



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 L_{1,3} 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.

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



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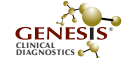


Table 2. Comparison of Multiple Assay Systems for the Detection of *Chlamydia trachomatis*.

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

† = 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

Azithromycin 1 g orally in a single dose **OR**

Doxycycline ^a 100 mg orally twice a day for 7 days

Alternative Regimens

Erythromycin base^b 500 mg orally four times a day for 7 days **OR**

Erythromycin ethylsuccinate^c 800 mg orally four times a day for 7 days **OR**

Levofloxacin^d 500 mg orally once daily for 7 days **OR**

Ofloxacin^e 300 mg orally twice a day for 7 days

^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.

* Complete treatment guidelines are available from the CDC <http://www.cdc.gov/std/tg2015/default.htm> (Accessed August 26, 2015).

* Indicates update from the 2010 CDC Guidelines for the Treatment of Sexually Transmitted Diseases.

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

Recommended Regimens

Azithromycin^f 1 g orally in a single dose

Alternative Regimens

★ **Amoxicillin** 500 mg orally three times a day for 7 days **OR**

Erythromycin base^{b,e} 500 mg orally four times a day for 7 days **OR**

Erythromycin base 250 mg orally four times a day for 14 days **OR**

Erythromycin ethylsuccinate 800 mg orally four times a day for 7 days **OR**

Erythromycin ethylsuccinate 400 mg orally four times a day for 14 days

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