



Trichomonas vaginalis

by Real-time PCR (Reflex to metronidazole resistance)

T. vaginalis is a flagellated, anaerobic protozoan and is the most common non-viral sexually transmitted pathogen. Approximately half of female *T. vaginalis* infections are asymptomatic, as are most male infections (1). Symptomatic infections manifest as Trichomoniasis with symptoms of discharge (yellow, green or gray, sometimes frothy), odor, itching, and pain during urination and/or intercourse. Signs of infection include small red ulcerations on the vagina and/or cervix, positive amine (whiff) test and elevated pH. Wet-mount microscopy of a vaginal swab often reveals white blood cells and rapidly motile trichomonads. However, detection of trichomonads by microscopy has a sensitivity of only 60%-75%, whereas, polymerase chain reaction (PCR) can detect *T. vaginalis* with a sensitivity of 85%-100% (2,3). Trichomoniasis is associated with a number of serious clinical complications, as pregnant women with Trichomoniasis are at increased risk for pre-term labor and delivery of low birth weight neonates (4,5). In addition, Trichomoniasis is associated with HIV transmission (6, 7). Patients are normally treated with a single oral dose of metronidazole, an antibiotic used to treat infections caused by anaerobic bacteria and parasites. Although generally effective, some *T. vaginalis* strains are resistant to metronidazole. If metronidazole treatment fails, the only other approved treatment for Trichomoniasis is the related drug tinidazole. Therefore, identifying Trichomoniasis resistance to metronidazole can help guide clinicians in prescribing effective therapy for Trichomoniasis patients.

Epidemiology

- There are more than seven million cases of Trichomoniasis each year in the United States (3).
- The overall prevalence of *T. vaginalis* among American women is 3.2%, but varies dramatically by race, from 1.3% for non-hispanic white women to 13.3% for non-hispanic black women (8).
- Most sexually-transmitted infections are more prevalent among adolescents and young adults; however, Trichomoniasis has a similar prevalence among sexually active women of different age groups (3).
- Although metronidazole treatment is reported to be 85%-95% effective, recent reports suggest that between 2.5% and 10% of clinical *T. vaginalis* isolates exhibit some degree of metronidazole-resistance (9-11).

Pathogenesis

- *T. vaginalis* attaches to the vaginal epithelium. Several *T. vaginalis* adhesins, substances that enable the attachment to epithelial surfaces, have been identified that mediate this binding (12).
- After binding, *T. vaginalis* triggers detachment of cells through proteolytic activity, cytotoxicity and apoptosis (3).
- Patients infected with *T. vaginalis* produce circulating (IgG) and secreted (IgA) antibodies that recognize adhesins and prevent parasite adhesion; however, protection is only short-term as re-infection rates as high as 30% have been observed (3).

Laboratory Diagnosis

- A cervico-vaginal specimen can be submitted for laboratory testing to detect *T. vaginalis*. Detection of trichomonads by PCR has a sensitivity of 85%-100% (3).
- Currently, only the Centers for Disease Control and Prevention (CDC) can determine metronidazole susceptibility for *T. vaginalis*. A viable culture of *T. vaginalis* must be received, using a specialized collection and transport device.
- Medical Diagnostic Laboratories, L.L.C. (MDL), can now detect metronidazole resistance in a subset of *T. vaginalis* specimens by Real-Time PCR. Our current assay detects a mutation that encodes a K80STOP change in the Tvnr6 protein and has 40% sensitivity, 96% specificity, and a 91% positive predictive value (PPV) for the detection of *T. vaginalis* metronidazole resistance. This test was developed using 100 well-characterized *T. vaginalis* isolates from the CDC.
- **Test 111 *Trichomonas vaginalis* by Real-Time PCR (Reflex to metronidazole resistance)** developed by MDL, offers a valuable diagnostic tool for the reliable detection of genetic determinants of antibiotic resistance, thereby predicting antibiotic susceptibility of *T. vaginalis* in a given clinical specimen. This test delivers a prognostic recommendation for antibiotic therapy in a personalized manner.
- Currently, MDL is the only medical laboratory in the United States to offer a reflex assay for metronidazole resistance at no additional charge.

Clinical Benefits of Testing

- This testing is currently available utilizing the **OneSwab[®]**, **UroSwab[®]** (males and females), and **ThinPrep[®]** specimen collection platforms for the detection of *T. vaginalis* and associated metronidazole resistance in cervico-vaginal specimens.
- Detection of metronidazole resistance can assist clinicians in administering effective treatment for Trichomoniasis patients.

Treatment Considerations

Table 2. Current Recommendations from the CDC for persistent or recurrent *T. vaginalis* Infection (15).

Recommended Regimens

Metronidazole^a 2 g orally in a single dose **OR**

Tinidazole^b 2 g orally in a single dose

Alternative Regimens

Metronidazole^a 500 mg orally twice a day for 7 days

If This Regimen Fails

Metronidazole 2g orally twice a day for 7 days **OR**

Tinidazole 2 g orally for 7 days

^a Pregnant patients can be treated with 2 g single dose.

^b Randomized controlled trials comparing single 2 g doses of metronidazole and tinidazole suggest that tinidazole is equivalent to, or superior to, metronidazole in achieving parasitologic cure and resolution of symptoms.



- Patients should avoid alcohol during metronidazole or tinidazole treatment, as well as for 24 hours after the end of metronidazole treatment and 72 hours after the end of tinidazole treatment.
- In asymptomatic pregnant women, clinicians should counsel patients regarding the potential risks and benefits of treatment and communicate the option of therapy deferral until after 37 weeks' gestation
- All symptomatic pregnant women should not only be considered for treatment regardless of pregnancy stage, but be provided careful counseling regarding condom use and the continued risk of sexual transmission.
- If treatment is still unsuccessful, contact the CDC for a consultation.
- The CDC recently reported an increase in treatment success for women with Trichomoniasis that previously failed metronidazole therapy by utilizing susceptibility testing to tailor subsequent treatment (16).
- All *T. vaginalis* positive results for specimens collected using the MDL **OneSwab**[®], **UroSwab**[®] and ThinPrep[®] platforms are further tested for metronidazole resistance at no additional charge. This additional assay also serves to confirm the initial positive result. This information assists clinicians in administering an effective diagnosis and treatment for their patients and is especially useful for those patients presenting with recurring trichomoniasis. Information about how the assay is performed, assay interpretation, and the CDC 2015 STD Treatment Guidelines are distributed (15-17).
- Complete treatment guidelines for Trichomoniasis are available from the CDC <http://www.cdc.gov/std/treatment/2010/vaginal-discharge.htm>. (Accessed March 28, 2012). The guidelines include alternative treatment therapy for cases of metronidazole treatment failure.

References

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Frequently Asked Questions (FAQ)

- **What does a positive result mean for the detection of the Tvnr6 K80STOP mutation?**

A positive result indicates a >90% likelihood that the *T. vaginalis* present in the specimen exhibits some degree of resistance to metronidazole. It is not known if this level of resistance is associated with clinical failure to metronidazole treatment.

- **What does a negative result mean for the detection of the Tvnr6 K80STOP mutation?**

As our current assay only detects 40% of resistant *T. vaginalis* isolates; a negative result is inconclusive. It does not mean that the *T. vaginalis* in question is susceptible or resistant to metronidazole.

