COMPARATIVE ANALYSIS OF METHODS FOR SEROLOGICAL DIAGNOSIS OF MYCOPLASMOSIS CAUSED BY *MYCOPLASMA HOMINIS* Kyrylenko Y.¹, Klochko V.^{1,2} ¹Igor Sikorsky Kyiv Polytechnic Institute, kyrylenko.yuliia@lll.kpi.ua ²Zabolotny Institute of Microbiology and Virology, NAS of Ukraine

Abstract

This article presents serological diagnostic methods for detecting antibodies to Mycoplasma hominis and identifies the advantages and disadvantages of the most promising of them. Also, the classes of immunoglobulins that are formed in the human body as a reaction to inflammatory processes caused by M. hominis are indicated.

Keywords: Mycoplasma hominis, serological diagnostics, mycoplasmosis.

Introduction. Bacteria of the genus *Mycoplasma* are represented by both parasitic and saprophytic species, which are carried by 60-80% of the population. Asymptomatic carriage of mycoplasmas is the main danger, as the disease can develop for a long time without visible signs and manifest itself in a decrease in the overall resistance of the body. In the future, this can cause a sharp increase in the number of mycoplasmas, and, accordingly, mycoplasmosis. The *Mycoplasma hominis* species belongs to opportunistic bacteria and is capable of causing asymptomatic, prolonged and chronic infection of the genitourinary tract, as well as extragenital infections, including neonatal infections, which are considered a threat to human health [1]. In view of the above, there is a need for rapid diagnosis for the correct detection of urogenital mycoplasmas and timely treatment to prevent exacerbation of the disease and its transition to a chronic form.

There are three main methods of diagnosis: microbiological, molecular genetic, and serological. The first two methods have certain disadvantages. For example, the microbiological method is time-consuming, labor-intensive, and suboptimal for determining further treatment steps. Molecular genetic testing cannot be performed without specialized and expensive equipment and pre-trained qualified personnel [2]. At the same time, the serologic method does not have these disadvantages of other methods. The serologic method is characterized by high specificity, availability and allows to obtain results within a few hours.

The aim of this research was to analyze the literature data on serological diagnostic methods for the determination of antibodies to *M. hominis*, and to compare the methods.

Materials and methods. The paper uses modern scientific literature on the study of methods of serological diagnosis of mycoplasmosis caused by M. *hominis*. A critical analysis of this literature was carried out, which identified the main advantages and disadvantages of the methods of serological diagnosis of *M. hominis*.

Results and discussion.

Various biochemical mechanisms of *M. hominis* lead to inflammation of tissues, the development of chronic infection, and, ultimately, damage to host tissues [1]. In parallel with these processes in the human body, an increase in antibody titers in the

blood serum is observed, which gives grounds for concluding that the pathogen is directly involved in the development of pathological processes.

Determination of antibody levels in human serum using serological diagnostic methods is based on the principle of specific antigen-antibody binding. Antibodies to M. hominis belong to several classes, so the direct presence of a certain type of immunoglobulin (Ig) in the blood allows determining the stage of the disease.

IgA antibodies to *M. hominis* are always a component of the first pronounced immune response and appear in human serum from the first days (1-4) after the beginning of the disease caused by *M. hominis*. IgM immunoglobulins correspond to an acute infection, while the presence of IgG antibodies reflects the overall pattern of the immune response due to a prolonged or previous infection. IgG antibodies appear in the serum in the first week of the disease and persist throughout the disease [3].

Many different serological methods have been adapted to detect antibodies to mycoplasmas: complement fixation (CF), growth inhibition (GI), enzyme-linked immunosorbent assay (ELISA), microimmunofluorescence (MIF), indirect hemagglutination (IHA), Western blot, and metabolic inhibition (MI). Studies by various researchers have found that some of these methods are not optimal for rapid laboratory diagnosis. In particular, MI requires a lot of time to obtain the results, and therefore the diagnosis itself is a complex procedure; GI and CF are considered to be quite insensitive and thus should not be used to detect antibodies to *M. hominis* [4, 5].

Four serologic methods are among the most promising for the detection of antibodies to M. *hominis*: enzyme-linked immunosorbent assay, microimmunofluorescence, indirect hemagglutination, and Western blot. The advantages and disadvantages of each of these methods are shown in Table 1.

Method	Advantages	Disadvantages
Western blot	- High specificity;	- More often used for confirmation
	- Accurate and easy to interpret	rather than quantitative detection;
	results;	- A laborious and expensive method;
	- Determination of the molecular	- Requires the use of long-lasting
	weight of the target protein [6].	substrates (8-24 hours) or fluorescent
		dyes, otherwise the detection signal
		will decrease rapidly;
		- The method is more sensitive to
		impurities in sample preparation [6].
Microimmuno-	- High selectivity and sensitivity;	- Labor-intensive method;
fluorescence	- Detection of antibodies in the early	- Low objectivity of measurements
	stages of the disease [7].	(reproducibility and interpretation of
		results) [8].
Enzyme-linked	- Fast method;	- Special storage conditions;
ımmunosorbent	- High specificity and sensitivity;	- False-positive or false-negative
assay	- Minimal sample preparation;	results due to technical errors,
	- Analysis of multiple samples	sample contamination, use of low-
	simultaneously;	quality reagents, etc [6].
	- Possibility of automation;	
	- No expensive reagents are used;	
	- Detection of low concentration of	
	antibodies [6].	

 Table 1. Advantages and disadvantages of the most promising methods of serological diagnosis of mycoplasmosis

Continued Table 1

Method	Advantages	Disadvantages
Indirect	- Sensitivity;	- Labor-intensive;
hemagglutination	- Reproducibility;	- False negative results due to the
	- Specificity [5].	circulating immune complexes and
		other inhibitory factors in the blood
		serum [5].

Due to the ease of implementation, cost-effectiveness, time spent on the analysis and, in fact, the overall ratio of advantages/disadvantages, the enzyme-linked immunosorbent assay is one of the most effective methods for diagnosing the mycoplasmosis pathogen *M. hominis*.

Conclusions. Serologic diagnostic methods are widely used in the standard clinical diagnosis of mycoplasmosis worldwide. This group of methods is characterized by high sensitivity, specificity, and the ability to perform rapid tests. Many methods have been developed for the detection of antibodies to *Mycoplasma hominis*, but not all of them are able to provide adequate sensitivity in the laboratory, combined with the speed and ease of diagnosing the pathogen.

Therefore, after analyzing the advantages and disadvantages of the above methods, it can be argued that enzyme-linked immunosorbent assay is the best method for detecting antibodies to *M. hominis*, as in addition to sensitivity, selectivity and speed, this method can be automated, which greatly simplifies the process. Moreover, enzyme-linked immunosorbent assay is used as a comparison for most studies.

References:

1. Ahmed J, Rawre J, Dhawan N, et al. Mycoplasma hominis: an under recognized pathogen. Indian journal of medical microbiology. 2020. URL: https://doi.org/10.1016/j.ijmmb.2020.10.020.

2. Zarucheynova O. V. The methods of laboratory diagnostics of urogenital infections associated with Mycoplasma hominis and ureaplasma spp. Journal of infection and immunity. 2015. Vol. 4, no. 4. P. 331–338. URL: https://doi.org/10.15789/2220-7619-2014-4-331-338.

3. Ernø H., Thomsen A. C. Immunoglobulin classes of urinary and serum antibodies in mycoplasmal pyelonephritis. Acta pathologica microbiologica scandinavica section C immunology. 1980. Vol. 88C, no. 1-6. P. 237–240. URL: https://doi.org/10.1111/j.1699-0463.1980.tb00100.x.

4. Mardh P. A., Westrom L. Antibodies to Mycoplasma hominis in patients with genital infections and in healthy controls. Sexually transmitted infections. 1970. Vol. 46, no. 5. P. 390–397. URL: https://doi.org/10.1136/sti.46.5.390.

5. Möller B. R. Comparison of serological tests for detection of Mycoplasma hominis antibodies in female grivet monkeys with experimentally induced salpingitis. Acta pathologica microbiologica scandinavica section B microbiology. 2009. Vol. 89B, no. 1-6. P. 7–11. URL: https://doi.org/10.1111/j.1699-0463.1981.tb00145_89b.x.

6. ELISA vs western blot: when to use each immunoassay technique. Life in the Lab. URL: https://www.thermofisher.com/blog/life-in-the-lab/western-blot-or-elisa/.

7. Bradbury J. M., McCarthy J. D., Metwali A. Z. Microimmunofluorescence for the serological diagnosis of avian mycoplasma infections. Avian pathology. 1990. Vol. 19, no. 2. P. 213–222. URL: https://doi.org/10.1080/03079459008418674.

8. Ciervo A, Petrucca A, Visca P, et al. Evaluation and optimization of ELISA for detection of anti-Chlamydophila pneumoniae IgG and IgA in patients with coronary heart diseases. Journal of microbiological methods. 2004. Vol. 59, no. 1. P. 135–140. URL: https://doi.org/10.1016/j.mimet.2004.05.011.