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 uroculo-urogenital disease, and Lp 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.

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

Table 1: Summary of Clinical Manifestations.

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).
### 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>Aptima Combo 2</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>BD Probe Tec</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>GEN-PROBE (Pace 2)</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>
</tbody>
</table>

* = Unless otherwise noted, all specimens are swabs
Ϯ = 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**

- **Azithromycin**: 1 g orally in a single dose OR
- **Doxycline**: 100 mg orally twice a day for 7 days

**Alternative Regimens**

- **Erythromycin**: base
  - 500 mg orally four times a day for 7 days OR
  - 800 mg orally four times a day for 7 days OR

- **Levofloxacin**: 500 mg orally once daily for 7 days OR

- **Ofloxacin**: 300 mg orally twice a day for 7 days

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**References:**