DIAGNOSIS OF CHLAMYDIA TRACHOMATIS INFECTION – CULTURE VERSUS SEROLOGY


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The diagnostic value of different laboratory methods in detecting Chlamydia trachomatis infections in high risk groups was analysed. The efficiency of a direct specimen test was compared with serology (IgG and IgM ELISA) and culture in L929 cells, stained either with fluorescein conjugated monoclonal antibodies or with iodine. Patients (no. = 1041) with localized genital infections attending a STD clinic, sexual contacts and patients with ascending infections from urological and gynecological clinics were examined. Chlamydia trachomatis was detected in 225 patients: 210 (93.3%) were reactive in the direct test (smears stained with monoclonal antibodies), whereas culture missed only 5 (sensitivity 97.8%) when stained by the same method. Cultures stained with iodine produced the lowest recovery rate (73.8%), but this rate increased to 80.9% when a second passage was performed. In addition the prevalence of Neisseria gonorrhoeae, Mycoplasma hominis, Ureaplasma urealyticum, Candida albicans and Trichomonas vaginalis was investigated.

In patients with non-gonococcal urethritis (no. = 331) and cervicitis (no. = 353), Chlamydia trachomatis was isolated in 32.3% and 12.8% respectively. However, this pathogen could be isolated in only 3 (15.8%) out of 19 patients with epididymitis and 15 (14%) out of 107 patients with adnexitis, although 66.7% and 93.3% respectively had specific IgG antibodies. Specific IgM could by detected with a sandwich ELISA in patients with adnexitis (46.7%), epididymitis (33.3%), cervicitis (22.2%), non-gonococcal urethritis (14%) and in the sexual partners of patients with genital infections (35.7%).

The direct specimen test with monoclonal antibodies is the method of choice for the diagnosis of a C. trachomatis infection in patients with urethritis and cervicitis. In ascending infections of the genital tract, however, even culture may give negative results, and serology may be of diagnostic assistance.

INTRODUCTION

Chlamydia trachomatis serovar D-K is now recognized as one of the most common sexually transmitted pathogens. Interest in Chlamydia trachomatis has increased in recent years because this agent is known to be responsible for a variety of genital tract infections with an expanding clinical spectrum in both men and women (20, 39, 17). The organisms may also persist in the body for many years without causing inflammation and then multiply and produce disease symptoms (31, 27, 11, 10).

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The diagnosis is based primarily on the isolation of the agent in cell culture (29, 42). Sensitivity and specificity of the conventional cell culture techniques have been improved recently by the use of monoclonal antibodies against species specific \textit{Chlamydia trachomatis} antigen (22, 35, 37). Cost, technical complexity and particular transport requirements, however, limit the use of these techniques in routine examinations.

Antigen detection techniques, which do not require the presence of viable microorganisms, have several advantages over culture methods (34, 38). Highly specific results can be obtained in half an hour by the use of specific fluorescein isothiocyanate labeled monoclonal antibodies on smears.

The demonstration of a specific antibody response may support the diagnosis and may also, within certain limits, be indicative of the activity of infection (26, 16). Therefore a simple and sensitive serological test could be of considerable clinical and epidemiological value and also decrease the cost of screening. The established serologic tests for the identification of chlamydial antibodies are the complement fixation test, indirect inclusion immunofluorescence, the immunoperoxidase assays and the microimmunofluorescence test (28, 25, 3).

The complement fixation test is capable of detecting chlamydial group antibodies only. High titers might indicate active disease, but generally, the method is of limited value due to its limited sensitivity (40).

The microimmunofluorescence test is more sensitive but requires highly skilled personnel; it reacts specifically with the different serovars of \textit{Chlamydia trachomatis}.

The enzyme-linked immunosorbent assay is a simple and sensitive system using material from a L2 strain for antigen (7, 14, 30). This test can be applied as a routine method.

This report analyzes the diagnostic value of different laboratory methods in the detection of chlamydial infections in certain high-risk groups of patients attending dermatological, urological and gynecological clinics as well as sexually transmitted disease (STD) outpatient facilities in Austria.

A modified enzyme-linked immunosorbent assay is applied for the detection of \textit{Chlamydia trachomatis} specific IgG and IgM in the serum. The reliability of the enzyme-linked immunosorbent assay results was assessed by comparison with direct smear tests as well as with standard culture techniques. Furthermore, the prevalence of \textit{Neisseria gonorrhoea}, \textit{Mycoplasma hominis}, \textit{Ureaplasma urealyticum}, \textit{Candida albicans} and \textit{Trichomonas vaginalis} was investigated since these organisms may also be involved in urogenital disorders among persons at high-risk for STD infection.

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**MATERIAL AND METHODS**

**Origin of samples**

Patients attending the venereal disease clinic of the dermatological or urological department of the hospital of Lainz, Vienna, the University clinic for gynecology and obstetrics of Vienna, or the gynecological and dermatological departments of the hospital of Salzburg with diagnoses of nongonococcal urethritis (n=331), gonococcal urethritis (n=136), cervicitis (n=353), epididymitis (n=19), Reiter's syndrome (n=20), adnexitis (n=107) and a few sexual contacts (n=21) were screened for \textit{Chlamydia trachomatis}, \textit{Mycoplasma hominis}, \textit{Ureaplasma urealyticum}, \textit{Candida albicans} and \textit{Trichomonas vaginalis}. Patients receiving antimicrobial chemotherapy within the previous four weeks were excluded. Volunteers attending the clinic for routine check-ups but without history of chlamydial infection served as controls.

**Collection of specimens**

Specimens for \textit{Chlamydia trachomatis} cultures and smears were taken with metal wire shaft cotton-tipped swabs (Medical wire and equipment Co. Ltd. Corsham, Wiltshire, England). In men, the swab was inserted into the urethra, rotated and withdrawn. In women, the exocervix was first cleaned with a cotton swab to remove mucus and exudate. The collection swab was inserted into the endocervical canal, rotated and removed avoiding contact with the vaginal surface. The initial swab from each site (women: cervix and urethra, men: urethra) was used to prepare a smear by rolling it over a circular area on a glass slide for \textit{Chlamydia trachomatis} direct specimen test. The smears were then air dried, fixed with acetone and stored at $-20^\circ C$ until they were stained. The cotton tip of the second swab was immediately cut and dropped into a vial containing 2ml of a 0.2M sucrose-phosphate transport medium (23) supplemented with gentamicin (10µg/ml), vancomycin (25µg/ml) and nystatin (25U/ml) as well as 2% fetal calf serum, quick frozen within an hour and stored in liquid nitrogen. Sera were obtained from all patients and stored at $-70^\circ C$ until testing.

**Direct specimen test**

The MicroTrak™ Merck/Syva reagent contains fluorescein-conjugated monoclonal antibodies against the chlamydial major outer membrane protein antigens (reactive with all 15 known human serovars of \textit{Chlamydia trachomatis}) and a counterstain (Evans blue). Staining of the acetone fixed smears was performed using the standard