Original Article

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Review on the laboratory diagnosis of *Mycoplasma pneumoniae* infection

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Abstract: *Mycoplasma pneumoniae* (MP) is an important pathogen of community-acquired pneumonia in children. As a type of self-limited disease, most MP infections cause mild clinical symptoms, but they can also lead to severe pneumonia or extrapulmonary complications. The resistance rate of MP has increased in recent years. Early and rapid diagnosis of MP infection is important for the treatment and prognosis of the disease. Current methods for diagnosing MP infection include isolation culture, serological diagnosis, and molecular biological diagnosis. This review summarizes the recent research progress in the internal and external laboratory diagnoses of MP infection both at home and abroad and the advantages and disadvantages of various diagnostic methods.

Keywords: *Mycoplasma pneumoniae*, infection, diagnosis

Mycoplasma pneumoniae (MP) belongs to the class Mollicutes, which is distinguished by the absence of a cell wall. Among prokaryotes, MP has the smallest cell biology with the minimum volume and the shortest gene fragment and can be replicated autonomously. MP has a tip-like structure that strongly adsorbs host cells, mainly adhering to the lung epithelial cells of the upper respiratory tract. It provides the nutrients needed for survival relying on host cells. The interaction of the host and the pathogen on the surface of the respiratory tract determines whether it is cleared by the host, and the amount of residual bacteria determines whether it causes symptoms of clinical infection. MP infection only causes disease in the human body. It spreads through droplets and can survive in the respiratory tract for several weeks or several months with 1–3 weeks of incubation [1]. MP infection is a seasonal disease and is frequently acquired in the fall. Most MP infections cause mild clinical symptoms and feature self-limiting characteristics. MP has a common antigen with the myocardium, lung, and brain tissue of human beings. After being infected, the body produces an antibody that can generate cross-antigen antibody reaction with human myocardium, liver, lung, and brain tissue to form an antigen–antibody complex, causing lesions of extrapulmonary tissues, such as pneumonia, arthritis, hemolytic anemia, liver necrosis, and damage to nerve tissue. MP infection can occur in all ages, usually with the lowest incidence among the elderly people, followed by adults, and with the highest incidence among school-age children and adolescents. MP infection is an important pathogen of community-acquired pneumonia (CAP), accounting for about 10%–30% of children's CAP [2-3]. MP infections occur in densely populated areas, such as home, school, institution, camping area, and military base, and outbreak every 3–7 years, up to 1 year. Gotoh *et al.* [2] showed that the asymptomatic carrying rate of MP is high in children of all ages. Meyer *et al.* [1] reported that the incidence of extrapulmonary injury in patients with *Mycoplasma pneumoniae* pneumonia (MPP) is as high as 25%. Currently, drug-resistant MP is the main pathogen causing MP infection in children, and drug-resistant MPP has spread worldwide. It is highly prevalent in East Asian countries, such as China, Japan, and South Korea, and relatively low in North America and Europe. Early, rapid, and accurate diagnosis of MP and drug-resistant MP plays a crucial role in guiding the clinical medication, prognosis, and outcome of the disease.

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The current methods for diagnosing MP infection are divided into three categories: MP isolation culture and identification, serological diagnostic method, and molecular biological method.

1 MP isolation, culture, and identification

1.1 Isolation, culture, and identification

MP isolation and culture is the gold standard for the diagnosis of MP infection [4]. MP is usually isolated from the throat, nasopharynx, pleural effusion, or body fluid of patients with pneumonia. MP can grow on an animal artificial medium containing cholesterol, can survive for several hours at 37°C, and multiply in a two-split form with a slow speed and long culture period (10–14 days or longer). Specimens are inoculated into a microbial culture medium of pleurisy and mixed and placed in a 5% CO_2 incubator at 37°C, and negative and positive controls are prepared (MP standard strain M129). MP ferments the amylaceum in the medium to produce acid. A decrease in medium pH and yellowing phenol red indicator indicate MP growth.

1.2 Methodological evaluation

MP isolation and culture require a special medium and laboratory condition. The sensitivity of the diagnosis of MP infection is only 60% due to various factors, such as sample source, preservation, transportation mode, MP content in the sample, and technical level of the laboratory technician [5]. This approach has insufficient significance for early clinical diagnosis and is often used for retrospective diagnosis and research.

2 Serological diagnosis

In China, the serological method detects changes in MP-specific antibody titers in serum and is thus often used to diagnose MP infection. Test methods include specificity tests, including complement fixation test (CFT), gelatin agglutination (PA) test, enzyme-linked immunosorbent assay (ELISA), non-specific test, and cold agglutination test. At present, a standard for the diagnosis of MP infection by serological test remains to be established. In paired serum samples in the acute and recovery phases, MP infection is diagnosed when the MP-specific antibody titer is increased or decreased by four times or more [6]. However, the paired serum test has poor feasibility and lacks early diagnostic value. A significant increase in single serum-specific IgM antibody is the main laboratory basis for the clinical diagnosis of MP infection. In recent years, the PA method has been used to measure IgM antibodies in clinical practice. In general, MP-IgM ≥1:160 has a high diagnostic value.

2.1 Serological diagnostic method

CFT is the standard method for detecting MP infection. It mainly detects early IgM responses and is insensitive to IgG. However, this method has poor overall sensitivity and specificity and cannot distinguish the type of antibody [7]. The lipid antigen used in the test exists in human tissues and certain bacteria, but it is rarely used clinically because it is prone to cross-reaction and false-positive results. When CFT is used to detect the titer of serum IgM antibody, the sensitivity is only 58.8% [8].

The sensitivity and specificity of the PA test are significantly better than those of the CFT. Thus, the PA test is widely used in clinical practice in some countries. The positive rate achieved with the PA test has a high diagnostic value but, similar to CFT, this test cannot distinguish the type of antibody. The higher the PA titer, the greater the likelihood of positive IgM, but the PA titer is not significantly associated with positive or

negative IgG. When the PA test takes an antibody titer of 1:80 or 1:160 as the diagnostic standard, then it has a good consistency with the PCR results. A previous study reported that the use of the PA test to detect paired serum can provide a basis for the diagnosis of MP infection [5].

ELISA is the commonly used method in the current serological test for the diagnosis of MP infection in China. It can detect IgM and IgG separately, and the sensitivity and specificity can reach a high level. Many commercial kits are available, but large-scale standardized clinical trial evaluations are lacking, and only few of these kits have been certified by the US Food and Drug Administration (FDA). Beersma *et al.* [9] compared the sensitivity and specificity of 12 commercial kits with real-time quantitative PCR as the gold standard and found that the highest sensitivity and specificity were 77% and 92%, respectively, and the worst sensitivity was only 35%. The sensitivity of commercial kits for detecting MP infection is affected by factors such as patient's age, condition of laboratory hardware, laboratory staff's technique, specimen collection time, acute collection period, and paired serum during recovery. Low-quality commodity kits can result in non-uniform diagnostic criteria. Thus, large-scale clinical trials are conducted to reassess their quality, uniform diagnostic criteria are established, and appropriate detection reagents are selected to diagnose and treat MP infection.

The cold agglutination (CA) test is a common method for diagnosing MP infection with about 50% of positive rate of detection. Infections caused by adenovirus, cytomegalovirus, and EB virus can also induce the production of serum-condensed agglutinin. The CA test is a non-specific diagnosis and currently only serves as the reference for MP infection.

2.2 Methodological evaluation

Serum-specific antibody IgM generally appears from 7th to 14th days after MP infection, reaching a peak from 3rd to 4th weeks, which can last for several months [8]. The IgM antibody immune response is fast and thus can be used as an indicator for early diagnosis. In the acute phase, the IgM detected in the serum is considered a recent infection. It takes a certain time for the body to be infected from MP to the immune response. The blood sampling time has a great influence on the test results. False-negative results may arise when serum samples are collected too early and when the IgM changes may not be detected. To detect the IgM antibody titer for the diagnosis of MP infection, the positive rate of detection of serum antibody titer in the acute phase is between 14% and 45%, whereas that in the acute phase can be increased to 39%–88%. To detect the IgG antibody titer for the diagnosis of MP infection, the positive rate of detection of serum antibody titer in the acute phase is between 39% and 45%, whereas that in the acute phase can be increased to 73%–82% [10].

IgM levels among MP-infected children are significantly higher than those among healthy children, peaking in the middle school period, decreasing with age, and may only produce IgG in adulthood [11]. This observation may increase the diagnostic value of IgM antibody level for childhood MP infection, indicating poor sensitivity of adult infections to the diagnosis. Beersma *et al.* [9] used the FDA-approved ImmunoCard kit for the diagnosis of childhood MP infection, which has a high sensitivity and can be only used to detect IgM antibody level. The IgM antibody level alone can also be used to diagnose MP infection in children. Talkington *et al.* [10] reported that about 20% of adults infected with MP do not show an increase in IgM antibody titers and that IgM alone is less effective in diagnosing MP infection in adults than in children.

Specific IgG is later than IgM, usually peaking from 4th to 6th weeks, with a slower rate of increase and longer duration in the body (up to 4 years). Owing to the immunological characteristics of IgM antibodies, IgM detection for adults may cause an erroneous diagnosis. At present, China has adopted the simultaneous detection of IgG and IgM antibody levels to confirm the diagnosis of MP infection.

Specific IgA production time is similar to that of IgM and is higher in adults, especially in the elderly people. Increased levels of IgA antibodies may be an important indicator of acute MP infection. IgM is faster than IgM and may serve as a supplemental reference for infections in certain adult MPs that do not produce IgM. When elderly people are infected with MP, the elevated IgA antibody titers may be more conducive to diagnosis [11]. Using IgA antibody titers to carry out the serological tests are often more reliable in patients with acute exacerbation of chronic obstructive pulmonary disease with MP infection [12].

Given the immunological characteristics of serum antibodies, the serological test for MP infection has certain limitations. Single serodiagnostic MP infection is less sensitive even with a recent and rapid serum diagnostic (enzyme immunoassay) technique.

The sensitivity of the serological test is also considerably lower than that of molecular biological diagnosis [5]. Clinically diagnosed MP infections often require the simultaneous detection of paired serum, which must be collected once every 2 weeks. This approach is applicable for epidemiological investigations but not for an early, rapid diagnosis. On the basis of Taiwanese literature, the collection rate of paired serum clinically is less than 10% [13]. In summary, serological diagnosis should not be used as a method for the rapid diagnosis of early MP infection.

3 Molecular biological diagnosis

The molecular biological method allows early and rapid diagnosis with a high sensitivity and specificity. Commonly used experimental methods are traditional PCR, nested PCR, real-time quantitative PCR, nucleic acid sequence-based amplification (NASBA), loop-mediated isothermal amplification (LAMP) technology, and RNA simultaneous amplification and testing (SAT). On the basis of the basic principles of these experimental methods, domestic and foreign scholars have developed a composite technology that can simultaneously detect multiple pathogens. The target genes frequently amplified in the experiment include P1 protein coding gene, 16S rRNA, card gene, ATPase gene, etc. The types of clinical specimens available are pharyngeal swab, throat swab, sputum specimen, alveolar lavage fluid, serum, whole blood, and cerebrospinal fluid. Domestic and foreign scholars have employed various methods to diagnose MP infection under different laboratory conditions, experimental purposes, and research directions.

3.1 Molecular biological diagnostic method

3.1.1 Nested PCR

Compared with traditional PCR, the nested PCR reaction system contains two primers. Even if the first amplification produces an error fragment, the probability of primer pairing and amplification on the wrong fragment for the second time is extremely low and therefore has a high specificity. In the study by Ursi *et al.* [14], the sensitivity of MP infection detected by nested PCR in three laboratories ranged from 87.2% to 97.4% and the specificity ranged from 89.2% to 97.4%. A high sensitivity and specificity can provide a reference for the early, rapid diagnosis of MP.

3.1.2 Real-time quantitative PCR

Real-time quantitative PCR is a new quantitative test technology that involves using a fluorescent dye or a fluorescently labeled specific probe to label and trace the PCR products. The reaction process is monitored in real time, and the products are analyzed based on the corresponding software; finally, the initial concentration of the sample template to be tested is calculated [15]. Compared with traditional PCR, real-time quantitative PCR has a higher sensitivity and can be used for quantitative analysis, which can be used for early diagnosis and provides data for the prognosis and outcome of disease treatment. When the antibody titer change in paired serum IgG is taken as the gold standard, the sensitivity of real-time quantitative PCR is 81.8% and the specificity is 98.6%. When the IgM antibody titer in the acute phase changes, the sensitivity of real-time quantitative PCR is 91.7%. Thus, real-time quantitative PCR can be used as a diagnostic tool for MP infection [16]. Other studies considered specimen collection time to have an impact on real-time quantitative PCR results. As the disease worsens, the sensitivity of PCR detected by real-time quantitative PCR also decreases [17].

3.1.3 LAMP technology

LAMP is a new technology developed in recent years. It has a high sensitivity and specificity and requires only a simple equipment. The experimental conditions of constant temperature are easy to control and can meet the needs of grassroots and on-site investigation. The LAMP commercial kit has been utilized in Japan, and the pharyngeal swab is an FDA-approved usable specimen. Ratliff *et al.* [18] used the LAMP method to detect alveolar lavage fluid and sputum and to diagnose MP infection, and obtained satisfactory results. Compared with serological diagnostic methods, enzyme immunoassay has a higher sensitivity and specificity of 38.7% and 76.9%, respectively, when used to detect IgM antibody titer and diagnose MP infection. When the PA method is used to diagnose MP infection, the sensitivity and specificity are 19.4% and 93.1%, respectively. LAMP used to diagnose MP infection shows sensitivity and specificity of 96.8% and 100%, respectively, which are significantly superior to those of the serological diagnostic method [19-20]. Taking culture as the gold standard, when LAMP is used to detect MP infection, the sensitivity and specificity are 99.0% and 100%, respectively [18]. If a large-scale standardized clinical trial is conducted to evaluate its clinical value and establish a reasonable fee standard, then it is expected to become a new method for MP diagnosis.

3.1.4 NASBA

NASBA is an RNA-enhancing technique and an enzymatic reaction that is isothermally amplified by a pair of primer-mediated, uniform, *in vitro*-specific nucleotide sequences. The advantage of this method is the amplification of RNA. RNA is less stable than DNA and is more easily disintegrated. Amplified RNA can better reflect the viability of pathogens.

NASBA and real-time quantitative PCR have a good agreement for the diagnosis of MP infection [21].

3.1.5 SAT technology

SAT is a new technology that detects MP based on RNA template. It can reflect the proliferative status and activity of MP in the human body in real time and provides reference for disease staging.

3.1.6 Compound PCR

The method of simultaneously amplifying several gene fragments with multiple sets of primers in the same reaction is called multiplex PCR. This method [17] can simultaneously detect several mycoplasmas or MP and other pathogens that cause lung diseases. Khanna *et al.* [17] reported that the multiplex PCR hybridization method can simultaneously detect five typical pathogens of pneumonia, including MP, with a sensitivity and specificity of 100% and 98.5%, respectively. The authors believe that multiplex PCR hybridization can be taken as a method for screening of MP infection. Loens *et al.* [22] reported that the sensitivity of multiplex PCR detection is less than that of single PCR detection, but the sensitivity of multiplex PCR detection is acceptable, and large-scale clinical trials are required to verify the detection rate of the clinical strain.

3.2 Selection of target gene

Zhou *et al.* [3] used the nested PCR method to compare the significance of P1 encoding gene and 16S rRNA for detecting MP infection. The sensitivity and positive rate of the 16S rRNA gene (54.5%) are higher than those of the P1 protein coding gene (45.5%). Winchell *et al.* [23] reported that in the epidemic of MP outbreak in Atlanta, USA, the sensitivities of the card gene, mp3 gene, and mp7 gene were tested by real-time quantitative PCR. The results confirmed that the sensitivity of the card gene is higher than that of the two genes.

3.3 Selection of specimens

Räty *et al.* [24] studied the PCR method using 16S rRNA as the target gene and detected mp DNA in sputum, pharyngeal swab, and throat swab specimens and diagnosed MP infection. The positive rates were 69.0%, 50.0%, and 37.5% respectively. In general, sputum specimen is superior to pharyngeal and throat swabs. Waites *et al.* [4] found that among serologically diagnosed MP-infected young people, although sputum specimens are superior to nasopharyngeal swab and throat swabs, many children and adults did not have sputum after MP infection. Based on clinical practice, nasopharyngeal and throat swabs are superior to sputum in terms of specimen acquisition. Winchell *et al.* [23] considered that the sensitivity and specificity of nasopharyngeal swab and laryngeal swab are equal. In all aspects, simultaneous detection of nasopharyngeal and throat swabs could improve the diagnostic rate.

Xu *et al.* [25] used the nested PCR method to detect DNA content in throat swab and alveolar lavage fluid to determine the type of specimens suitable for the diagnosis of MP infection. The sensitivity and specificity of throat swab are 78.6% and 63.4%, respectively, whereas those of alveolar lavage fluid are 70.3% and 58.7%, respectively. Considering economic factors, clinical significance, and the degree of injury to the children, we conclude that throat swab is more suitable for the diagnosis of MP infection than alveolar lavage.

3.4. Molecular biological diagnostic method

Although the molecular biological method is useful in the acute phase of MP infection, the MP carrying rate in a healthy population ranges from 0.1% to 13.5% [16], and DNA could exist in the upper respiratory tract for 7 weeks and 7 months [26]. Even if mp DNA can be detected by molecular biology, it cannot be diagnosed as MP infection or MP carrier. Diagnosis should be made in conjunction with clinical symptoms and other diagnostic methods. Saraya *et al.* [27] used PCR to detect throat swab and alveolar lavage fluid to diagnose the MP infection rate in CAP and obtained positive rates of 48.5% and 40.9%, respectively, which are higher than the reported 10.0%–30.0% in most studies. This phenomenon may be caused by the asymptomatic carrying of healthy people or the persistence of mp DNA after infection.

A diagnosis of MP infection based on PCR result alone may produce false-positive results, causing overdiagnosis. At present, laboratories use their own methods to detect MP infection, but a standard method remains to be developed, which complicates evaluation of the laboratory diagnostic method. Chang *et al.* [13] used the same method to detect MP infection in three laboratories. Although the three laboratories were comparable in terms of experience and experimental conditions, the results were still inconsistent. This phenomenon may be caused by inconsistent diagnostic criteria. Thus, multicenter experiments must be conducted, and experimental methods must be evaluated to find the optimal diagnostic method and laboratory condition. Furthermore, a standard for the diagnosis of MP infection must be established.

Nested PCR based on respiratory samples is widely used for the rapid diagnosis of MP infection. However, many factors influence the diagnosis of MP infection by PCR in different individuals. These factors include the age of the patient, the interval between symptoms, the time of specimen collection, the method of sample collection, the target gene of PCR, and the skill level of the experimenter. The immune response to MP infection in patients less than 3 years old is immature, and molecular biological diagnosis may be more accurate than serological diagnosis. In addition, different periods of disease result in different immune statuses and amounts of infectious bacteria. Different diagnostic methods also affect the accuracy of diagnosis. In the early stages of disease, culture and nucleic acid amplification are desirable diagnostic methods due to the time required for the body's immune response. In the later stages, serological diagnosis may be preferable because of the low load of MP in the airway. Some scholars recommend the use of culture and nucleic acid amplification in the early stages and serological test in the later stages of the disease [28-29].

4 Brief summaries

Different diagnostic methods, including isolated culture, serological test, and molecular biochemical test, have their own limitations. At present, two or more diagnostic methods should be used together to improve the accuracy of diagnosis [29]. In the future, we will analyze the inconsistencies in the results of different diagnostic methods, make the necessary adjustments, and comprehensively consider the clinical, economic, and operable issues.

Exploration and development of a rapid, accurate, and economical diagnostic method that is suitable for clinical practice will certainly promote the further development of clinical diagnosis and treatment of MP infection.

In conclusion, other diagnostic methods that are suitable for clinical diagnosis must be explored, and the type of infection and stage of disease must be determined accurately based on clinical symptoms to develop a reasonable treatment plan and select the right drug for achieving the desired therapeutic effect.

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References

[1] Meyer Sauteur P.M., van Rossum A.M., Vink C., *Mycoplasma pneumoniae* in children: carriage, pathogenesis, and antibiotic resistance, CurrOpin. Infect. Dis., 2014, 27(3), 220-227.
[2] Gotoh K., Nishimura N., Ohshima Y., Arakawa Y., Hosono H., Yamamoto Y., et al., Detection of *Mycoplasma pneumoniae* by loop-mediated isothermal amplification (LAMP) assay and serology in pediatric community-acquired pneumonia, J. Infect. Chemother., 2012, 18(5), 662-667.
[3] Zhou Z., Li X., Chen X., Yao L., Pan C., Huang H., et al., Comparison of P1 and 16S rRNA genes for detection of *Mycoplasma pneumoniae* by nested PCR in adults in Zhejiang, China, J. Infect. DevCtries, 2015, 9(3), 244-253.
[4] Waites K.B., Balish M.F., Atkinson T.P., New insights into the pathogenesis and detection of *Mycoplasma pneumoniae* infections, Future Microbiol., 2008, 3(6), 635-648.
[5] Blanco S., Fuenzalida L., Bas A., Prat C., Ramírez A., Matas L., et al., Comparison of 2 molecular assays and a serologic test in diagnosing *Mycoplasma pneumoniae* infection in paediatrics, Diagn. Microbiol. Infect. Dis., 2011, 71(4), 463-466.
[6] Infectious Group, Respiratory Disease Society, Chinese Medical Association. Expert Consensus on Diagnosis and Treatment of Adult Pneumonia and *Mycoplasma Pneumoniae*, Chin. J. Tuberc. Respir. Dis., 2010, 33(9): 643-645.
[7] Infectious Group, Respiratory Disease Society, Chinese Medical Association. Editorial Board of *Chinese Journal of Pediatrics*. Guidelines for the Management of Children Community Acquired Pneumonia (2013 Revision) (I), Chin. J. Pediatr., 2013, 51 (10), 745-752.
[8] Loens K., Goossens H., Ieven M., Acute respiratory infection due to *Mycoplasma pneumoniae*: current status of diagnostic methods, Eur. J. Clin. Microbiol. Infect. Dis., 2010, 29(9), 1055-1069.
[9] Beersma M.F., Dirven K., van Dam A.P., Templeton K.E., Claas E.C., Goossens H., Evaluation of 12 commercial tests and the complement fixation test for *Mycoplasma pneumoniae*-specific immunoglobulin G (IgG) and IgM antibodies, with PCR used as the "gold standard", J. Clin. Microbiol., 2005, 43(5), 2277-2285.
[10] Talkington D.F., Shott S., Fallon M.T., Schwartz S.B., Thacker W.L., Analysis of eight commercial enzyme immunoassay tests for detection of antibodies to *Mycoplasma pneumoniae* in human serum, Clin. Diagn. Lab Immunol., 2004, 11(5), 862-867.
[11] Watkins-Riedel T., Stanek G., Daxboeck F., Comparison of SeroMP IgA with four other commercial assays for serodiagnosis of *Mycoplasma pneumoniae* pneumonia, Diagn. Microbiol. Infect. Dis., 2001, 40(1-2), 21-25.
[12] Lieberman D., Lieberman D., Ben-Yaakov M., Shmarkov O., Gelfer Y., Varshavsky R., et al., Serological evidence of *Mycoplasma pneumoniae* infection in acute exacerbation of COPD, Diagn. Microbiol. Infect. Dis., 2002, 44(1), 1-6.
[13] Chang H.Y., Chang L.Y., Shao P.L., Lee P.I., Chen J.M., Lee C.Y., et al., Comparison of real-time polymerase chain reaction and serological tests for the confirmation of *Mycoplasma pneumoniae* infection in children with clinical diagnosis of atypical pneumonia, J. Microbiol. Immunol. Infect., 2014, 47(2), 137-144.

[14] Ursi D., Ieven M., Noordhoek G.T., Ritzler M., Zandleven H., Altwegg M., An interlaboratory comparison for the detection of *Mycoplasma pneumoniae* in respiratory samples by the polymerase chain reaction, J. Microbiol. Methods, 2003, 53(3), 289-294.
[15] Guo D., Hu W., Li D., Li J., Li S., Wu Z., et al., Establishment of the method for detecting *Mycoplasma Pneumoniae* through fluorescence quantitative polymerase chain reaction, Inf. Infect. Dis., 2016, 29(1), 52-56
[16] Medjo B., Atanaskovic-Markovic M., Radic S., Nikolic D., Lukac M., Djukic S., *Mycoplasma pneumoniae* as a causative agent of community-acquired pneumonia in children: clinical features and laboratory diagnosis, Ital. J. Pediatr., 2014, 40, 104.
[17] Khanna M., Fan J., Pehler-Harrington K., Waters C., Douglass P., Stallock J., et al., The pneumoplex assays, a multiplex PCR-enzyme hybridization assay that allows simultaneous detection of five organisms, *Mycoplasma pneumoniae*, Chlamydia (Chlamydophila) pneumoniae, Legionella pneumophila, Legionella micdadei, and Bordetella pertussis, and its real-time counterpart, J. Clin. Microbiol., 2005, 43(2), 565-571.
[18] Ratliff A.E., Duffy L.B., Waites K.B., Comparison of the illumigene Mycoplasma DNA amplification assay and culture for detection of *Mycoplasma pneumoniae*, J. Clin. Microbiol., 2014, 52(4), 1060-1063.
[19] Thurman K.A., Walter N.D., Schwartz S.B., Mitchell S.L., Dillon M.T., Baughman A.L., et al. Comparison of laboratory diagnostic procedures for detection of *Mycoplasma pneumoniae* in community outbreaks, Clin. Infect. Dis., 2009, 48(9), 1244-1249.
[20] Kakuya F., Kinebuchi T., Fujiyasu H., Tanaka R., Kano H., Genetic point-of-care diagnosis of *Mycoplasma pneumoniae* infection using LAMP assay. Pediatr Int, 2014, 56(4), 547-552.
[21] Templeton K.E., Scheltinga S.A., Graffelman A.W., Van Schie J.M., Crielaard J.W., Sillekens P., et al., Comparison and evaluation of real-time PCR, real-time nucleic acid sequence-based amplification, conventional PCR, and serology for diagnosis of *Mycoplasma pneumoniae*, J. Clin. Microbiol., 2003, 41(9), 4366-4371.
[22] Loens K., Beck T., Ursi D., Overdijk M., Sillekens P., Goossens H., et al., Development of real-time multiplex nucleic acid sequence-based amplification for detection of *Mycoplasma pneumoniae*, Chlamydophila pneumoniae, and Legionella spp. in respiratory specimens, J. Clin. Microbiol., 2008, 46(1), 185-191.
[23] Winchell J.M., Thurman K.A., Mitchell S.L., Thacker W.L., Fields B.S., Evaluation of three real-time PCR assays for detection of *Mycoplasma pneumoniae* in an outbreak investigation, J. Clin. Microbiol., 2008, 46(9), 3116-3118.
[24] Räty R., Rönkkö E., Kleemola M., Sample type is crucial to the diagnosis of *Mycoplasma pneumoniae* pneumonia by PCR, J. Med. Microbiol., 2005, 54(Pt 3), 287-291.
[25] Xu D., Li S., Chen Z., Du L., Reply to "how to diagnose *Mycoplasma pneumoniae* etiology in a child with pneumonia", Eur J Pediatr, 2012, 171(3), 595-596.
[26] Waites K.B., Xiao L., Paralanov V., Viscardi RM, Glass J., Molecular methods for the detection of Mycoplasma and ureaplasma infections in humans: a paper from the 2011 William Beaumont Hospital Symposium on molecular pathology. J Mol Diagn, 2012, 14(5):437-450.
[27] Saraya T., Kurai D., Nakagaki K., Sasaki Y., Niwa S., Tsukagoshi H., et al., Novel aspects on the pathogenesis of *Mycoplasma pneumoniae* pneumonia and therapeutic implications. Front Microbiol, 2014, 5, 410.
[28] Thurman K.A., Walter N.D., Schwartz S.B, Mitchell S.L., Dillon M.T., Baughman A.L., et al. Comparison of laboratory diagnostic procedures for detection of *Mycoplasma pneumoniae* in community outbreaks, Clin. Infect. Dis., 2009, 48(9), 1244-1249.
[29] Qu J., Gu L., Wu J., Dong J., Pu Z., Gao Y., et al., Accuracy of IgM antibody testing, FQPCR and culture in laboratory diagnosis of acute infection by *Mycoplasma pneumoniae* in adults and adolescents with community-acquired pneumonia, BMC Infect. Dis., 2013, 13:172.