97% and 98% respectively (Madico et al., 1998). The same study found the sensitivities of wet mount microscopy and culture to be 36% and 70% respectively, illustrating the improvement in sensitivity offered by PCR. A study comparing the sensitivity of two real-time fluorescence resonance energy transfer (FRET) hybridisation probe based PCR assays specific to the beta-tubulin gene and 18S rRNA gene found assay sensitivities of 96% and 100% respectively (Simpson et al., 2007). The *T. vaginalis* genome harbours a number of conserved repeated DNA sequences, and these are attractive targets for nucleic acid amplification tests (NAATs), as they provide a higher copy number per cell, and improve detection limits and sensitivities (Bandea et al., 2013). The sequencing of the ~160 Mb *T. vaginalis* genome identified 59 common repeat families that make up ~39 Mb of the complete sequence (Carlton et al., 2007). The majority of the repeat sequences have a copy number of >100, with the average being 660 copies. Importantly, these repeats show a high level of homogeneity, with sequence variation identified between repeats of the same family in only 2.5% of repeats. This provides a stable, high copy number target for molecular assays. Single parasite detection has been demonstrated for PCR assays using these repeated sequences as targets (Kengne et al., 1994), and the improved sensitivity has enable testing from non-invasive urine samples, which typically contain a lower organism load than the swab samples more frequently used (Bandea et al., 2013).

The overwhelming majority of NAATs available for *T. vaginalis* identification are PCR based, however novel isothermal diagnostic methods have been applied to the detection of this organism. The commercially available APTIMA *T. vaginalis* assay (Gen-Probe, US), relies on transcription mediated

amplification technology, in combination with a target capture specimen processing system, to provide a highly sensitive assay for *T. vaginalis* detection (Chapin and Andrea, 2011). The assay is designed to be used on one of the automation systems available from Gen-Probe, such as the TIGRIS. The assay is approved for use in the US by the Federal Drug Administration (FDA), and is approved for use in the UK. The approval only relates to the use of the assay with a number of sample types from female patients, including urine samples, endocervical swabs, and vaginal swabs. GenProbe also manufactures AMPTIMA assays for other sexually transmitted pathogens, including *N. gonorrhoeae*, human papillomavirus (HPV), and a combined *C. trachomatis* and *N. gonorrhoeae* assay (APTIMA COMBO 2 assay). A large scale study of 933 symptomatic and asymptomatic female patients attending an STI clinic found the APTIMA assay to have the following sensitivity and specificity, respectively, in the following samples types; 100% and 99.0% for vaginal swabs, 100% and 99.4% for endocervical swabs, and 95.2% and 98.9% in urine specimens (Schwebke et al., 2011).

Another commercially available molecular diagnostic test for *T. vaginalis* infection is the Affirm VPIII *Trichomonas vaginalis* assay (Becton Dickinson, US), which relies on RNA probe hybridisation to detect target DNA. The test has been shown to be more sensitive than wet mount microscopy (Brown et al., 2004), but lacks sensitivity compared to NAATs, as the target DNA is not amplified before detection, which results in a higher starting copy number being required in order to generate signal. One study compared the Affirm VPIII assay with the APTIMA assay, and found sensitivities of 63.4% and 100% respectively (Chapin and Andrea, 2011). The test is fully automated, and takes 45 minutes to run, including 2 minutes “hands on time”, potentially enabling point of care testing.

POC testing for sexually transmitted infections, including trichomoniasis, could be of great benefit in the control of these diseases (Tucker et al., 2013). Testing at the POC enables consultation, testing, and the provision of appropriate treatment to all be carried out in the same day, at the same site. This removes the risk of patients neglecting to return for results and medication, and also reduces the possibility of transmission by sexual contact during the delay before treatment is instigated (Tucker et al., 2013). Currently, it is possible to carry out testing via wet mount microscopy at POC, although this is insensitive, low throughput, and effected by the experience and skill of the technician. Also, the immunochromatographic OSOM Trichomonas Rapid Test (Sekisui Diagnostics, US) is able to provide results within 10 minutes, is easy to use and read, and has higher sensitivity than non-molecular methods (82% compared with PCR), making it a very good option for providing a POC diagnostic (Huppert et al., 2007). The current goal of POC diagnostic research is to provide the sensitivity of NAAT diagnostics, whilst eliminating hands-on processing, and decreasing the time-to-result, enabling maximally sensitive testing at the point of care (Craw and Balachandran, 2012). The

majority of POC NAAT systems in development rely on an automated nucleic acid procedure, rapid target amplification, and detection of reaction products, typically by optical detection of fluorescence (Niemz et al., 2011). These processes are often carried out in a disposable single use cartridge or chip, preventing contamination of the machine during sample handling (Niemz et al., 2011). One such system currently in development is the Atlas Io PoC (Atlas Ltd, UK) platform, which is aiming to release a *Trichomonas vaginalis* test in 2014. The test involves automated nucleic acid extraction, followed by amplification of a multi-copy DNA repeat sequence target, and novel electrochemical endpoint detection. A small scale lab evaluation of the test, comparing its performance with that of the APTIMA *T. vaginalis* test (Hologic Gen-Probe, USA), over 90 clinical samples, found that the sensitivity and specificity of the assay were 95.5% and 97.5% respectively (Pearce et al., 2013). The most widespread commercially available POC system is the GeneXpert (Cepheid, USA), a platform for processing real-time PCR based assays with fully automated sample preparation, amplification and detection on disposable assay-specific cartridges (Helb et al., 2010). Currently there are FDA approved GenExpert assays available for *Mycobacterium tuberculosis* (Marlowe et al., 2011), *Clostridium difficile* (Babady et al., 2010) and a combined *C. trachomatis* and *N. gonorrhoeae* assay (Tabrizi et al., 2013), all of which have high sensitivities and specificities, and provide results within 90 minutes. Cepheid has announced that it plans to release a *T. vaginalis* assay in the 2014-2015 product range, enabling testing for trichomoniasis to be carried out using this platform.

The use of POC testing for trichomoniasis could be of particular benefit in sub-Saharan Africa, where both *T. vaginalis* and HIV infection are highly prevalent. The improved control of *T. vaginalis* could potentially reduce HIV transmission, and significantly impact on morbitity and mortality in the region (Johnston and Mabey, 2008). POC testing has been regarded as being particularly well suited to developing countries, as the automated POC systems reduce the need for skilled technicians, or well equipped centralised laboratories, which may not be widely available (Pai et al., 2012). Additionally, the ease of transport, and lack of additional equipment needed by these systems, enables the testing of remote communities, far removed from traditional hospital based healthcare. However, concerns remain over whether the expense of POC NAAT systems will prevent their widespread use in developing countries, with studies examining the prospective cost of implicating widespread POC NAAT testing in Africa highlighting the increased cost of diagnosis (Meyer-Rath et al., 2012). Field testing of the Cepheid GenExpert *C. trachomatis* assay in South Africa has given promising results, demonstrating high clinical sensitivity in combination with being well suited for use outside of the traditional laboratory environment (Jenson et al., 2013), and the GenExpert *M. tuberculosis* assay is already being widely used in sub-Saharan Africa (Osman et al., 2014; Lawn et al., 2013). As use of the

GenExpert system is becoming more widespread in developing countries, especially for detecting M. tuberculosis, it is possible that healthcare providers may take advantage of the platform to diagnose *T. vaginalis*, upon the predicted release of the assay in 2015.

**Male patients**

The diagnostic testing for *T. vaginalis* infection in male patients is rarely undertaken, for a number of reasons. *T. vaginalis* infection in men is rarely symptomatic, and male partners of women who have received a positive diagnosis are treated concurrently without any confirmatory testing (Schwebke and Lawing, 2002). Microscopy of urethral discharge, if present, has a poorer sensitivity with male samples than females. Culture can be undertaken from male samples, and the optimal sample type is considered to be a combination of urethral swabbing and collection of urine sediment however, as with microscopy, sensitivity is poor (Krieger et al., 1993). The low organism loads encountered in male patients mean that sensitive, molecular methods are required in order to provide an acceptable level of sensitivity, if testing for *T. vaginalis* infection is going to be carried out.

In the past *T. vaginalis* infection in males has often been considered to be a nuisance infection, due to the fact that the infection in males is self-limiting, typically clears without intervention, and the belief that infection in males rarely results in any serious sequelae (Van Der Pol, 2007). There is growing evidence to the contrary; that infection in males can lead to chronic colonisation (Lee et al., 2012), and that it may be a causative agent in prostate cancer (Sutcliffe et al., 2006). It has been shown conclusively that *T. vaginalis* increases the transmission of sexually transmitted viruses, including HIV (Sorvillo and Kerndt, 1998; Mavedzenge et al., 2010), which in itself makes the control of this organism in males a non-trivial matter. The increasing realisation that this organism is associated with disease states with high morbidity in both men and women, and can have serious detrimental effects on reproductive health has lead to an increased interest for diagnosis and treatment of infections (Soper, 2004), and the treatment of males inevitably will play a part in this process. This is driving the need for sensitive, molecular based approaches for the detection of this organism.

**Treatment**

The standard treatment for *T. vaginalis* infection, in the UK and worldwide, is a single 2g oral dose of metronidazole or tinidazole (Muzny and Schwebke, 2013). The most commonly administered drug in the UK is metronidazole, in part due to the low associated cost(Sherrard et al., 2014). These antibiotics from the 5-nitroimidazole family exert their antimicrobial activity by disrupting the redox system of the parasite, with metabolic products of the drug binding to proteins in the thioredoxin

mediated redox network and inhibiting thioredoxin reductase (Leitsch et al., 2009). The antimicrobial properties of these drugs rely on reduction at the nitro group, which occurs after passive diffusion of the drug into the hydrogenosome of the cell, generating nitroradical anions, and further reduced reactive intermediates (Dunne et al., 2003).Whilst in bacterial cells these nitroradicals would cause DNA damage, and cell death, the precise mechanism of by which they damage eukaryotic microorganisms is poorly understood (Kulda, 1999).

Of the available 5-nitroimidazole drugs, tinidazole is considered the optimal antimicrobial agent, with numerous studies showing that tinidazole therapy has either an equal or lower failure rate than metronidazole therapy (Bachmann et al., 2011). A large scale study reviewing the outcomes of female patients receiving different therapies found the clinical failure rate of those taking metronidazole to be 14.8%, compared with a 3.7% failure rate for those receiving tinidazole (Bachmann et al., 2011). This is partly explained by the ~12.5 hour half-life of tinidazole being ~70% longer than that of metronidazole, and its higher serum concentration (Bachmann et al., 2011).

Resistance to 5-nitroimidazole class drugs has been encountered in clinical *T. vaginalis* isolates, in particular resistance to metronizadole, which is the antimicrobial that has historically seen the greatest use in the treatment of this organism. The overall prevalence of resistance is low; a study of 538 isolates from a number of clinical sites in the US found low level metronizadole resistance in 4.3% of isolates, and no resistance to tinidazole (Kirkcaldy et al., 2012). The prevalence of 5-nitroimidazole resistance in developing countries does not appear to be any greater than in developed countries; studies in Africa have found the prevalence to be 6% (Rukasha et al., 2013). Due to the lack of effective alternative approved treatments for *T. vaginalis* infection, the only available treatment for a metronizadole resistant infection is to increase the dosage, to a potentially toxic and side effect inducing level, or alternatively use tinidazole (Cudmore et al., 2004).

**Conclusion**

The pathogenic effect caused by colonisation of the genital tract by *T. vaginalis* is caused by damage to the epithelia of the genital tract, which the parasite causes in a variety of ways, including mechanical damage, the secretion of apoptosis inducing proteases, the disturbance of the junctional complexes in the monolayer, and the instigation of an inflammatory response. The virulence of the infection, and disease severity, is similarly complex, and governed by a range of factors. Infection of the female genital tract is capable of significant modification of the vaginal microbiome, potentially resulting in bacterial vaginosis, worsening the symptoms of the infection (Fichorova et al., 2013). As well as organisms present in the vaginal environment, *T. vaginalis* infection can be impacted by the

presence of the intracellular symbionts *M. hominis* or TVV, which modulate *T. vaginalis* gene expression in a process thought to increase virulence (Fichorova et al., 2013; Fiori et al., 2013; Fraga et al., 2012).

*T. vaginalis* infection is most common non-viral STI worldwide; only genital human papillomavirus (HPV) is more prevalent (Bruni et al., 2010) . There are an estimated total number of 276.4 million cases per year world-wide, more than the 106.1 million new cases of *N. gonorrhoeae* infection, and 105.7 new *C. trachomatis* infections combined (World Health Organisation, 2012). *T. vaginalis* infection is a widespread, global concern, prevalent in Europe (5.8%), the Americas (22%) and Africa (20.2%). The high prevalence of *T. vaginalis* infection in Africa is of a particular concern, as the parasite has been implicated in increasing the likelihood of both becoming infected by, and transmitting, HIV (Mavedzenge et al., 2010). Despite the high frequency, and ubiquitous geographic spread of this disease, it has received a much smaller public health response than the next most prevalent curable STI's, *C. trachomatis* and *N. gonorrhoeae* (Van Der Pol, 2007). This has been due in part to the consideration of trichomoniasis as a mild "nuisance" infection, compared with the more serious tubal infertility risk associated with chlamydia infections in women, and the obvious symptoms caused by gonorrhoea. Growing evidence that *T. vaginalis* infection can increase the risk of disease states associated with high morbidity in both male and female patients is increasing the interest in the detection and treatment of this parasite. The development of sensitive NAAT tests for *T. vaginalis* has opened up the possibility of testing asymptomatic patients, who often have low organism loads, undetectable with less sensitive diagnostic methods. In the UK, the cost of offering this service in a sexual health screen is thought to outweigh the benefit of detecting these asymptomatic infections, due to the relatively low prevalence of this organism in the general population (Ng and Ross, 2012). However, in the US, where the prevalence is much higher, this could be a viable strategy. The testing of males, who tend to have a lower organism load, by traditional methods such as microscopy or culture, is less sensitive than in females, and the increased sensitivity afforded by NAATs allows for the sensitive testing of this group. The continuing development and improvement of POC NAAT testing, and the imminent release of *T. vaginalis* assays for existing POC platforms, will provide the opportunity for the provision of sensitive rapid testing for this organism, with all the inherent benefits of testing at the POC.

**Declaration of interest**

The authors report no declaration of interest.

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**Tables**

| WHO Region | Incidence (per 1000) | | Prevalence (%) | |
|---|---|---|---|---|
| | Female | Male | Female | Male |
| Africa | 146.0 | 164.8 | 20.2 | 2 |
| The Americas | 177.7 | 180.6 | 22.0 | 2.2 |
| South East Asia | 40.3 | 50.1 | 5.6 | 0.6 |
| European | 51.7 | 48.4 | 5.8 | 0.6 |
| Eastern Mediterranean | 64.0 | 66.1 | 8.0 | 0.8 |
| Western Pacific | 45.6 | 47 | 5.7 | 0.6 |

**Table 1.** Global incidence and prevalence of *T. vaginalis* infection, according to 2012 WHO estimates. Data taken from World Health Organisation, 2012.