International Journal of Clinical and Experimental Medicine Research, 2022, 6(3), 216-221
https: //www.hillpublisher.com/journals/ijcemr/
ISSN Online: 2575-7970
ISSN Print: 2575-7989

Immunological and Molecular Diagnostic Techniques for Leptospirosis: An Update

Mahendra Pal[1,*], Adugna Girma Lema[2], Dagmawit Atalel[3]

[1]Narayan Consultancy on Veterinary Public Health and Microbiology, Anand, Gujarat, India.
[2]Yemalog Walal Woreda Livestock and Fishery Development and Resource Office, Kellem Wollega Zone, Oromia, Ethiopia.
[3]Department of Veterinary Laboratory Technology, School of Veterinary Medicine, Ambo University, Ambo, Oromia, Ethiopia.

How to cite this paper: Mahendra Pal, Adugna Girma Lema, Dagmawit Atalel. (2022) Immunological and Molecular Diagnostic Techniques for Leptospirosis: An Update. *International Journal of Clinical and Experimental Medicine Research*, 6(3), 216-221.
DOI: 10.26855/ijcemr.2022.07.001

Received: April 6, 2022
Accepted: April 29, 2022
Published: May 10, 2022

***Corresponding author:** Mahendra Pal, Narayan Consultancy on Veterinary Public Health and Microbiology, Anand, Gujarat, India.
Email: palmahendra2@gmail.com

Abstract

A number of laboratory techniques are employed to establish the diagnosis of microbial diseases that cause significant morbidity as well as mortality in humans and animals throughout the world. Leptospirosis is an emerging and re-emerging enigmatic zoonotic disease of public health and economic importance. Currently, over 600,000 human deaths are attributed due to leptosprirosis annually in the world. However, there is a lack of information on *Leptospira* strains in remote parts of the world. The diagnosis of leptospirosis is challenging due to non-specific clinical feature. Thus, laboratory tests are necessary to confirm the diagnosis of disease due to its varied symptomatology. The microscopic agglutination test (MAT), which determines agglutinating antibodies in sera for various serovars of *Leptospira* species is considered the gold standard for the diagnosis of leptospirosis. Enzyme linked immunosorbent assay (ELISA) usually detects only the antibodies reacting with a broadly reactive genus specific antigen and thus gives no indication of the causative serovar or serogroup, which limits its application. Polymerase chain reaction on the other hand is considered sensitive and specific for the rapid detection of *Leptospira* in clinical samples. It is imperative to employ immunological and molecular techniques in order to make an unequivocal diagnosis of leptospirosis to institute immediate therapy to mitigate the suffering of the patients.

Keywords

Diagnostic methods, Immunological technique, leptospirosis, Molecular tool, Zoonosis

1. Introduction

Leptospirosis is one amongst the foremost widespread microbial zoonoses within the world. However, there is an absence of knowledge on spirochete strains in remote parts of the world [1]. Leptospirosis is predicted to become a lot of vital due to world climate changes and speedy urbanization in developing countries wherever slum settlements have created the conditions for epidemic rat borne transmission of the disease. In addition, the disease is of great significance in some rural regions because of the exposure to an oversized variety of animal reservoirs [1].

Leptospirosis has been classified emerging or re-emerging communicable disease by the Centers for Disease Control (CDC) and World Health Organization (WHO) [2]. Leptospirosis is often misdiagnosed as a result of its variable and non-specific presentation resembling several common microbial infections like cold, influenza, herpesviruses infections, Hantavirus infection and brucellosis and others [3].

The majorities of leptospirosis patients are not recognized or misdiagnosed due to different causes of an acute

symptom of disease. The shortage of adequate diagnostic assay has more contributed to below reportage of cases yet as deaths. Under recognition of the morbidity and mortality by leptospirosis is so common and has directly contributed to its neglected disease status [4]. The shortage of reliable estimates of the leptospirosis burden has hampered efforts to formulate the investment case to handle key barriers, like improved medical specialty, and determine effective hindrance, and management measures [5].

Timely diagnosis depends on an efficient laboratory diagnosis since the presentation of early-phase of leptospirosis is commonly nonspecific. Diagnosis confirmation is impractical for clinical deciding since it needs analysis of paired bodily fluid samples for correct interpretation and a reference laboratory to perform dark-field microscopy [6].

Comparatively few cases of leptospirosis are recorded from the African continent, chiefly due to difficulties in diagnosis in each human and animals; hence the sickness is not well investigated. However, the prevailing environmental condition and socio economic environments of the African continent are favorable for a high incidence of this disease [7]. Leptospires could also be visualized in clinical material by dark-field microscope or by technique or lightweight research once applicable staining. More or less 10^4leptospires/ml area unit are necessary for one cell per field to be visible by dark-ground microscope (DGM). The diagnosis of blood is valuable solely throughout the primary few days of the acute disease, where as leptospiremia occurs [8].

Leptospira infection in animals and humans could lead to fatal outcomes from the urinary organ and pneumonic infection. Designation of zoonosis is typically accomplished by medical science, because of culture needs each special media and incubation for many weeks. Molecular ways have recently been tried as an alternate to culture. Polymerase chain reaction (PCR) is found to be, specific and speedy technique for detection in addition as differentiation of spirochaete as infective or non-pathogenic compared to clinical methods [9].

Zoonotic importance of leptospirosis is below estimated due to lack of awareness and under recognition through an absence of correct use of diagnostic tools [10].This critical review is aimed to describe different immunological and molecular diagnostic methods of leptospirosis and discuss the relative advantage and limitation of each diagnostic test.

2. Diagnostic Methods for Leptospirosis

Few cases of leptospirosis have been reported from Africa, owing to problems in diagnosing the disease in humans and animals. As a result, the disease remains understudied. However, the African continent's current climatic and socioeconomic circumstances are conducive to a high prevalence of this disease [7]. There is a scarcity of data on circulating *Leptospira* strains around the world. This is especially true in some areas, such as Africa. To gain a better grasp of leptospirosis epidemiology, scientists must isolate *Leptospira* strains, which is difficult because the organisms are detectable in the bloodstream for just a few days first week after the onset of symptoms or transiently in the urine [1].

Culture is the gold standard for diagnosis, and isolation of the organism enables for the identification of the infectious serovar. Culture, on the other hand, is rather insensitive and necessitates several weeks of incubation, limiting its application in most laboratories [11]. According to the WHO guidelines, three types of laboratory tests can be employed to confirm a leptospirosis diagnosis: i) *Leptospira* isolation using culture methods, (ii) DNA detection using PCR, and (iii) antibody detection using MAT [3].

2.1. Immunological methods of diagnosing leptospirosis

The majority of leptospirosis patients are diagnosed using serology. Serologic tests can be performed with either serum or plasma; however, serum should be used more frequently. Around five to seven days after the onset of symptoms, antibodies are evident in the blood. Reactions might last months or even years. As a result, persistent antibodies allow for retrospective identification [8]. Medical science strategies are now required due to the decreasing likelihood of leptospires detection in blood. Immune globulin antibodies appear later than immune globulin during *Leptospira* infection, peaking after a few weeks of illness and remaining at a low level for the rest of the infection [12].

2.1.1. Microscopic agglutination test

The gold standard for leptospirosis diagnosis is the microscopic agglutination test (MAT), which evaluates agglutinating antibodies in sera for distinct serovars of *Leptospira* species [13]. For many years, the microscopic agglutination test (MAT) for leptospirosis antibodies has been used in both human and veterinary medicine to diagnose leptospirosis. Apart from a dark-field microscope, it is a straightforward procedure that distinguishes between

antibodies directed against distinct leptospiral serogroups [14].

The microscopic agglutination test uses a panel of leptospiral strains as antigens for detection of agglutinating antibodies. This assay requires significant expertise to perform, and inter laboratory variation in results is high. Despite these limitations, the MAT has epidemiological value, and it is often used to give an indication of the presumptive serovar or serogroup of leptospires involved in an infection [11]. The microscopic agglutination test requires live cultures of leptospiral bacteria as diagnostic antigens. As diagnostic reagents, these cultures are difficult to standardize. MAT may remain in used widely in countries with limited resources, some of which have wet tropical climates and, consequently, high prevalence of leptospirosis [14].

The results of the MAT can thus give an indication of the infecting serovar, and this is important both for diagnosis and in epidemiological studies [11]. Microspcopic agglutination test is performed according to the Office International des Epizooties, standards (OIE). The samples are tested for the presence of antibodies [15].

2.1.2. Indirect fluorescent antibody test

Specimens of blood, urine and parenchymatous organs are stained with light sera and examined under a fluorescent microscope. The substance protein advanced fluoresces brilliantly, and is visible beneath the magnifier. By this methodology living, dead or perhaps fragmented leptospires will be demonstrated [11]. Indirect fluorescent antibody test (IFAT) is very specific, however, wants visible radiation magnifier and so, cannot be used as a screening test [16].

2.1.3. Macroscopic slide agglutination

Macroscopic slide agglutination test (MSAT) was developed for the identification of leptospirosis. Stoenner and Davis have changed the preparation of plate substances for leptospirosis diagnostic and terminated that this antigen may well be utilized in fast tests, getting similar sensitivities with the MSAT in human, porcine and bovine sera [12]. This test is conducted with a dense suspension of leptospires that agglutinate into clumps visible to the eye. They are performed on slides or plates. Formol killed antigens square measure was mixed with liquid body substance to be tested and viewed with eye for presence or absence of agglutination [17].

The macroscopic slide blood method is inexpensive, can be done much more rapidly and easily than ELISA and MAT, and can be used by laboratories with little resources. It looks to be a simple test for detecting leptospirosis for the first time, with a high sensitivity in the acute part. For early protein identification during the course of the disease, it is far more sensitive than MAT and ELISA. The most significant disadvantages of this test are that it is ineffective for epidemiologic investigations, strain identification, determining the likely infecting serogroup, and confirming ill health for the public health surveillance [12].

2.1.4. Complement fixation test

Complement fixation method was widely used; however, the method was not standardized. Compliment fixation technique was applied to veterinary; however, species-specific variations were noted [15]. Complement fixation is performed victimization either whole leptospiral cells or soluble extracts. Complement fixation test is beneficial in detective work comparatively recent infection. Immunologic response tests have currently been replaced by ELISA method [17].

2.1.5. Indirect hemaggultination assay

Indirect hemaggultination assay could be a straightforward test used for screening however having low sensitivity on samples from acute infections [16]. Indirect hemaggultination assay developed at Center for Disease Control and Prevention was shown to possess a sensitivity of 92% and specificity of 95% compared with the MAT [8]. It will detect IgM and IgG antibodies. The sensitivity of the IHA in populations within which leptospirosis is endemic has varied from smart to poor [18].

2.1.6. Enzyme linked immune sorbent assay

Because of the quality of the MAT, speedy screening tests for leptospiral antibodies in acute infection are developed. Standard medical science strategies like enzyme-linked immunosorbent assay (ELISA) area unit wide used for the diagnosing of *Leptospira* infection [12]. It is a truly sensitive and specific check for the biological diagnosing of leptospirosis. It is of specific price as a medical science screening check due to its relative simplicity as compared with the MAT. ELISA sometimes detects solely the antibodies reacting with a broadly reactive genus-specific antigen and therefore provides no indication of the precipitating serovar or serogroup [17].

A broadly reactive thus known as genus-specific antigen is usually accustomed notice IgM, and sometimes IgG antibodies. The presence of IgM antibodies might indicate current or recent zoonosis, however IgM category anti-

bodies might stay detectable for many years [17]. ELISA is straightforward, safe and might detect IgM and IgG. It will be utilized in humans and animals. ELISA will be performed with less training and generally provides ends up in 2–4 time units. It has been counseled for the speedy diagnosing of leptospirosis in endemic areas [12].

ELISA test systems area unit less specific than the MAT. Weak cross reactions because of the presence of alternative diseases could also be ascertained. ELISA results ought to thus be confirmed by the MAT [17].

ELISA-based testing are less costly than MAT because it does not need the upkeep of live organisms and might be performed in any routine laboratory and more in contrast to the MAT does not need well trained personnel and might be quantitated and is not at risk of inter- observer and intra-observer errors as in the MAT. Therefore, enzyme-linked-immunosorbent serologic assay has been used as an alternate take a look at to the MAT for screening for leptospiral infection in humans and animals. Another advantage of enzyme-linked-immunosorbent serologic assay over the MAT is that the serological response of IgM and Ig will be detected separately [14].

Generally, ELISA serologic assay has the subsequent disadvantages: (i) solely one antigen is employed, specifically the genus-specific antigen that is shared by infective and saprophytic leptospires alike; (ii) since it is supported genus-specific antigen, the enzyme-linked-immunosorbent serologic assay take a look at doesn't offer a sign of infecting serovar [16].

2.1.7. Radioimmuno assay

The detection of leptospiral antigens in clinical material would provide larger specificity than dark-field microscopy whereas having the potential for larger sensitivity. Associate degree analysis of many ways finished that immunoassay might detect 10^4 to 10^5 leptospires/ml. Radioimmuno assay was more sensitive than dark-field research, however, less sensitive than culture [19].

2.2. Molecular Diagnostic Techniques

2.2.1. Polymerase chain reaction

The isolation of leptospires from the clinical specimens needs up to many weeks for growth and current serologic tests exhibit low sensitivity within the acute part that limits their contribution to early diagnosis, need paired sera for definitive serodiagnosis and up to date findings counsel low specificity of those tests to overcome of these limitations, Polymerase chain reaction (PCR) are used that is taken into account sensitive and specific for the fast detection of spirochete in clinical samples [12]. Polymerase chain reaction will speedily ensure the diagnosis within the early part of the infection; once bacterium could appear before protein titers at detectable levels. This technique can be applied to blood, urine, cerebrospinal fluid, and tissue samples [17].

In recent years, PCR has been well tried for the detection of leptospiral DNA in samples like urine and bodily fluid. The success of PCR depends on the method on DNA extraction [20]. The major advantage of PCR is the prospect of confirming the diagnosis during the early acute (leptospiremia) stage of the illness, before the appearance of immunoglobulin M (IgM) antibodies when treatment is likely to have the greatest benefit.

In cases the death occurs before seroconversion, PCR may be of great diagnostic value [11]. The main limitations of PCR-based methods are the need of special equipment, the relatively high cost of the reagents and the absence of automated and standardized procedures allowing the testing of large sets of samples and the inability of most PCR assays to identify the infecting serovar [17].

To gain detection capabilities, the next generation of PCR technology, real-time PCR, was used. In comparison to conventional PCR, real-time PCR has a faster turnaround time and higher sensitivity and fidelity [21]. Real-time PCR technology diagnosis is a relatively new approach of identification. This approach uses a melting curve to differentiate pathogenic *Leptospira* and provides a result considerably faster than convectional PCR and is less prone to contamination, making it a quick alternative for *Leptospira* species detection. A number of real-time PCRs have recently been introduced as a quick, sensitive, and specific technique for detecting leptospires, thus lowering the chance of false positives [22].

Convectional PCR analyses, particularly sequencing, are time consuming, which is problematic if rapid diagnosis is required. Medical and veterinary practitioners must be informed early in the case of infection [23]. The real-time RT-PCR produced 5.6 cycle threshold of positive detection earlier than convectional PCR when the same DNA samples were analyzed in parallel indicating enhanced detection by RT-PCR [21]. The efficacy of real time PCR for the daignosis of leptosprisos has been reported by Fornazari and co-investigators [24]. Recently, Perez and co-workes [25] mentioned that new multiplex qPCR is a dependable tool to diagnose *Leptospira* infections. In addition, this novel assay can be employed for the detection and differentiation of *Leptospira* strains from both pathogenic groups I and II.

2.2.2. In situ hybridization techniques

Molecular detection of leptospires comprises a number in situ hybridization using labeled whole genome DNA or specific DNA segments as probes. In situ hybridization, tissue samples is a useful approach for determining the infection in carriers or confirming leptospirosis in fatal cases but otherwise is not very helpful in diagnosis. The main drawback of DNA hybridization is the use of radio-active isotopes as a label. This requires special safety facilities, and well-equipped laboratories to perform the test [5].

2.2.3. Nucleic acid amplification techniques

Most molecular diagnosis tests rely on amplification of *Leptospira* specific nucleic acids from clinical samples that contain leptospires at an early acute phase of the disease. Such methods include isothermal amplification methods the nucleic acid sequence based amplification (NASBA) in the early 1990's and the recent loop-mediated isothermal amplification (LAMP). NASBA amplifies multicopy RNA, which provides the potential of a high diagnostic sensitivity [5].

The isothermal approach evading the use of expensive and complicated thermal cyclers and the possibility of reading results by eye was used to propagate the method as a simple and affordable diagnostic tool. The limitation LAMP cannot yet compete with the diagnostic sensitivity and specificity of PCR unlike NASBA [5].

The most important advantage of LAMP is the ability to amplify target DNA under isothermal conditions, usually between 60^0C and 65^0C with high specificity, efficiency, and fast speed [18].

2.3. Leptodri dot

Leptodri Dot is a new card agglutination test developed by the Dutch Royal Tropical Institute for rapid diagnosis of leptospirosis. The method does not require special place or sophisticated equipment and can be conducted by relatively low skilled personnel [26].

3. Conclusions

The diagnosis of leptospirosis has a challenge because organism isolation is frequently required, which takes time. Fast diagnostic tests of leptospirosis ought to ideally be correct, easy to use, comparatively cheap, straightforward to interpret, stable beneath extreme conditions with very little or no process and provides the results at intervals 1-2 hrs. The microscopic blood test (MAT) for leptospirosis antibodies may be an easy technique and needs very little valuable instrumentation, aside from a dark field microscope. MAT employed in indicating serovar that is concerned with leptospirosis; however, it is not standardized as a result of live *Leptospira* used as matter. ELISA has very sensitive and specific check for the biological diagnosis of leptospirosis, however, with some limitations including principally non-pathogen antigen was used, thus this test might not identify all infecting serovars.

The most important advantage of PCR is that the prospect of confirming the diagnosis throughout the first acute (leptospiremia) stage of the illness, before the appearance of immunoglobulin (IgM) antibodies once treatment is probably going to possess the best profit. Real time PCRs is additionally a fast, sensitive and specific tool for leptospires detection, reducing the danger of false positive results by carryover contamination. During conducting ELISA test, cross-reactions because of the presence of alternative diseases could also be discovered. Therefore, assay results ought to be confirmed by the MAT. It is emphasized that ELISA test is simple and rapid for *Leptospira* diagnosis besides its limitation in specification. However, real time PCR (RT PCR) assays have high sensitivity and specificity for diagnosis of leptospirosis so that they are preferably and commonly used widely.

Acknowledgements

We wish to thank to Prof. Dr. R. K. Narayan for his suggestions during the preparation of manuscript and Anubha Priyabandhu for computer help. The paper is dedicated to all the scientists who did pioneer and outstanding work in the field of leptospirosis.

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