Chlamydia trachomatis
by Real-Time PCR

“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 typically available within 24-48 hours.
- This test has been validated for detection of C. trachomatis using the OneSwab®, UroSwab®, ThinPrep®, and Endocell™ systems.

Laboratory Diagnosis

- Laboratory diagnosis of C. trachomatis infection is crucial for definitive diagnosis. This is due to the relatively poor sensitivity and specificity of other methods, including Gram-staining, Giemsa staining for inclusion bodies, and serology. The superiority of nucleic acid amplification tests such as Real-Time PCR has become the current standard-of-care.

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 fifteen distinct serovariants. Serovariants A-C are associated with Trachoma, D-K with urogenital disease, and L₁–L₅ 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 (6) in the serovar E, designated nvCT. 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 fifteen serovariants (Accession numbers: DQ06813 to DQ63827; GI: 73544092 to 7354418). The MDL assay is capable of identifying all fifteen 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 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) also should 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 restested 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).
- Lymphogranuloma Venereum (LGV) is caused by C. trachomatis serotypes L₁–L₅. MDL provides a specific real-time PCR test for this pathogen. Please refer to Test 136, Lymphogranuloma Venereum (LGV) by Real-Time PCR.
- The World Health Organization has reported that infections with C. trachomatis are responsible for about 3.6% of blindness cases in the world. (3)

Pathogenesis

Chlamydia trachomatis is an obligate, aerobic, intracellular parasite of eukaryotic cells. In fact, humans are the only known natural host for C. trachomatis. The life cycle of Chlamydia trachomatis consists of two stages.

1. Elementary body – This is the dispersal form and is about 0.3 μm in diameter. Electron microscopy has demonstrated the presence of hexagonally organized surface projections arranged regularly with a center to center spacing of approximately 50 nm. C. trachomatis induces its own endocytosis upon exposure to target reproductive epithelial cells. It is this form that prevents phagocytosis, which then allows for intracellular survival of the bacteria. Upon entrance into the host cell, the chromosome
begins immediate chromosomal decondensation, and within 15 minutes transcriptional activity can be detected within the EB which rapidly differentiates into the metabolically-active reticulate body. Thus, the metabolically inactive elementary body is able to become the reticulate body.

2. Reticulate body – Within the host cell, the elementary body germinates into the reticulate body as a result of the glyogen that is produced within the host cell. It can be detected by light microscopy as an inclusion in the infected cell. The RB is about 1.0 µm in diameter and the cytoplasm appears granular with diffuse, fibrillar nucleic acids, in contrast with the highly condensed nucleic acid content of the EB. After division, the reticulate body transforms back to the elementary form and is released by the cell by exocytosis. One reticulate body usually produces 100-1,000 elementary bodies. The multiplying reticulate bodies then become elementary bodies again and burst out of the host cell to continue the infection cycle. Infection of the reproductive epithelial cells and macrophages results in the production of chemokines, cytokines and activation of innate immune receptors and Toll-like 2 receptors. (7)

Figure 1. Life Cycle of C. trachomatis in Human Urogenital Host Cells [Nature Reviews Immunology 5, 149-161 (February 2005)].

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 risk assessment of exposure. However, a number of clinical syndromes require further evaluation for C. trachomatis infection.

- Definitive diagnosis of C. trachomatis infections for all conditions is obtained with Nucleic acid amplification tests.

- Persons who are diagnosed with C. trachomatis infection should be tested for other STD’s, including Neisseria gonorrhoeae.

Table 1: Summary of Clinical Manifestations.

<table>
<thead>
<tr>
<th>Clinical condition</th>
<th>Signs and Symptoms</th>
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<tr>
<td>Cervicitis</td>
<td>75% asymptomatic, mucopurulent discharge, bleeding.</td>
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<tr>
<td>Salpingitis (PID)</td>
<td>Adnexal, lower abdominal pain on direct palpation and cervical motion tenderness.</td>
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<tr>
<td>Urethritis (Urethral Syndrome)</td>
<td>Dysuria, urgency, frequency, pyuria, no hematuria, Reiter’s syndrome.</td>
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<tr>
<td>Nongonococcal Urethritis (NGU)</td>
<td>Dysuria, urgency, frequency, pyuria, Reiter’s syndrome.</td>
</tr>
<tr>
<td>Postgonococcal Urethritis (PGU)</td>
<td>Same as NGU.</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>Pain tenderness, swelling, fever presence of NGU.</td>
</tr>
<tr>
<td>Proctitis</td>
<td>Rectal pain, bleeding, discharge.</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>Ocular pain, redness, discharge in association with urogenital C. trachomatis infection.</td>
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<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>
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<tr>
<td>Pneumonia</td>
<td>Staccato cough, lung hyperinflation, eosinophilia.</td>
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Table 2. Current Recommendations from the CDC for Uncomplicated C. trachomatis Infection of the Genito-Urinary Tract (2).

**Recommended Regimens**

- Azithromycin 1 g orally in a single dose
- Doxycycline 100 mg orally twice a day for 7 days

**Alternative Regimens**

- Erythromycin base 500 mg orally four times a day for 7 days
- Erythromycin ethylsuccinate 800 mg orally four times a day for 7 days
- Levofloxacin 500 mg orally once daily for 7 days (contraindicated in pregnant patients)
- Ofloxacin 300 mg orally twice a day for 7 days

References: