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LABORATORY METHODS FOR DIAGNOSING UROGENITAL CHLAMYDIA

Kudratova Z. E.
PhD, Assistant of the Department of Clinical Laboratory Diagnostics of SamSMU

Shamsiddinova M. Sh.
Student of 5-th Course SamSMU
Samarkand State Medical University, Samarkand, Uzbekistan

Abstract
All laboratory methods of diagnostics of chlamydial infections can be divided into the following groups: cytological methods, culture methods of chlamydia diagnostics, serological methods, molecular genetic methods (PCR) [1,2].

Cytological Methods

Cytological methods are microscopic examination of clinical material. Scrapings from the mucous membranes of the urethra and cervix are taken with a Folkman spoon, a special brush or a probe, observing the rules of antisepsis. The urethra and cervical mucus plug are removed with a swab. Scrapings from deep within the urethra and cervical canal are made from all four quadrants. Epithelial cells should be present. The presence of discharge and blood masks the chlamydia and makes diagnosis difficult. In extragenital lesions, scrapings are obtained in the same way. The basis of these methods is the detection of inclusion elements, based on the use of different staining methods (Giemsa or iodine). The sensitivity of this method is low. Cytoplasmic methods of chlamydia identification are used to detect the characteristic intraplasmic inclusions of semilunar shape, which are pathognomonic features of chlamydia. Lugol's solution staining allows to reveal the glycogen matrix of developing inclusions of dark brown colour on the pale background of cells. In recent years, direct immunofluorescence (DIF) has become more widely used. Direct MIF is comparable to the conventional cell culture method, especially with iodine staining and immunofluorescence. In cell culture, C.trachomatis is identified by the presence of inclusion bodies, whereas in smears taken directly from C.trachomatis-infected body sites (cervix, rectum, urethra), typically arranged extracellular elementary cells are pathognomonic. Most staining methods do not allow the detection of elementary cells, so the diagnosis of chlamydial infection has been based on cell culture, which is technically difficult and time-consuming. Direct MIF allows the detection of elementary cells directly in smears obtained from patients, which provides an easy and rapid diagnosis of chlamydial infection [3,4,5,6].

Culture Methods

Due to its complex life cycle, C.trachomatis is more difficult to isolate than most bacteria. In order to optimise all steps of the culture method, it is necessary to have close links with the laboratory performing the test; from sample collection to transport (preferably by cold storage) and subsequent laboratory examination. All these factors make this method expensive, but it has a high sensitivity (minimum 90%) and specificity. Specificity can be increased by increasing the number of samples tested. In this method of diagnosis of chlamydia infection, the methods of collection and transport systems have a significant impact on the results of culture tests. A central problem in implementing such a method of chlamydia detection becomes the sensitivity of monolayer cell culture, as well as the procedure for identifying cellular changes. In general, the sensitivity of culture methods for the diagnosis of chlamydial infections has not been determined, and their specificity is

probably very high (95-99%), especially when performed by experienced personnel. However, these methods are quite expensive. Chlamydiae do not multiply on artificial nutrient media. Their parasitism is due to a pronounced metabolic dependence on the host cell. The bacteriological method of diagnosis is based on the isolation of chlamydia from the material under study by infecting primary or transplanted cell cultures. In the process of cultivation, the pathogen is identified and sensitivity to antibiotics is determined. Infection of the yolk sacs of chicken embryos and white mice is currently not used in routine diagnosis and is used for the management of laboratory strains [7,8]. When examining preparations of infected cell cultures, chlamydia are detected in the form of characteristic cytoplasmic inclusions coloured according to the method. Detection of at least one cytoplasmic inclusion with specific colouring, shape and structure is sufficient to establish the fact of chlamydial infection in the object under study. It should be noted that the results of culture tests performed in different laboratories vary considerably. The sensitivity of the method ranges from 9 to 93% (Persson K., 2005). The method of diagnostic isolation of Chlamydia in cell culture can be used during the entire period of illness, except for the period of antibiotic therapy and for one month afterwards. However, at present, the culture method is mainly used in cure control to identify viable chlamydiae capable of performing a complete developmental cycle [9,10].

Serological Methods

Serological methods of diagnosis - have obvious advantages The evaluation of serological reactions used for diagnostic purposes is based on the following criteria: a) detection of seroconversion, which means that antibody titres are not detected in the early stages of the study, but increase to > or = 8 during the course of the disease and during recovery; b) detection of a more than fourfold increase in the titre of antibodies related to Ig G immunoglobulins; c) detection of Ig M antibodies by RSC, a radioimmunoprecipitation method, which is more than 20 times more sensitive than RSC and comparable to the microimmunofluorescence method, which can determine the serotype of chlamydia. Antibodies to C.trachomatis can be detected by microimmunofluorescence (MIF). Chlamydiae obtained from cell culture or chicken embryos are used for this purpose. Antibodies of classes G, A or M to surface antigenic determinants are determined. In recent years, peptide-based ELISA tests have been widely used in many countries for the detection of chlamydial antibodies and may eventually replace MIF. Antibodies to C.trachomatis are used as markers of chlamydial infection in several studies on late complications. These studies have established an association between chlamydial infection and ectopic pregnancy and infertility due to fallopian tube obstruction. Antibodies to chlamydial heat shock protein were found to be an independent sign of fallopian tube damage due to chlamydial infection. However, serological methods are currently not a reliable method for the diagnosis of UGC. It was shown that IgG antibodies 80 were detected in the serum of 40-100% of women whose cervical scrapings revealed Chlamydia by culture and nucleic acid amplification tests, while IgG antibodies were also detected in 16-87% of women who tested negative for C.trachomatis [11,12]. Thus, the prognostic value of this test is very low. Serological tests should not be used as a cure control test, as the antibody titre remains quite high for several months after treatment, but they are well applicable in the differential diagnosis of chlamydia. The value of this method is particularly high in chronic asymptomatic forms of chlamydial infection of the pelvic organs (prostatitis, epididymitis, salpingitis, salpingo-ophoritis). Sensitivity and specificity of test systems for the determination of antibodies to chlamydiae are not less than 95%. Nucleic acid amplification methods (molecular genetic methods). Tests based on nucleic acid amplification (NATT - nucleic acid analysis tests) can be carried out by different methods: polymerase chain reaction (PCR), ligase chain reaction (LCR), SSD (single strand displacement) and TMA (transcript-mediated assay) methods. These methods have advantages

over others because of their high sensitivity and specificity. The sensitivity of nucleic acid amplification methods (NAMA) is 90-95% and is determined by the type of sample. In vitro testing of different dilutions of C.trachomatis strains shows that MANK gives a positive result in the presence of 10-100 microorganisms, culture method - 1000-10000 microorganisms, ELISA - at higher quantities of C.trachomatis. Another advantage is that any type of clinical material, including urine, can be used for testing. Non-living C.trachomatis can also be detected using these methods. NATT is an expensive method, but due to the cost of labour in man-hours, these technologies are cost-effective [13,14,15].

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