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# DEMONSTRATION OF DIFFERENT ENDOCERVICAL STAINING METHODS AND THEIR USEFULNESS IN THE DIAGNOSIS OF THE CHLAMYDIAL INFECTION IN EXFOLIATED CELLS ADVANTAGES AND DISADVANTAGES

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## ABSTRACT

Microscopic demonstration of chlamydial inclusions within cells offered the first laboratory procedure supporting the clinical diagnosis of chlamydial infection.

Our aim is to evaluate the usefulness of different endocervical staining methods in diagnosis of Chlamydia trachomatis (CT) infection within exfoliated cells of the endocervix. The cytological test for the detection of chlamydial inclusions in genital tract infection, though not as sensitive and specific as isolation in the cell culture monolayers, is still of the diagnostic value.

The present study discusses the collection of clinical smears for microscopic examination, their preparation; fixation and staining of slides by a variety of staining methods that have been used to detect Chlamydia in clinical smears and biopsies. Most of these methods such as Giemsa stain, Papanicolaou, iodine, and immunofluorescence (IF) using monoclonal antibodies, are based on the combination of dyes designed to obtain optimum differentiation of the various structures.

The utilization of different endocervical smear stains together with the clinical information can be used to identify women at high risk for CT infection.

**Key words:** Endocervical Stain; Giemsa, Papanicolaou, Iodine, Immunofluorescence, Chlamydia Trachomatis.

## INTRODUCTION

Bacterial-chlamydial genital infections are commonly included in the group of Sexually Transmitted Diseases-the second generation (STDs). Chlamydia trachomatis (CT) as bacterial agent is a cause of the huge number of genital infections spreading particularly among younger people. Almost 4 (four) million new cases of the chlamydial infections /genital tract/ are yearly registered in the USA, and 3 (three) million in the Europe [1].

Reports on the incidence of these infections demonstrate an increased number of the registered cases, which might be explain as a result of the increased number of performed tests and registrations, as well [2]. Epidemiological investigations of the large number of

women in the USA and Scandinavia confirm Chlamydia to be the most prevalent STD in developed countries. In view of the prevalence of chlamydial infections, their serious consequences and huge treatment costs, screening methods that include high-risk persons have been implemented in the USA:

- Persons with anamnestic history of STDs;
- Young persons, sexually active;
- Promiscuous persons;
- Males with lymphogranuloma infection;
- Newborns;
- Reiter's syndrome diagnosed in younger males.

Throughout years 2001 and 2002, one case of chlamydial infection was reported per a year in Bosnia and Herzegovina, while in 2003 one case was reported in July 2003 [3, 4, 5]. These data are not valid because they do not illustrate the real situation in the region, although chlamydial infections are at the list of notifiable diseases.

CT is the etiological agent of cervicitis/urethritis, which are oligosymptomatic, asymptomatic, chronic and persistent infections with different complications [6]. About 70% percent of all chlamydial cervicitis are without symptoms, and 20-30% women with diagnosed chlamydial cervicitis have no clinical signs, which is important fact for the medical practitioners. Morphologically, chlamydiaceae are coccoid, small Gram-negative bacteria, non-motile, and there are obligate intracellular parasites, energy defect.

All chlamydias are placed into their order-*chlamydiales*, family-*chlamydiaceae*, genus- **Chlamydia** consisted of 4 recognized species:

- C. trachomatis,**
- C. psittaci,**
- C. pneumoniae,**
- C. pecorum.**

They possess a unique developmental cycle consisting of metabolically inactive infectious elementary bodies

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(Ebs), sized about 300 nanometres, and metabolically active but non-infectious reticulate bodies (Rbs), sized about 1 micrometer.

There are different ways of chlamydial infection spreading: sexual, perinatal; although not exclusive the other ways of infection transmission include chloride water in the swimming pools, wet towels, intrahospital infections and gynaecological exams when the necessary protection measures are not applied [7]. It is supposed according to statistics, that about 10% of women in reproductive age are infected with CT. Similarly to infection with the human papilloma virus, chlamydial infection is a very essential provoking agent of the cervical intraepithelial neoplasm with consequent bad Papanicolaou test result, salpingitis with incomplete or complete obstruction, and finally possible extrauterine pregnancy and sterility. During pregnancy, CT causes disorders and ruptures of the fertile membranes with consequent delayed spontaneous abortion or the earlier delivery.

Target population is women in reproductive age, men and adolescents. Because of that, early diagnosis and therapeutic treatment, medical education of population, especially younger, present the most important way in prevention chlamydial infection.

The aim of this study was to find a modus, one completely operative diagnostic procedure, with possibility to achieve a more applicable screening method in detection of CT in reproductively active population.

## PATIENTS AND METHODS

There are four different laboratory procedure types for CT detection:

- Direct microscopic slide of exfoliated cells according to typical intracytoplasmatic inclusions;
- Microorganism isolation in tissue culture, method of choice, "gold standard";
- Detection of chlamydial antigens or nucleic acids with immunological or hybridisation methods;
- Detection of immune response in sera or in secrets.

Our patients were women in reproductive age divided into two groups: moderate risk - *experimental group*, and low risk - *control group*. Moderate risk group consisted of women having some symptoms of genitourinary infections, while low risk group consisted of women without any infection symptoms. The study included 120 patients. Three endocervical swab specimens for three different screening laboratory methods (based on antigen detection using monoclonal and polyclonal antibodies) were collected per each patient. Each specimen was analysed according to following methods:

- **RIA - Rapid Immunoassay;**
- **DFA - Direct Fluorescence Assay,**
- **EIA - Enzyme Immunoassay.**

### RIA - use and principle

- Rapid qualitative immunoassay based on immunocromatography;
- Intended for "in vitro" diagnosis Chlamydia antigen from endocervical swab specimen;
- If the swab specimen is positive (consist Chlamydia antigen), reaction is perceptible (a visible complex antigen - specific antibody- antibody);
- Interpretation of the results: if *the test is positive*, we have two same pinkie lines in the test and control regions;
- Interpretation of the results: if *the test is negative*, there is no visible line in the test region, but it is present in the control region;
- Interpretation of the results: if *the test is invalid*, there are no visible lines both in the test and control regions, the test is to be repeated;
- Do not interpret results after 15 minutes;
- The test does not make any difference among *C. trachomatis*, *C. psittaci*, and *C. pneumoniae*.

### DFA - use and principle

- Intended for direct detection antigen Chlamydia cell from swab specimens in the period of acute genital chlamydial infection;
- It is based on the use fluorescent, labelled monoclonal antibodies directed to the Major Outer Membrane Protein (MOMP) that reacts to all CT serotypes;
- Chlamydial cells appear like an intensive green spots called "green *apple*" on dark background illustrated by fluorescence microscope ("*phenomena of starry sky*");
- Test is positive if there are ten or more intensive green coloured elementary bodies on/in the red coloured epithelial cells on background;
- Every other fluorescent material or particles of irregular shapes or sizes that emitted yellow or red colour are considered as artefact and need to be thrown away.

### EIA - use and principle

- Enzyme immunoassay is used for qualitative detection of chlamydial antigens from the endocervical swab specimens;
- It uses polyclonal antibodies directed on lipopolysaccharide antigen (LPS) that are not strictly specific for the CT species;
- Accounting value of negative control, which is

divided to the whole value for three negative controls to number 3 (three);

- Presence or absences of Chlamydia antigens are established in the comparison of absorption values swab specimens with value of *cut off*;
- Swab specimens with absorption values same or higher than cut off is considered as *positive*.

## STATISTICS

True positive results were those when two of three test methods were coincided.

We used descriptive statistics to determine validity of screening tests as specificity, sensitivity, and predictive values [8].

## RESULTS

**Table 1.** Detection of Chlamydia trachomatis using Rapid Immunoassay (RIA) in experimental and control groups of the examined female population

Examined groups	Total number	Percent (%)	Rapid test (+)	Rapid test (-)
Experimental group	60	50.00	4	56
Control group	60	50.00	3	57
TOTAL	120	100.00	7	113

**Table 2.** Detection of Chlamydia trachomatis using Direct Florescence Assay (DFA) in experimental and control groups of the examined female population

Examined groups	Total number	Percent (%)	DFA (+)	DFA (-)
Experimental group	60	50.00	5	55
Control group	60	50.00	3	57
TOTAL	120	100.00	8	112

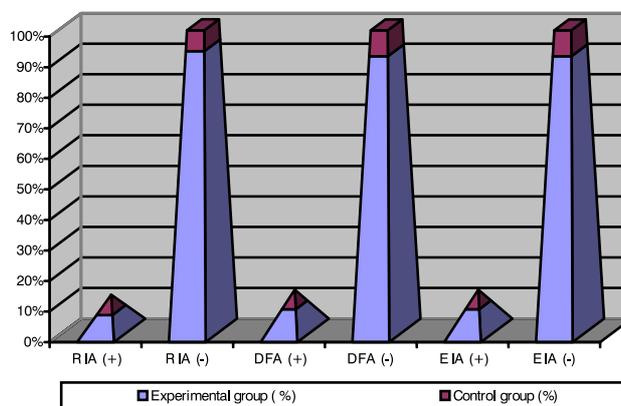
**Table 3.** Detection of Chlamydia trachomatis using Enzyme Immunoassay (EIA) in experimental and control groups of the examined female population

Examined groups	Total number	Percent (%)	EIA (+)	EIA (-)
Experimental group	60	50.00	5	55
Control group	60	50.00	3	57
TOTAL	120	100.00	8	112

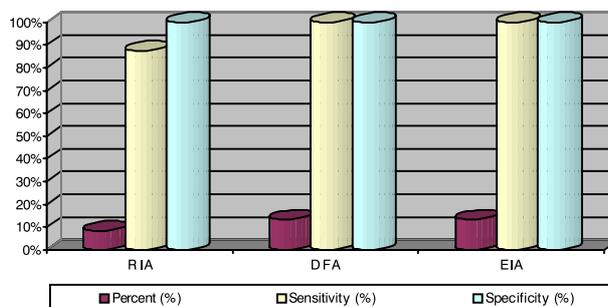
**Table 4.** Prevalence and predictive values (PPV, NPV) for Rapid Immunoassay (RIA), Direct Florescence Assay (DFA), and Enzyme Immunoassay (EIA) in both groups of patients

Assay test	PPV (%)	NPV (%)	Prevalence (%) (experimental and control groups)
RIA	100.00	99.10	5.83
DFA	100.00	100.00	6.66
EIA	100.00	100.00	6.66

**Graph 1.** Comparison of the results received from Rapid Immunoassay (RIA), Direct Fluorescence Assay (DFA) and Enzyme Immunoassay (EIA) in experimental and control groups of the examined female population



**Graph 2.** Comparison of the numbers and percents of positive results of received applicable assays in sensitivity and specificity (examined female population)



## DISCUSSION AND CONCLUSION

In our study, we tried to examine which of applicable test methods allow more rapid and supreme CT diagnosis in endocervical swab specimens collected from examined female population divided into two groups: experimental and control. The choice of the most appropriate diagnostics of chlamydial test depends on the local possibilities, proficiency, and clinical population, as well. Slides dyed by different staining method such as Gram, Giemsa, Lugol, and Papanicolaou showed a low specificity in diagnosis of chronic chlamydial infections (15-41%), so monoclonal antibodies are more frequently used.

Despite the fact that chlamydial infections are asymptomatic in 70% women (without specific symptoms of disease), gynaecological examination plus adequate laboratory treatment are the only possible ways to recognize, treat and stop chlamydial infections from spreading [6]. It is known that chlamydial infections are "enemy of the fertility" and bio-contaminant level 2 (BCL2). People infected with CT represent target population for HIV infections.

Results obtained from experimental group of women are in percentile lower than results reported by other investigators. Reasons are probably in the anamnestic data of examined patients (absence of STDs, the choice and sam-

ple size and timely period of the investigations) [9, 10]. Achieved data of the prevalence value of examined control group coincide in percentile with the results stated by other investigators. Analytical values of the applicable test methods reported by mentioned authors were between 82% (EIA) - 100% (isolation) versus 91.3% (EIA) - 95% (isolation).

According to our results we conclude that in laboratory diagnostics of CT, in our conditions, the best choice is to use DFA, having in mind its high sensitivity and specificity [11]. It is simple in valuation of the sample quality using the specific monoclonal antibodies directed at the MOMP. This test is also a confirmed and approved by World Health Organization (WHO).

DFA is non-invasive test method in the diagnostics of chlamydial infection, which enables the unique possibility of identification both asymptomatic and symptomatic patients.

We put the accent on DFA because of its already stated advantages (use of the monoclonal antibodies, the most acceptable screening test, reference for the practical work, 100% sensitivity, 100% specificity, species specificity), having in mind the high sensitivity of EIA, and specificity of two other test methods (RIA, EIA).

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## REFERENCES

- (1) Centres for Disease Control. Chlamydia trachomatis. Genital Infection-United States. Morbid Mortal Weekly Rep 1997; 46 (09): 193-198.
- (2) WHO Scientific working group. Laboratory tests for the detection of reproductive Tract Infections. 1999, WHO.
- (3) SFOR Communicable Diseases Annual Report for 2001
- (4) SFOR Communicable Diseases Annual Report for 2002
- (5) SFOR Communicable Diseases Bulletins Number 01-07/2003
- (6) Pechere J.C. Ur. Intracellular bacterial infections. Cambridge Medical Publications 1996: 59-64.
- (7) Schonvald S. Lijecenje spolno prenosivih bolesti. Medicus 2000; 9 (2): 201-208.
- (8) Greenberg Raymond S., et al. Medical Epidemiology. 2nd Ed. A Lange medical book, 1996.
- (9) Tilton R.C., Judson F.N., Barnes R.C., et al. Multicentre comparative evaluation of two rapid microscopic methods and culture for detection of Chlamydia trachomatis in patient specimens. J Clin Microbiol 1998, 26 (2): 167-170.
- (10) Raymond R.W., Kwasnik I., Steingrimsson O., et al. Rapid detection Chlamydia trachomatis by an Enzyme Immunoassay Method. Diagn Microbiol Infect Dis 1986; 5: 225-234.
- (11) Mahmutovi} S. Komparativna evaluacija dijagnosti-kih vrijednosti izabranih metoda u detekciji Chlamydia trachomatis. Magistarski rad. Sarajevo: Medicinski fakultet Univerziteta u Sarajevu, 2002.