CANADIAN STI BEST PRACTICE LABORATORY GUIDELINES

The laboratory diagnosis of Trichomonas vaginalis

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Trichomonas vaginalis, a parasitic protozoa that causes the sexually transmitted infection trichomoniasis, is the sexually transmitted infection with the largest annual incidence, exceeding 170 million cases per year. The disease can be difficult to diagnose due to its heterogeneous presentation and problems with diagnostic testing. All diagnostic tests are fraught with imperfections, but the old, reliable wet mount examination (in trained hands), and the newer InPouch method may be advantageous due to simplicity in technology and cost. The present article reviews the pros and cons of culture, antibody and nucleic acid-based technologies that may point to future diagnostic advances.

Key Words: Diagnostic tests; STI; Trichomonas vaginalis; Vaginitis

Turogenital tract of both women and men worldwide. vichomonas vaginalis is a parasitic protozoan that infects the Trichomoniasis, which is caused by T vaginalis, is the most common sexually transmitted infection (STI) today, with an annual incidence of more than 170 million cases worldwide. Over eight million women are infected with T vaginalis in North America alone (1). Although once considered a nuisance infection, T vaginalis in women has since been associated with an increased incidence of prolonged postpartum fever and endometritis (2), premature rupture of membranes (3) and cytological changes in cervical cell morphology (4). Although usually an asymptomatic disease in men, T vaginalis has been associated with 5% to 15% of nongonococcal urethritis cases (5). These figures may be higher in men with nongonococcal urethritis who have failed tetracycline and erythromycin therapy. T vaginalis has also been associated with epididymitis, prostatitis (6) and balanitis. The organism has now been

Le diagnostic en laboratoire du Trichomonas vaginalis

Le *Trichomonas vaginalis*, un protozoaire parasite responsable de la trichomonase, une infection transmise sexuellement, est l'infection transmise sexuellement à l'incidence annuelle la plus élevée, avec plus de 170 millions de cas par année. La maladie peut être difficile à diagnostiquer en raison de sa présentation hétérogène et des problèmes reliés aux épreuves diagnostiques. Toutes les épreuves diagnostiques sont pleines d'imperfections, mais l'examen sur préparation humide, ancien et fiable (par des personnes exercées), et la méthode InPouch, plus récente, peuvent être avantageux en raison de la simplicité de leur technologie et de leur coût. Le présent article porte sur le pour et le contre des technologies des cultures, des anticorps et des acides nucléides qui pourraient annoncer de futurs progrès diagnostiques.

associated with a significantly higher risk of HIV transmission (OR 2 to 2.5) (7), and it is suggested that this parasite may increase maternal-to-infant transmission in HIV. This increased transmission in females is believed to be due to the denution of the cervicovaginal epithelium along with the accumulation of CD4 lymphocytes and macrophages at the site of infection, which could provide pools of HIV-susceptible or HIV-infected cells (8). Twenty-five per cent of university students in Nigeria tested positive for T vaginalis and up to 20% of pregnant women in the United States (US) are culture positive (9) – these data support the prevalence of *T* vaginalis. Because of the impact on premature rupture of membranes and low birth rate, T vaginalis is considered to be a significant cause of neonatal morbidity in the US (10). The incidence of T vaginalis in Canada is unknown. It appears to be subject to underdiagnosis and misdiagnosis in clinical practice because the symptom complex can overlap with other causes of

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vaginitis. As well, conventional diagnostic tests are often not readily available.

CLINICAL PRESENTATION

The appropriate use of diagnostic testing is dependent on appropriate specimen collection. However, specimens are only taken when a diagnosis is considered. The classic presentation of *T vaginalis* is that of a purulent, foul-smelling vaginal discharge which is associated with pruritis, dysuria and dyspareunia. The discharge usually exceeds pH 6.0, and a 'strawberry' cervix, which is characterized by punctate hemorrhagic lesions, is described. These classic symptoms are only seen 20% of the time (11), which often leads to women with these vaginal symptoms being empirically treated for yeast infections or bacterial vaginosis. The majority of men who have had sexual contact with a woman with *T vaginalis* will be completely asymptomatic or only have mild dysuria even though the organism can be found on culture.

Microscopy

DIAGNOSTIC TESTS

The diagnosis of trichomoniasis has traditionally depended on the microscopic observation of motile protozoa from vaginal or cervical samples and from urethral or prostatic secretions. This technique was first described in 1836 by Donné (12). T vagi*nalis* can be differentiated on the basis of its characteristic jerky movements. On occasion, flagellar movement can also be noted. The sensitivity of this test varies from 38% to 82% and is dependent on the inoculum size because fewer than 10^4 organisms/mL will not be seen. As well, the need for the specimen to remain moist and the experience of the observer are important variables. The size of the trichomonad is approximately the same as that of a lymphocyte (10 µm to 20 µm) or a small neutrophil; when not motile, a trichomonad can be difficult to differentiate from the nucleus of a vaginal epithelial cell. Motility is very dependent on the temperature of the specimen. At room temperature in phosphate-buffered saline, the organism will remain alive for more than 6 h; however, the motility of the organisms becomes significantly attenuated. This wet mount examination is clearly the most cost-effective diagnostic test, but the lack of sensitivity contributes to the underdiagnosis of the disease. Because viable organisms are required, delay in transport and evaporation of moisture from the specimen reduces motility and, consequently, diagnostic sensitivity.

Culture

Broth culture technique has been the gold standard for T vaginalis for the past 40 years. The inoculum size required is only in the range of 10² organisms/mL and the growth of the organism is easy to interpret. The standard broth is Diamond's TYI medium in glass tubes (13). Incubation periods ranging from two to seven days are required to identify T vaginalis in culture. Contamination with bacteria is a major problem, even with broth cultures spiked with antibiotics to eliminate vaginal flora. Passage of the culture after two to three days to reduce the bacterial contamination may be required to definitively identify the T vaginalis culture. The organism has the capacity to enter lag growth and, even in well-established axenic culture, can sometimes have attenuated growth for 24 h to 48 h before re-establishing its characteristic log/day growth. Because of the expense, culture techniques have not been readily available but would be the most effective way of establishing the true epidemiology of *T vaginalis*, particularly where STI clinics are remote from the clinical laboratory. To circumvent these problems, the InPouch system (BioMed Diagnostics, USA) and similar culture systems have been developed whereby the specimen is put into a two-chambered bag, allowing sampling for immediate wet mount microscopy and incubation for culture (14). In some studies (14), this has been shown to be at least as effective as Diamond's medium in glass vials. *T vaginalis* is an anaerobic organism that grows more slowly under aerobic conditions. Thus, CO₂ incubation has been recommended for optimal recovery.

Cultivation on cell cultures is more sensitive, enabling the observation of *T vaginalis* from an inoculum containing as few as 3 organisms/mL. However, cell culture is expensive, inconvenient and even more prone to vaginal bacterial contamination. This technique requires pretreatment of the specimen with antibiotics using Diamond's TYI medium as a transfer medium, followed by subsequent passage onto the cell cultures. A combination of TYI and cell culture medium (2:1) supports both the monolayer and *T vaginalis* growth. Despite its high sensitivity, this method has not been used outside a limited number of studies (13).

To improve the sensitivity of microscopic evaluations and the speed in comparison with culture results, staining techniques have been used. The use of acridine orange and periodic acid-Schiff, among other techniques, have been shown to be more sensitive in some investigators' hands. Other laboratories have not found this technique to be as helpful (15). The Papanicolaou smear also has considerable appeal because it is routinely used in gynecological screening and especially in women with a history of exposure to sexually transmitted pathogens. However, the T vaginalis organism is predominantly found in the vagina and, therefore, the endocervix is not the optimal location for sampling. This technique is fraught with both false-positive and false-negative results. The difficulties with staining techniques include the elimination of motility due to the fixative and the fact that T vaginalis does not always have its characteristic pear shaped form. Thus, staining in most cases is best used in conjunction with direct wet mount motility observation (16).

Nucleic acid detection

Recombinant DNA technology has been adapted over the past decade as a diagnostic tool. A variety of primers have been tested, including primers initially reported by Riley et al (17). This has subsequently been commercialized into the Affirm VP system (Becton, Dickinson and Company, USA). Depending on the studies performed, the highest sensitivity of this technique is less than 90%, and false positives have been reported post-treatment because of DNA from dead organisms (18). A recent article by Crucitti et al (19) indicated that in an African population, some primers were more effective than others when compared with culture-based techniques. Other studies have shown a higher sensitivity rate, which also appears to be dependent on which specimens are being used for testing. In general, urine sampling for T vaginalis in women is less effective than supervised vaginal swabs, and self-administered vaginal swabs have had variable results depending on the population tested (20). Specificity with this method can be less than with wet mount due to false-positive tests.

Dot blot hybridization has also been used, employing a 2.3 kb $T\ vaginalis$ DNA fragment as a probe. Unfortunately, this probe

has been shown to be unstable as a radioactive probe but can be improved with a fluorescent-labelled technique (21). Its role in determining asymptomatic carriers has not been established.

Nucleic acid detection of *T vaginalis* is not generally available in Canada (even in reference laboratories).

Identification of T vaginalis in men

Because the majority of men are asymptomatic carriers, the diagnosis of T vaginalis is usually not made, and the male partner is identified and treated with metronidazole at the same time that the female partner is treated. In the small number of males who are symptomatic, urethral discharge collected with a swab would provide the best results using the broth culture technique, with sampling ideally done before first-voided morning urine. Using the urine-based polymerase chain reaction (PCR)-ELISA technique described by Kaydos-Daniels et al (22), it has been shown that in a Malawian population, PCR on first-catch urine had a sensitivity of 92.7% and a specificity of 88.6%. They determined that where urethral swabs and cultures are not feasible, this technique would be a reasonable way of diagnosing T vaginalis. The incidence of T vaginalis was 9% in members of the Malawian population who had urethritis, but only 3.5% in those without urethritis.

Antibody based technique

T vaginalis has an estimated eight serotypes and, in immunoblot studies (23), a wide variety of antigenic markers have been seen. This work also showed that the serological response to T vaginalis is variable among different people who could react to different parasitic antigens (23,24). A variety of techniques, including complement fixation, hemagglutination, gel diffusion, fluorescent antibody and ELISA, have been used to determine the presence of trichomonal antibodies. However, these are certainly not specific in determining recent from remote infection. As well, in low incidence populations, positive antibody could reflect interaction with nonpathogenic trichomonads.

Monoclonal antibodies derived from specific 62 kDa and 65 kDa proteins have been effective in identifying *T vaginalis* from clinical specimens (25); however, these techniques have essentially been abandoned for PCR-based technology.

Antimicrobial susceptibility testing

The mainstay of treatment against *T vaginalis* is metronidazole, which is usually given as a single 2 g oral dose. Because most organisms are susceptible, susceptibility testing is not routinely

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done; however, women are increasingly found to have clinically resistant *T vaginalis*. Susceptibility testing can be performed in microtitre wells or in shell vials to determine the minimal inhibitory concentration of the antibiotic. Interestingly, correlates with clinical resistance cannot be made if the susceptibility is performed under anaerobic conditions; correlation is found when the organism is grown aerobically (26). In general, susceptibility testing is not performed routinely, there are no standardized methods, and no proficiency testing is available (27).

Quality control and proficiency testing

Examination for T vaginalis is most often done as part of the investigation of vaginal discharge. It is recommended that the examination is included as part of any routine examination for vaginitis. pH evaluation with pH paper can help to rapidly differentiate T vaginalis from yeast at the bedside. The vaginal pH, which is normally pH 4.5, is not altered by yeast infection but is elevated in bacterial vaginosis and often rises above pH 6 in florid trichomoniasis. Quality concerns include the need to have the microscopic examination done immediately after specimen collection; even then, keeping the sample moistened with saline or phosphate-buffered saline is key. Alternatively, the specimen can be collected into an InPouch kit or into Diamond's medium to ensure survival of the organism during transportation to the laboratory. Where specimen transportation is suboptimal, the report of a negative finding should include a reference to the possibility of a false-negative result. Stained preparations can be difficult to read; therefore, the laboratory must take the steps necessary to ensure the ongoing competence of its microscopists. Cultures and positive specimens can be used to familiarize staff with the characteristic movement of motile T vaginalis. The laboratory should maintain communication with its clinicians to ensure that specimens submitted for the diagnosis of trichomoniasis are vaginal and not cervical.

SUMMARY AND CONCLUSIONS

Although T vaginalis is one of the most prevalent STIs, it is nonetheless an orphan area relying on old technology, clinical suspicion and empirical management. The impact of the disease appears to be quite pervasive on the transmission of other sexually transmitted pathogens and on the unborn child.

Vaginal swab culture either in broth tubes or using the InPouch system is an important diagnostic procedure that has a particular role in identifying women who do not have clinically overt disease.

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