Leukorrhea Panel

What is Leukorrhea?

- Leukorrhea means increased vaginal discharge; however, the term is often used loosely to include any abnormal vaginal discharge, even blood-tinged.
- A symptom rather than a disease, leukorrhea is one of the most common complaints for which a patient seeks help, particularly if the discharge is accompanied by itching and burning.
- Microscopic examination revealing pus cells (>10 White Blood Cells/ High power field) can confirm the diagnosis of leukorrhea (1).
- There are two classifications of Leukorrhea:
  - **Pathologic leukorrhea** is usually due to infections of the upper and lower female genital tract. The most common sexually transmitted pathogens associated with leukorrhea are *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*. Leukorrhea may be the only presenting sign in women infected with these pathogens (1-3).
  - **Physiologic leukorrhea** is caused by congestion of the vaginal mucosal membranes due to hormonal stimulation. This may occur during ovulation and pregnancy.
- Medical Diagnostic Laboratories, L.L.C. (MDL), is currently the only clinical laboratory in the United States that offers this panel of testing which not only detects these pathogens but also provides reflex antibiotic resistance testing at no additional charge.

Test 121 Leukorrhea Panel (*Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis*) by Real-Time PCR (Reflex to antimicrobial resistance Profile)

Includes:

- *Chlamydia trachomatis*
  - Azithromycin
  - Ciprofloxacin
  - Ceftriaxone
  - Spectinomycin
  - Cefuroxime
- *Neisseria gonorrhoeae*
  - Azithromycin
  - Ciprofloxacin
  - Ceftriaxone
  - Spectinomycin
  - Cefuroxime
- *Trichomonas vaginalis*
  - Metronidazole

- Test 105: *Chlamydia trachomatis* by Real-Time PCR (Reflex to azithromycin resistance by Pyrosequencing). This assay detects a single nucleotide polymorphism (SNP) associated with azithromycin resistance utilizing pyrosequencing. This technology can accurately detect the presence or absence of this SNP, thus providing additional molecular evidence for resistance to azithromycin. This reflex test is performed at no additional charge on all OneSwab®, UroSwab® and ThinPrep® specimens that test positive for *C. trachomatis*.

- Test 167: *Neisseria gonorrhoeae* by Real-Time PCR (Reflex to antibiotic resistance by Molecular Analysis). This assay provides a simple method of determining gonorrhea infections and the assessment of *N. gonorrhoeae*-specific genetic markers of resistance to six classes of antibiotics. This assay does not involve the isolation of live bacterial cells from the specimen. Instead, it screens for *N. gonorrhoeae* specific genes and mutations from DNA extracted from the OneSwab®, UroSwab® and ThinPrep® collection systems. This reflex test is performed at no additional charge on all OneSwab®, UroSwab® and ThinPrep® specimens that test positive for *N. gonorrhoeae*.

- Test 111: *Trichomonas vaginalis* by Real-Time PCR (Reflex to metronidazole resistance). 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. 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 Tvntr6 protein, and has 40% sensitivity, 96% specificity, and a 91% positive predictive value (PPV) for the detection of *T. vaginalis* metronidazole resistance. This reflex test is performed at no additional charge on all OneSwab®, UroSwab® and ThinPrep® specimens that test positive for *T. vaginalis*.

REFERENCES:


Upd: 3/2016
Chlamydia trachomatis
by Real-time PCR (Reflex to azithromycin resistance by Pyrosequencing)

“The sensitivity and specificity of the nucleic acid amplification tests (NAATs) are clearly the highest of any of the test platforms for the diagnosis of chlamydial and gonococcal infections. Since accurate diagnosis is the goal, there is no justification for the ongoing use of other technologies”(1, 2). - Centers for Disease Control and Prevention (CDC)

- MDL provides detection of C. trachomatis by Real-Time PCR, one of the most powerful and sensitive gene analysis techniques available.
- Sensitivity and specificity up to 99%.
- Test results are typically available within 24-48 hours.
- This test has been validated for detection of C. trachomatis using the OneSwab®, UroSwab® (males and females), and ThinPrep®.

Molecular Microbiology

- C. trachomatis has a genome that consists of 1,042,519 nucleotide base pairs and has approximately 894 likely protein coding sequences. C. trachomatis strains have an extrachromosomal plasmid, which was sequenced to be a 7,493 base pair plasmid (4). There are 15 distinct serovariants. Serovariants A-C are associated with Trachoma, D-K with oculo-urogenital disease, and L1-3 with LGV.
- Human C. trachomatis isolates are highly conserved with one another, having a reported 1% variation in their nucleotide sequences.
- In 2006, a spontaneous variant of the cryptic plasmid was discovered in Sweden in the serovar E, designated nVCT (6). The change consisted of a 377 base pair deletion within the coding sequence of CDS 1.
- The target DNA of the MDL Real-Time PCR for C. trachomatis Assay is the ORF8 of the cryptic plasmid pLGV440 which is found in all 15 serovariants (Accession numbers: DQ06813 to DQ63827; GI: 73544092 to 7354418). The MDL assay is capable of identifying all 15 serovariants, including the recently discovered Swedish variant, nVCT (based upon an analysis of the published genomic information).
- Azithromycin resistance- A single nucleotide polymorphism (SNP) was identified in domain V of the 23s rRNA of C. trachomatis consistently associated with resistance to azithromycin. This substitution of thymine to guanine (T → G) occurs at the position 2611 (T2611G) (12,13). MDL has developed a test to detect this SNP utilizing pyrosequencing which can accurately discover the presence or absence of this SNP, thus providing additional molecular evidence for resistance to azithromycin. This is provided as a reflex test at no additional charge. Currently, MDL is the only medical laboratory in the United States offering this service.

Epidemiology

- Urogenital infections with C. trachomatis are amongst the most common sexually transmitted reportable diseases in the United States and the world. In women, the most serious complications are Pelvic Inflammatory Disease (PID), ectopic pregnancy, and infertility (2). In the United States, 1,244,180 cases of C. trachomatis urogenital infection were reported to the CDC in 2009 (3). However, many infections are not detected, and an estimated 2.8 million infections occur in the United States annually (3).

- Annual screening of all sexually active women aged ≤25 years is recommended, as is screening of older women with risk factors (2).
- All pregnant women should be routinely screened for C. trachomatis during their first prenatal visit. Women aged ≤25 years and those at increased risk for chlamydia (e.g., women who have a new or more than one sex partner) should also be retested during the third trimester to prevent maternal postnatal complications and chlamydial infection in the infant. Women found to have a chlamydial infection during the first trimester should be retested within approximately 3–6 months, preferably in the third trimester.
- The screening of sexually active young men should also be considered in clinical settings with a high prevalence of C. trachomatis, such as adolescent clinics, correctional facilities, and STD clinics (2).
- The World Health Organization has reported that infections with C. trachomatis are responsible for about 3.6% of cases of blindness in the world (3).

Clinical Significance

- C. trachomatis is transmitted through infected secretions only. It infects mainly mucosal membranes, such as the cervix, rectum, urethra, throat, and conjunctiva. It is primarily spread via sexual contact and manifests as a sexually transmitted disease. Symptoms and physical findings are usually nonspecific.
- Up to 50% of men with chlamydial urethral infections, and up to 75% of women with cervicitis, are asymptomatic. The history may be crucial for the risk assessment of exposure. However, a number of clinical syndromes require further evaluation for C. trachomatis infection.
- Definitive diagnosis of C. trachomatis infection for all conditions is obtained with nucleic acid amplification tests.
- Persons who are diagnosed with C. trachomatis infection should be tested for other STDs, including Neisseria gonorrhoeae.

Table 1: Summary of Clinical Manifestations.

<table>
<thead>
<tr>
<th>Clinical condition</th>
<th>Signs and Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
</tr>
<tr>
<td>Cervicitis</td>
<td>75% asymptomatic, mucopurulent discharge, bleeding</td>
</tr>
<tr>
<td>Salpingitis (PID)</td>
<td>Adnexal, lower abdominal pain on direct palpation and cervical motion tenderness.</td>
</tr>
<tr>
<td>Urethritis (Urethral Syndrome)</td>
<td>Dysuria, urgency, frequency, pyuria, no hematuria, Reiter’s syndrome.</td>
</tr>
<tr>
<td>Nongonococcal Urethritis (NGU)</td>
<td>Same as NGU.</td>
</tr>
<tr>
<td>Men</td>
<td></td>
</tr>
<tr>
<td>Postgonococcal Urethritis (PGU)</td>
<td>Same as NGU.</td>
</tr>
<tr>
<td>Epididymitis Orchitis</td>
<td>Pain tenderness, swelling, fever presence of NGU.</td>
</tr>
<tr>
<td>Proctitis</td>
<td>Rectal pain, bleeding, discharge.</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>Ocular pain, redness, discharge in association with urogenital C. trachomatis infection.</td>
</tr>
<tr>
<td>Neonates</td>
<td></td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>Consider in all neonates with conjunctivitis aged ≤ 30 days, especially if the mother has a history of untreated C. trachomatis infection.</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>Staccato cough, lung hyperinflation, eosinophilia.</td>
</tr>
</tbody>
</table>
Table 2. Comparison of Multiple Assay Systems for the Detection of Chlamydia trachomatis.

<table>
<thead>
<tr>
<th>Test</th>
<th>N †</th>
<th>Prevalence (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>1000</td>
<td>12.9</td>
<td>98</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>(14)</td>
</tr>
<tr>
<td><strong>AmpliCaur</strong></td>
<td>2254</td>
<td>7.5 *</td>
<td>96.9</td>
<td>98.6</td>
<td>84.9 *</td>
<td>99.7 *</td>
<td>(15)</td>
</tr>
<tr>
<td><strong>Aptima Combo 2</strong></td>
<td>1389</td>
<td>15.0</td>
<td>94.2</td>
<td>97.6</td>
<td>87.4</td>
<td>99.0</td>
<td>(16)</td>
</tr>
<tr>
<td><strong>BD Probe Tec</strong></td>
<td>1419</td>
<td>9.9</td>
<td>98.7</td>
<td>97.8</td>
<td>84.8</td>
<td>99.1</td>
<td>(17)</td>
</tr>
<tr>
<td><strong>GEN-PROBE (Pace 2)</strong></td>
<td>940</td>
<td>3.9</td>
<td>75.5</td>
<td>97.0</td>
<td>50.5</td>
<td>99.0</td>
<td>(18)</td>
</tr>
</tbody>
</table>

† = Unless otherwise noted, all specimens are swabs
§ = Calculated data

Treatment

Table 3. Current Recommendations from the CDC for Uncomplicated C. trachomatis Infection of the Genito-Urinary Tract (19).

**Recommended Regimens—Adults & Adolescents**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>1 g orally in a single dose</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>* 100 mg orally twice a day for 7 days</td>
</tr>
</tbody>
</table>

**Alternative Regimens**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin base</td>
<td>b 500 mg orally four times a day for 7 days</td>
</tr>
<tr>
<td>Erythromycin ethylsuccinate</td>
<td>a 800 mg orally four times a day for 7 days</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>a 500 mg orally once daily for 7 days</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>a 300 mg orally twice a day for 7 days</td>
</tr>
</tbody>
</table>

a Should not be administered during pregnancy, lactation, or to children <8 years of age.
b If patient cannot tolerate high-dose erythromycin base schedules, change to 250 mg 4x/day for 14 days.
c If patient cannot tolerate high-dose erythromycin ethylsuccinate schedules, change to 400 mg orally 4 times a day for 14 days.
d Contraindicated for pregnant or lactating women.
e Clinical experience and published studies suggest that azithromycin is safe and effective.
f Erythromycin estolate is contraindicated during pregnancy.

Table 4. Current Recommendations from the CDC for C. trachomatis Infection in pregnant women (2).

**Recommended Regimens**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>1 g orally in a single dose</td>
</tr>
</tbody>
</table>

**Alternative Regimens**

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>500 mg orally three times a day for 7 days</td>
</tr>
<tr>
<td>Erythromycin base</td>
<td>b 500 mg orally four times a day for 7 days</td>
</tr>
<tr>
<td>Erythromycin base</td>
<td>c 250 mg orally four times a day for 14 days</td>
</tr>
<tr>
<td>Erythromycin ethylsuccinate</td>
<td>d 800 mg orally four times a day for 7 days</td>
</tr>
<tr>
<td>Erythromycin ethylsuccinate</td>
<td>e 400 mg orally four times a day for 14 days</td>
</tr>
</tbody>
</table>

a Should not be administered during pregnancy, lactation, or to children <8 years of age.
b If patient cannot tolerate high-dose erythromycin base schedules, change to 250 mg 4x/day for 14 days.
c If patient cannot tolerate high-dose erythromycin ethylsuccinate schedules, change to 400 mg orally 4 times a day for 14 days.
d Contraindicated for pregnant or lactating women.
e Clinical experience and published studies suggest that azithromycin is safe and effective.

References:

Complications that can occur during pregnancy include: amniotic

Due to the fact that gonorrhea can have serious consequences

Clinical manifestation in men usually includes symptomatic

Gonorrhea is the second most commonly reported bacterial

Epidemiology

• Gonorrhea is the second most commonly reported bacterial STD in the United States with an estimated 700,000 new N. gonorrhoeae infections occurring each year (7).

• Due to the fact that gonococcal infections among women are frequently asymptomatic, targeted screening of young women at increased risk for infection is a primary component of gonorrhea control in the United States (7).

Pathogenesis

• Neisseria gonorrhoeae, a Gram-negative diplococci, is the causative agent of gonorrhea.

• Due to its affinity for columnar or pseudo stratified epithelium, it is most commonly detected in the genital tract with the primary site of involvement being the endocervical canal and transition zone of the cervix.

• N. gonorrhoeae’s unique ability to alter surface structures allows increased pathogenicity, facilitates epithelial surface attachment, and enables evasion of the host’s immune response.

• Transmission of N. gonorrhoeae occurs almost exclusively through sexual contact, though it can also be transmitted via the passage of a neonate through an infected mother’s birth canal or via autoinoculation from the hands of an infected person to their eye.

• Incubation time for this infection is typically 3-5 days and transmission more frequently occurs from male to female.

• Some risks factors for infection include: low socioeconomic status, early onset of sexual activity, unmarried status, a history of previous gonorrhea infection, illicit drug abuse, and prostitution.

Clinical Significance

• Clinical manifestation in men usually includes symptomatic urethritis; however, pharyngeal, anorectal, and disseminated infections are also possible.

• In women, infections are often asymptomatic; however, when manifested, symptoms may include: vaginal discharge, dysuria, intermenstrual bleeding, menorrhagia, pelvic discomfort, infection of the perirectal glands, Bartholin glands, and anorectum.

• Due to the fact that gonorrhea can have serious consequences for both mother and neonate, it is crucial to screen pregnant women for infection who reportedly have an incidence of gonorrhea during pregnancy as high as 10%.

• Complications that can occur during pregnancy include: amniotic infection syndrome, premature rupture of the membranes, chorioamnionitis, premature birth, intrauterine growth retardation, neonatal sepsis, and postpartum endometritis.

• During vaginal delivery with an infected mother, 30% to 35% of neonates will acquire Neisseria gonorrhoeae which, if left untreated, can progress to corneal ulceration and scarring, as well as blindness called gonorrheal ophthalmia neonatorum.

Laboratory Diagnosis

• Diagnosis of infections with N. gonorrhoeae has traditionally relied upon Gram stain, culture, and immunochemical techniques. Although culture techniques may be highly specific, sensitivity is greatly impacted by the adequacy of the clinical specimen and transport conditions, particularly when transporting to off-site facilities.

• Due to the genome plasticity of N. gonorrhoeae strains circulating in the population, this bacterium has developed resistance to multiple classes of antimicrobial agents, resulting in decreased efficacy for gonorrhea therapy. An increase of ceftriaxone-resistant N. gonorrhoeae demonstrated a similar pattern to previous reports in Japan and Southeast Asia that prompted the CDC to remove ciprofloxacin from the treatment guidelines as a primary antibiotic (1-3).

• In August 2012, the CDC called for Ceph-R NG surveillance through N. gonorrhoeae antibiotic susceptibility testing for patients that have failed treatment (3). Although susceptibility testing by culture remains the standard for antibiotic susceptibility determination in clinical microbiology, there are inherent growth-related issues that can delay results by as much as three days or more.

• Known mechanisms of antibiotic resistance in N. gonorrhoeae are linked to mutations in the chromosomal DNA as well as the presence of plasmid-borne genes. Surveillance of genetic markers of antibiotic resistance is important for the prediction of clinical resistance as the antibiotic susceptibility signatures of individual N. gonorrhoeae strains differ.

![Diagram](attachment:neisseria_gonorrhoeae.png)

**Figure 1:** Gonococcal isolate surveillance project (GISP)-penicillin, tetracycline, and ciprofloxacin resistance among GISP isolates, 2010 (7).

**Test 167 Neisseria gonorrhoeae by Real-Time PCR (Reflex to antibiotic resistance by Molecular Analysis)**

Development of MDL's Reflex test for gonorrhea and Neisseria gonorrhoeae (Reflex to antibiotic resistance by Molecular Analysis) allows for rapid and accurate detection of antibiotic resistance in clinical specimens. The test utilizes Real-Time PCR technology to detect N. gonorrhoeae directly from clinical specimens and provides a rapid report of antibiotic resistance patterns.

**Key Points:**

- The test is highly sensitive and specific, with a detection rate of 99%.
- Results are available within 24-48 hours.
- Antibiotic resistance patterns are determined using molecular analysis.
- The test is suitable for use in both diagnostic and research settings.

**Technical Details:**

- **Sample Type:** Clinically submitted whole blood, plasma, urine, and other body fluids.
- **Test Methodology:** Real-Time PCR using SYBR green technology.
- **Antibiotic Resistance Testing:** Includes detection of penicillin-resistant, tetracycline-resistant, and quinolone-resistant N. gonorrhoeae strains.
- **Data Interpretation:** Results are reported in a standardized format, allowing for easy comparison with previous isolates.

**Advantages:**

- Rapid detection of N. gonorrhoeae and antibiotic resistance.
- Increased patient throughput and reduced turnaround time.
- Reduced costs associated with delayed treatment.

**Limitations:**

- The test is not suitable for the detection of gonococcal infection in neonates.
- False-negative results may occur in cases of low bacterial load.

**Conclusion:**

The Reflex test for gonorrhea and Neisseria gonorrhoeae provides a valuable diagnostic tool for the reliable detection of antibiotic resistance in clinical specimens. Its implementation in clinical laboratories can significantly improve patient outcomes by facilitating prompt and appropriate antibiotic therapy.
Table 1: Comparison of Multiple Assay Systems for the Detection of Neisseria gonorrhoeae.

<table>
<thead>
<tr>
<th>Test</th>
<th>N</th>
<th>Prevalence (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>100</td>
<td>7.8</td>
<td>100</td>
<td>99.4</td>
<td>93.4</td>
<td>100</td>
<td>(16)</td>
</tr>
<tr>
<td>Amplicor</td>
<td>2238</td>
<td>5.2 *</td>
<td>96.3</td>
<td>98.7</td>
<td>80.2 *</td>
<td>99.8 *</td>
<td>(17)</td>
</tr>
<tr>
<td>Aptima Combo 2</td>
<td>1479</td>
<td>8.6</td>
<td>99.2</td>
<td>98.7</td>
<td>88.1</td>
<td>99.9</td>
<td>(18)</td>
</tr>
<tr>
<td>BD Probe Tec</td>
<td>1411</td>
<td>8.1</td>
<td>97.2</td>
<td>99.4</td>
<td>91.6</td>
<td>99.6</td>
<td>(19)</td>
</tr>
<tr>
<td>GEN-PROBE (Pace 2)</td>
<td>1750</td>
<td>8.7</td>
<td>97.1</td>
<td>99.1</td>
<td>90.6</td>
<td>99.8</td>
<td>(20)</td>
</tr>
<tr>
<td>Culture</td>
<td>866</td>
<td>4.5</td>
<td>50.0</td>
<td>97.1</td>
<td>40.0</td>
<td>98.0</td>
<td>(21)</td>
</tr>
</tbody>
</table>

† = Unless otherwise noted, all specimens are swabs.  ♦ = Calculated data.

Screening

Table 2: Summary of screening for N. gonorrhoeae infection by nucleic acid amplification testing (NAAT) (derived from 7).

**Women**
- Annual routine screening for all sexually active women at risk for infection.
- Screening at the first prenatal visit for all pregnant women at risk or living in a high prevalence area.
- In women with cervicitis via either vaginal, cervical, or urine samples.

**Men who have sex with men (MSM)**
- Screening for urethral infection via nucleic acid amplification testing (NAAT) of urine in all men who have had insertive intercourse the preceding year regardless of condom use.
- Screening for rectal infection via nucleic acid amplification testing (NAAT) of a rectal swab in all men who have had receptive anal intercourse during the preceding year.
- Screening for pharyngeal infection via nucleic acid amplification testing (NAAT) in all men who have had receptive oral intercourse during the preceding year.

**Both Men and Women**
- Newly diagnosed HIV infection.

Treatment

Table 3: Current Recommendations from the CDC for adults, adolescents & children > 45 kg: uncomplicated infection of the cervix, urethra and rectum (16).

**Recommended Regimens**
- Ceftriaxone 250 mg IM in a single dose **PLUS**
- Azithromycin * 1 g orally in a single dose

**Alternative Regimens: If ceftriaxone is not available**
- Cefixime 400 mg orally in a single dose **PLUS**
- Azithromycin * 1 g orally in a single dose

**Alternative Regimens: If cephalosporin allergy**
- Gemifloxacin 320 mg orally in a single dose **PLUS**
- Azithromycin 2 g orally in a single dose **OR**
- Gentamicin 240 mg IM single dose **PLUS**
- Azithromycin 2 g orally in a single dose **PLUS**

* Clinical experience and published studies suggest that azithromycin is safe and effective.

- Due to the concerns for developing patterns of antimicrobial resistance, most current recommendations for treatment should be followed. Guidance can be obtained from the CDC website (http://www.cdc.gov/std/gisp) and state and local health departments.
- If treatment is still unsuccessful, contact the CDC for a consultation.

References:

22. CDC. 2015. Sexually Transmitted Diseases, Treatment Guidelines, 2015. MMWR 64.72-75.
**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 ulcers 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 Tvntr6 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 persistant or recurrent **T. vaginalis** Infection (15).**

<table>
<thead>
<tr>
<th>Recommended Regimens</th>
</tr>
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<tbody>
<tr>
<td>Metronidazole * 2 g orally in a single dose OR</td>
</tr>
<tr>
<td>Tinidazole ** 2 g orally in a single dose</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alternative Regimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole * 500 mg orally twice a day for 7 days</td>
</tr>
</tbody>
</table>

**If This Regimen Fails**

| Metronidazole 2g orally twice a day for 7 days OR |
| Tinidazole 2 g orally for 7 days |

* Pregnant patients can be treated with 2 g single dose.

** Recommended Patients:**

- *Randomized controlled trials comparing single 2 g doses of metronidazole and tinidazole suggest that tinidazole is equivalent to, or superior to, metronidazole in achieving a 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).


Frequently Asked Questions (FAQ)

• What does a positive result mean for the detection of the Tvntr6 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 Tvntr6 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.

References


MDL-developed molecular assays undergo a rigorous validation process to assure tests of the highest quality. We believe that using molecular methods such as PCR to both detect the pathogen and identify mutations and genes strongly associated with antibiotic resistance in difficult-to-culture organisms such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Trichomonas vaginalis* is important. Additionally, our reflex antibiotic susceptibility genotyping assays provide confirmation of the initial positive PCR result. This tailored medicine molecular approach of pathogen detection and reflex antibiotic susceptibility genotyping provides clinicians with additional information in addition to a significant decrease in turn-around-time compared to culture-based antibiotic susceptibility testing, so that they can make their decision regarding the best course of treatment in a timely manner.

**Test 105: Chlamydia trachomatis by Real-Time PCR (Reflex to azithromycin resistance by Pyrosequencing)**

- The direct and indirect costs of treatment of the more than 3 million infections with *Chlamydia trachomatis* per year, have stretched into billions of dollars annually (1).
- The incidence of azithromycin treatment failures have been on the rise and the current prevalence rate is >5% in all patients, about 8% in women, and 23% in male non-gonococcal urethritis infections (1).
- MDL has developed an assay which:
  - Can identify a A2058C mutation within the 23S ribosomal RNA sequence through molecular sequencing that has specifically been associated with azithromycin resistance in *Chlamydia trachomatis* (2,3).
  - Is performed at no additional charge on all positive Chlamydia trachomatis tests ordered on the OneSwab®, UroSwab® and ThinPrep® platforms.
  - Confirms the initial Chlamydia trachomatis positive result.

**Test 167: Neisseria gonorrhoeae by Real-Time PCR (Reflex to antibiotic resistance by Molecular Analysis)**

- An increase of ceftriaxone-resistant *N. gonorrhoeae* (Ceph-R NG) demonstrated a similar pattern to previous reports in Japan and Southeast Asia that prompted the CDC to remove ciprofloxacin from the treatment guidelines as a primary antibiotic (1-3).
- In August 2012, the CDC called for Ceph-R NG surveillance through *N. gonorrhoeae* antibiotic susceptibility testing for patients that have failed treatment (3).
- MDL developed an assay which:
  - Examines 31 genetic markers strongly associated with antibiotic resistance in the *N. gonorrhoeae* chromosomal and plasmid genes.
  - Provides antibiotic susceptibility information for six antibiotics which can be used for Ceph-R NG infections including: cefixime, penicillin, ciprofloxacin, tetracycline, azithromycin, and spectinomycin.
  - Does not require the bacterium to be viable.
  - Includes genetic markers associated with ceftriaxone non-susceptibility (5).
  - Is performed at no additional charge on all positive *N. gonorrhoeae* tests ordered on the OneSwab®, UroSwab® and ThinPrep® platforms.
  - Will greatly improve the clinician’s ability to more easily comply with this CDC Public Health Response Plan.
  - Provide physicians with the ability to identify effective treatment alternatives (4-7).
  - Has been submitted as a patent application to the United States Patent & Trademark Office.
  - Is described in an article that has been published in a high-impact peer-reviewed journal (8).

**Test 111: Trichomonas vaginalis by Real Time PCR (Reflex to metronidazole resistance)**

- Although metronidazole treatment is thought to be 90% to 95% effective, recent reports suggest that between 2.4% and 9.6% of *Trichomonas vaginalis* isolates exhibit metronidazole resistance (1-5).
- 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 (1).
- MDL has developed an assay which:
  - Was developed in conjunction with the CDC.
  - Can identify *T. vaginalis* resistance to metronidazole.
  - Is performed at no additional charge on all positive *T. vaginalis* tests ordered on the OneSwab®, UroSwab® and ThinPrep® platforms.
  - Confirms the initial *T. vaginalis* positive result.
  - Assists clinicians in administering an effective diagnosis and treatment to their patients and is especially useful for patients that present with recurring Trichomoniass.
  - Has been submitted as a patent application to the United States Patent & Trademark Office.
  - Is described in an article that has been published in a high-impact peer-reviewed journal (6).

References: