

*Originalni članci/  
Original articles*

LABORATORY DIAGNOSIS OF CHLAMYDIA TRACHOMATIS INFECTIONS DETECTED BY DIRECT FLUORESCENT ANTIBODY AND POLYMERASE CHAIN REACTION

LABORATORIJSKA DIJAGNOSTIKA INFEKCIJA IZAZVANIH CHLAMYDIA TRACHOMATIS DETEKTOVANIH DIREKTNOM IMUNOFLUORESCENCIJOM I LANČANOM REAKCIJOM POLIMERAZE

**Correspondence to:**

**Mr sc. Dejana Savić**  
Institute for microbiology,  
Military Medical Academy,  
Belgrade, Serbia  
E-mail: dejsav@yubc.net

Dejana Savić<sup>1</sup>, Dragutin Jovanović<sup>1</sup>, Danijela Đurić Petković<sup>1</sup>, Ljiljana Radojčić<sup>1</sup>, Gora Miljanović<sup>2</sup>, Elizabeta Ristanović<sup>1</sup>, Zorica Lepšanić<sup>1</sup>

<sup>1</sup> Military Medical Academy, Belgrade, Serbia

<sup>2</sup> Medical college of vocational studies, Belgrade, Serbia

*Abstract*

*Key words*

Chlamydia trachomatis infections; polymerase chain reaction; direct fluorescent antibody; endocervical swab

*Ključne reči*

Chlamydia trachomatis, infekcije; lančana reakcija polimeraze; direktna imunofluorescencija; cervikalni bris

*Chlamydia trachomatis* is the commonest bacterial sexually cause of transmitted disease worldwide. The majority (at last 85 percent) of women infected at the cervix have neither sign nor symptoms, which is the rationale for routine annual screening of young sexually active women. Early correct diagnosis of infection with *C. trachomatis* is essential to prevent long-term sequelae associated with prolonged infection. Nonculture assays, such as direct florescent antibody staining of direct patient material and enzyme immunoassays, have been replaced by molecular tests called nucleic acid amplification tests (NAATs), which are currently the tests of choice. **Aim:** The present study was carried out to diagnose genital *Chlamydia trachomatis* infection among outpatient women of reproductive age, who took a routine gynecological examination in Military Medical Academy, by use direct fluorescent antibody (DFA) and polymerase chain reaction (PCR). **Specimens:** This study was performed at the Military Medical Academy, in the Department of Gynecology and Microbiology laboratory. Study materials were consisted of 109 samples of young sexually active women on gynecological examination, during August and September 2010. Specimens were endocervical swabs. Results: *C. trachomatis* was detected in 9 out of 109 samples with DFA, 5 were doubtful and rest of them were negative. PCR for *C. trachomatis* detected 7 positive and in 18 samples PCR inhibition was detected. On comparing the level of DFA positives with PCR results, we fortified that no one sample which was positive in DFA, was positive in PCR, and inverse. Two of 18 samples that had PCR inhibition, were DFA positive, but 1 of 5 doubtful DFA was PCR positive. **Conclusion:** Without regard on high percent of positive samples by DFA and high percent of inhibition by PCR, PCR results were more reliable.

*INTRODUCTION*

*Chlamydia trachomatis* is the commonest bacterial sexually cause of transmitted disease worldwide [1]. The majorities of affected persons are asymptomatic, especially in the early stages of the disease, and thus provide an ongoing reservoir for infection and symptoms which can vary among

individuals. However, serious permanent damage to the reproductive tissues and infertility can occur even in the absence of symptoms.

Symptoms generally begin to occur one to three weeks after exposure. In women, the cervix is the most commonly infected anatomic site [2], and a proportion of women may also have infection of the urethra. The majority (at last 85

percent) of women infected at the cervix have neither sign nor symptoms, which is the rationale for routine annual screening of young sexually active women [3]. When symptoms do occur, they are highly nonspecific, and can easily be confused with vaginitis or endometrial pathology. Undiagnosed infections often present as pelvic inflammatory disease, leading to ectopic pregnancy infertility or other adverse health outcomes in women [4]. For these reasons, early correct diagnosis of infection with *C. trachomatis* is essential to prevent long-term sequelae associated with prolonged infection.

Cell culture have long been considered the gold standard for diagnosis of *C. trachomatis* infections because of its absolute specificity [5]. However, due to its labor-intensive methodology, turnaround time, cost, and requirements for infrastructure and technical expertise, cell culture facilities were limited to specialized research laboratories only [6]. Nonculture assays, such as direct fluorescent antibody staining of direct patient material and enzyme immunoassays, have been replaced by molecular tests called nucleic acid amplification tests (NAATs) [7], which are currently the tests of choice [8]. Infections detected by NAATs may be up to 80% higher than those found by the use of older technology [8]. Several NAATs are available commercially (and one of these: Cobas Amlicor, Roche Molecular Diagnostics) [6, 9, 10, 11]. This methods have been found to have excellent sensitivity for detection of *C. trachomatis*, usually well above 90%, in genital specimens and urine specimens from adult man and women, while maintaining high specificity of 100% [7]. Such tests could eliminate the need for laboratory facilities and could be used in community settings [12]. Highly sensitive and specific NAATs are now the primary tests used to screen for *C. trachomatis* infections in younger age (in persons  $\leq 25$  years of age and older women with risk factors) [13, 14, 15].

The present study was carried out to diagnose genital *C. trachomatis* infection among women of reproductive age, attending Military Medical Academy, by use of direct fluorescent antibody (DFA) and polymerase chain reaction (PCR).

## MATERIALS AND METHODS

**Specimens:** This study was performed at the Military Medical Academy, in the Department of Gynecology and Microbiology laboratory. Study materials were consisted of 109 samples of young sexually active women on gynecological examination, during August and September 2010. Specimens were endocervical swabs, which were tested by both fluorescent antibody (DFA) and polymerase chain reaction (PCR) to diagnose genital *Chlamydia trachomatis* infections. Endocervical sampling was performed according to recommendations of Sood et al. [16].

**DFA (Direct Fluorescent Antibody):** For DFA slides were made on a clean glass and processed subsequently as per manufacturer's instructions. Slides were read using a fluorescent microscope (Leica)-a 40X objective was used for screening, and 100X objective was used for confirmation of morphology. Slides were examined for apple-green colored

elementary bodies contrasted against the reddish-brown background of the counterstained cells. The presence of  $>10$  such structures in a slide were taken to be positive. If elementary bodies were  $<10$  in number per slide, it was taken as doubtful positive while when no such structures were seen, the sample was considered negative [16].

**PCR for *Chlamydia trachomatis*:** For PCR, endocervical swabs were forwarded to the microbiological laboratory in a special chlamydial transport medium and they were tested by using COBAS AMPLICOR test. Specimens were prepared for PCR according to the manufacturer's instructions. PCR amplification and detection were performed by using COBAS AMPLICOR test [6, 9, 10, 11].

## RESULTS

There were 109 endocervical samples of women in the age of 16 to 40, enrolled in this study. By DFA, *C. trachomatis* was detected in 9 cases, another 5 were doubtful and rest of them were negative. PCR for *C. trachomatis* detected 7 positive and in 18 samples PCR inhibition was detected (Table 1).

On comparing the level of DFA positives with PCR results, we fortified that no one sample which was positive in DFA was positive in PCR, and inverse. There was no match. Two of 18 samples with PCR inhibition, were DFA positive. One of 5 doubtful DFA was PCR positive.

**Table 1.** Detection of *Chlamydia trachomatis* infections by DFA and PCR methods

Method	Positive (%)	DFA $\pm$ and PCR inhibition (%)	Negative (%)	Total
DFA	9 (8.3)	5 (4.6)	95 (87.1)	109
PCR	7 (6.4)	18 (16.5)	84 (77.1)	109

## DISCUSSION

Any sexually active individual with sign and symptoms consistent with the clinical syndromes associated with chlamydia should undergo diagnostic testing for *C. trachomatis*. About 80% of urogenital infections are usually asymptomatic. But in these females with symptoms, cervicitis, urethritis and salpingitis are the most frequent. Up to 40% of women with undiagnosed chlamydia develop pelvic inflammatory diseases and about 20% of these women become infertile.

There are several laboratory tests for diagnosis of *C. trachomatis* but the sensitivity of the tests depend on the nature of the disease, the site of specimen collection and the quality of the specimen. Since chlamydia is intracellular parasite, swabs of the involved sites rather than exudate must be submitted for analysis.

Tissue culture was long the gold standard for diagnosis of *C. trachomatis* [17, 18]. Many studies have indicated that culture sensitivity compared to molecular techniques can range from 50% to 100%, and is usually considered to average 85%, while specificity is considered to be 100% [5, 6]. But this method is too expensive, has low sensitivity, is labo-

rious and requires an experienced microscopist [19]. This has initiated search for alternative diagnostic strategies, such as direct fluorescent antibody staining (DFA), enzyme immunoassays (EIA) and molecular tests called NAATs. The nonculture tests which detect chlamydial antigens in clinical specimens (DFA and EIA), have specificities from 96% to 99% and sensitivity from 80% to 85%. DFA is relatively rapid (about 30 min) and do not require refrigeration of specimens during transport. However, these tests with high specificity yield a large number of false positives in population with a low disease prevalence [20]. This problem can be explained with bad made slides and unexperienced microscopists.

Many studies have approved that PCR is highly sensitive (89%-90%) and specific (100%) for detection of *C. trachomatis* in clinical specimens, compared to tissue culture, DFA and EIA [10, 16, 19, 21, 22]. In contrast, several recent studies, demonstrated that PCR has less or similar sensitivity as culture or antigen detection assays [23, 24, 25]. But without the difference, today PCR is considered for new „gold standard“ in diagnostic of *C. trachomatis*. Because, PCR test are designed to measure *C. trachomatis* DNA, while cell culture is designed to measure the presence of current *C. trachomatis* infection [26].

In this study, 9 out of 109 (8.26%) samples were DFA positive while 5 out 109 ( 4.59 %) samples were doubtful DFA positive. The remaining 95 samples (87.16%) were negative for *C. trachomatis*. In similar studies Sood and coauthors [16] detected 11 out of 97 (11.34%) samples DFA positive, and Agrawal at al [27] detected 36.6 % in symptomatic male patients by the same method.

Using PCR, 7 out of 109 (6.42%) samples were positive in our study, while 18 (16.51%) have inhibition of PCR. This high percent of inhibition can be explained with a presence of secretions and discharges that takes to reduction of amplification [28, 29, 30, 19]. Using swabs are less likely to induce bleeding, which in itself may also inhibit culture.

This study also compares the performances of an commercial PCR with reference to DFA and found that no one sample which was positive in DFA, was positive in PCR. This apparent discrepancy can be explained by the differences in criteria of microscopists [31, 32]. Two of 18 samples with PCR inhibition were DFA positive, which suggests that samples with PCR inhibition must be retested with properly new swabs without traces of secretions and blood. One sample out of 5 doubtful DFA was PCR positive. This result shows that the sensitivity of PCR is greater than that of DFA, that is in agreement with many studies [10, 16, 19].

All 7 PCR positive samples were DFA negative, and they must be considered as false negative because of high specificity (100%) of PCR. This results indicated that sensitivity of DFA in our study was not satisfactory.

Last, without regard on high percent of positive samples by DFA and high percent of inhibition by PCR, commercial PCR assays have become the test of choice for diagnosis of *C. trachomatis*, because they have internal control to monitor for amplification inhibitors, and Roche's Amplicor has one that is optional [33]. Furthermore, it was confirmed that detection of elementary bodies implying the presence of active, symptomatic *C. trachomatis* infection, but it can't be used like screening test for early detection of chlamydia infections, which PCR can.

### Sažetak

*Chlamydia trachomatis* je jedna od najčešćih uzročnika seksualno prenosivih bolesti u svetu. Veliki procenat (oko 85%) infekcija cerviksa kod žena je bez simptoma, što je uvrđeno u toku godišnjeg ginekološkog pregleda mladih, seksualno aktivnih žena. Rana dijagnostika infekcija izazvanih *C. trachomatis* je od izrazitog značaja u prevenciji nastajanja oštećenja nakon dugotrajne infekcije. Direktna imunofluorescencija (DIF), ELIS-a, kao i kultura tkiva, zamenjene su molekularnim metodama (PCR), koje su postale test izbora za detekciju hlamidija. **Cilj** ovog rada bio je dijagnostika infekcija izazvanih *C. trachomatis*, kod žena u reproduktivnom dobu, u Vojnomedicinskoj akademiji, upotrebom DFA i PCR-a. Testirano je 109 cervikalnih briseva uzetih u toku avgusta i septembra 2010. godine, primenom obe metode. *C. trachomatis* je detektovana DFA-om u 9 uzoraka, 5 je imalo graničnu vrednost, a ostali uzorci su bili negativni. PCR-om je detektovana *C. trachomatis* u 7 uzoraka, u 18 je došlo do inhibicije PCR. Upoređujući pozitivne rezultate kod obe metode, utvrdili smo da nijedan rezultat koji je bio pozitivan DFA-om, nije bio pozitivan PCR-om i obrnuto. Dva od 18 uzoraka sa inhibicijom PCR su bila pozitivna DFA-om, a jedan od pet sa graničnom vrednošću DFA-om je bio PCR pozitivan. Bez obzira na visok procenat pozitivnih uzoraka DFA-om i visok procenat inhibicije PCR, PCR rezultati su bili pouzdaniji.

## REFERENCES

1. Schachter J, Hook EW, Martin DH, Willis D, Fine P, Fuller D, et al. Confirming positive results of nucleic acid amplification test for Chlamydia trachomatis: All NAATs are not created equal. *J Clin Microbiol.* 2005;43: 1372-3.
2. Stamm WE. Chlamydia trachomatis infections of the adult. In *Sexually Transmitted Diseases*, 4th Ed. Holmes KK, Sparling PF, Mardh PA, et al (Eds), McGraw-Hill, New York, 2008, pp. 575.
3. Detels R, Green AM, Klausner JD, et al. The incidence and correlates of symptomatic and asymptomatic Chlamydia trachomatis and Neisseria gonorrhoeae infections in selected populations in five countries. *Sex Transm Dis.* 2011;38: 503.
4. Geoffray R, Swain S. Decision analysis: Point-of care Chlamydia trachomatis testing vs. laboratory based methods. *Clin Med Res.* 2004;2: 29-35.
5. Gaydos C. A. Chlamydial Infection: Molecular Testing Methodes. Black CM (ed): *Chlamydia Infection: A Clinical and Public Health Perspective.* Issues Infect Dis. Basel, Karger, 2013, vol7, pp. 78-88.
6. Gaydos C.A. Chlamydiae; in Spector S, Hodinka RL, Young SA, Wiedbrauk DI (eds): *Clinical Virology Manual*, ed 4. Washington, ASM Press, 2009, pp. 630-40.
7. Gaydos CA. Nucleic acid amplification tests for gonorrhea and chlamydia: practice and applications; in Zenilman JM, Moellering RC (eds): *Infectious Diseases Clinics of North America*, ed 19. Philadelphia, Elsevier Saunders, 2005, pp. 367-86.
8. APHL/CDC Panel Summary Reports: Laboratory Diagnostic Testing for Chlamydia trachomatis and Neisseria gonorrhoeae, Laboratory Diagnostic Testing for Treponema pallidum: Guidelines for the Laboratory Testing of STDs. 2009.
9. Campbel LA, Kuo C-C, Gaydos CA: Chlamydial infections; in Detrick B, Hamilton RG, Folds JD (eds): *Manual of Molecular and Clinical Laboratory Immunology*, ed 7. Washington, ASM Press, 2006, pp. 518-25.
10. Kuypers J, Gaydos CA, Peeling RW: Principles of laboratory diagnosis of STIs; in Holmes KK, Sparling PF, Stamm WE, Piot P, Wasserheit JN, Corey L, et al. (eds): *Sexually Transmitted Diseases*, ed 4. New York, McGraw Hill, 2008, pp. 937-57.
11. Gaydos CA, Cartwright CP, Colaninno P, Welsch J, Holden J, Ho SY, Webb EM, Anderson C, Bertuzis R, Zhang L, Miller T, Leckie G, Abravaya K, Robinson J: Performance of the Abbott RealTime CT/NG for the Detection of Chlamydia trachomatis and Neisseria gonorrhoeae. *Clin Microbiol.* 2010;48: 3236-43.
12. Hupert JS, Hesse E, Gaydos CA: What's the point? How point-of-care sexually transmitted infection tests can impact infected patients. *Point Care.* 2010;9: 36-46.
13. Geisler WM. Diagnosis and Management of Uncomplicated Chlamydia trachomatis Infections in Adolescents and Adults: Summary of Evidence Reviewed for the 2010 Centers for Disease Control and Prevention Sexually Transmitted Diseases Treatment Guidelines. *CID* 2011; 53 (Suppl 3): 592-8.
14. Sexually transmitted disease surveillance. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Division of STD Prevention, 2007.
15. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines 2006. *MMWR Recomm Rep* 2006; 55:1-94.
16. Sood S, Mukherjee A, Bala M, Satpathy G, Mahajan N, Sharma A, Kapil A, Sharma VK, Pandey RM, Samantaray JC. A pilot study for diagnosis of genital Chlamydia trachomatis infections by polymerase chain reaction among symptomatic Indian women. *Indian J Dermatol Venereol Leprol.* 2012;78, 4: 443-7.
17. Pearlman MD, McNeely SG. A review of the microbiology, immunology, and clinical implications of Chlamydia trachomatis infections. *Obstet Gynecol Surv.* 1992;47: 448-61.
18. Ward M, Ridgeway G. Chlamydia. In: Collins L, Balows A, Sussman M, editors *Topley & Wilson's Microbiology and Microbial Infections*. 9th ed. New York: Oxford University Press, 1998, pp. 968-98.
19. Stamm WE, Batteiger BE. Introduction to Chlamydia and Chlamydiae. In: Mandell GL, Bennett GE, Dolin R. *Principles and Practice of Infectious Diseases*. 7th ed. Churchill Livingstone Elsevier, 2010, pp. 2439-42.
20. Clinical Policy Bulletin: Chlamydia Trachomatis – Screening and Diagnosis, 2013. from: [www.aetna.com/cpb/medical/data/400\\_499/0433.html](http://www.aetna.com/cpb/medical/data/400_499/0433.html)
21. Khan ER, Hossain MA, Paul SK, Mahmud C, Hasan MM, Rahman MM, Nahar K, Kubayashi N. Comparison between ICT and PCR for diagnosis of Chlamydia trachomatis. *Mymensingh Med J.* 2012;21, 2: 190-4.
22. George JA, Panchatcharam TS, Paramasivam R, Balasubramanian S, Chakrapani V, Murugan G. Evaluation of Diagnostic Efficacy of PCR Methods for Chlamydia trachomatis Infection in Genital and Urine Specimens of Symptomatic Man and Women in India. *Jpn J Infect Dis.* 2003;56: 88-92.
23. Ostergaard L, Traulsen J, Birkelund S, Christiansen G. Evaluation of urogenital Chlamydia trachomatis infections by cell culture and the polymerase chain reaction using a closed system. *Eur J Clin Microbiol Infect Dis.* 1991;10: 1057-1061.
24. Palmer HM, Gilroy CB, Thomas BJ, Hay PE, Gilchrist C, Taylor-Robinson D. Detection Chlamydia trachomatis by the polymerase chain reaction using in swabs and urine from man with nongonococcal urethritis. *J Clin Microbiol.* 1991; 44: 321-325.
25. Wu C, Lee M, Yin S, Yang DM, Cheng SF. Comparison of polymerase chain reaction, monoclonal antibody based immunoassay, and cell culture for detection of Chlamydia trachomatis in genital specimens. *Sex Transm Dis.* 1992;19: 193-197.
26. Dendukuri N, Wang L, Hadgu A. Evaluating Diagnostic Test for Chlamydia trachomatis in the Absence of a Gold Standard: A Comparison of Three Statistical Methods. *Statistics in Biopharmaceutical Research.* 2011;3, 2: 385-397.
27. Agrawal SK, Reddy BS, Bhalla P, Kaur H. Utility of Direct Fluorescent Antibody Test for detected of Chlamydia trachomatis and its detection in male patients with non gonococcal urethritis in New Delhi. *Indian J Dermatol Venereol Leprol.* 2003;69, 2: 144-147.
28. Visser A. and Hoosen A. Diagnosis of Chlamydia trachomatis Infection. Chlamydia, Prof. Mihail Mars (Ed.), ISBN:978-953-51-0470-4, InTech, DOI: Available from: <http://www.interchopen.com/book/chlamydia/diagnosis-of-chlamydia-trachomatis>. 2012.
29. Embil J, Thiebaut H, MAnnual F, Pereira L, MacDonald S: Sequential cervical specimens and the isolation of Chlamydia trachomatis: factors affecting detection. *Sex Transm Dis.* 1983;10: 62-66.
30. Hobson D, Karayiannis P, Byng R, Rees E, Tait I, Davies J: Quantitative aspects of Chlamydia trachomatis of the cervix. *Br J Vener Dis.* 1980;56: 156-162.
31. Black C. Current methods of Laboratory Diagnosis of Chlamydia trachomatis Infections. *Clin Microbiol Rev.* 1997;10, 1: 160-184.
32. Woods G, Bryan J. Detection of Chlamydia trachomatis by direct fluorescent antibody staining, Results of the College of American Pathologists proficiency testing program, 1986-1992. *Arch Pathol Lab Med.* 1994;118: 483-488.
33. Chernesky MA. Chlamydia trachomatis diagnostics. *Sex Transm Infect.* 2002;78: 232-234.