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Review Article

Microbiology Section

Leptospirosis Diagnosis: Competancy of Various Laboratory Tests

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ABSTRACT

Leptospira can be found in virtually all tropical and temperate areas of the world and is presumed to be the most wide spread zoonoses in the world.Humans contact leptospirosis through mucosal or percutaneous exposure to leptospires in environments contaminated by the urine of chronically infected animal sources. Despite being common, the diagnosis of leptospirosis is often not made unless a patient presents with textbook manifestations of the so called Weil's disease, such as fever plus jaundice, renal failure and pulmonary haemorrhage. Leptospiral infection often has minimal or no clinical manifestations; of the cases in which fever develops, as many as 90% are undifferentiated febrile illnesses. Because of the variety of clinical symptoms seen in the symptomatic cases, leptospirosis at its onset is often misdiagnosed as aseptic meningitis, influenza, hepatic disease or fever (pyrexia) of unknown origin. Moreover, clinicians may fail to recognize that transmission of leptospirosis can occur in the urban setting because it is incorrectly perceived to be a rural disease. Therefore, diagnosis is based on laboratory tests rather than on clinical symptoms alone. In developing countries, laboratory facilities may be inadequate for diagnosis despite a high prevalence of the disease. Of substantial clinical importance, the syndrome of leptospiral pulmonary haemorrhage has emerged in recent years, in diverse places around the world.

Keywords: Leptospirosis, Weil's disease, PCR (Polymearase Chain Reaction), MAT (Microscopic Agglutination Test)

INTRODUCTION

Leptospirosis is presumed to be the most wide spread zoonoses in the world [1]. The disease leptospirosis is described as an occupationally transmitted disease. Humans contract leptospirosis through contaminated urine of chronically infected animal domestic or agricultural rodents, dogs, pigs and cattle [2]. Environmental conditions are an important influence on the incidence of leptospirosis; the disease is rare in deserts, common in warm, humid, tropical areas and seasonal rains and severe weather are associated with increased frequency of disease. Leptospirosis is found in a wide variety of environmental contexts, in industrialized and developing countries, and in urban and rural contexts [3]. In India, outbreaks have been reported related to heavy rainfall in various parts of the country. In South-India, suspected cases are reported between June and October due to heavy rains and floods. Leptospirosis has been consistently reported from the Andaman and Nicobar group of Islands (thus called 'Andaman Haemorrhagic Fever') West Bengal, Kerala and Coastal Karnataka, India [4,5].

DISCUSSION

Leptospirosis at its onset is often misdiagnosed as aseptic meningitis, influenza, hepatic disease or fever (pyrexia) of unknown origin [6]. Despite being common, the diagnosis of leptospirosis is often not made unless a patient presents with textbook manifestations of the so called Weil's disease, such as fever plus jaundice, renal failure and pulmonary haemorrhage. Leptospiral infection often has minimal or no clinical manifestations; of the cases in which fever develops, as many as 90% are undifferentiated febrile illnesses. Moreover, clinicians may fail to recognize that transmission of leptospirosis can occur in the urban setting because it is incorrectly perceived to be a rural disease. Therefore, diagnosis is based on laboratory tests rather than on clinical symptoms alone. In developing countries, laboratory facilities may be inadequate for diagnosis despite a high prevalence of the disease. Of substantial clinical importance, the syndrome of leptospiral pulmonary haemorrhage has emerged in recent years, in diverse places around the world.

Two important issues continue to confront clinicians regarding leptospirosis. The first is how to reliably establish the diagnosis. The most common way to diagnose leptospirosis is through serological tests either the Microscopic Agglutination Test (MAT) which detects serovar-specific antibodies, or a solid-phase assay for the detection of Immunoglobulin M (IgM) antibodies. Leptospira are present in the blood until they are cleared after 4-7 days following the production of Leptospira-specific antibodies, initially mainly of the IgM class [7,8]. However, the greatest drawback of IgM detection assays is that IgM antibodies can persist for many months raising the questions about whether a positive IgM result accurately identifies a current infection [9].

The MAT is the cornerstone of the serodiagnosis for leptospirosis, because this assay has a high sensitivity and allows for the detection of group specific antibodies [10]. Two major disadvantages of this test are that in regions where leptospirosis is common, there may be a substantial proportion of the population with elevated titres of MAT and secondly, the performance of MAT is restricted to laboratories that are capable of maintaining strains for the preparation of live antigens [11]. Therefore, serological tests remain suboptimal for clinical use in diagnosing leptospirosis as depicted in [Table/Fig-1]. The most promising diagnostic methods are those that demonstrate the presence of the organisms.

Culture of Leptospira is difficult for a variety of reasons. The process is very laborious, and can take up to three months [12]. Therefore, isolation and culture are primarily used for retrospective diagnosis. Moreover, to culture the organism from tissues or body fluids, knowledge of the stage of infection is critical. In the acute phase, which lasts for about 10 days, the leptospires can often be cultured from blood or cerebrospinal fluid (CSF). Usually, when a specific antibody response is detected (at approximately 10 days), leptospires disappear from the blood. During the second phase, which may last up to several months, bacteriuria is often intermittent.

Molecular techniques to detect the presence of leptospiral DNA in blood, urine or spinal fluid have shown to be sensitive and specific;

Test	Advantages	Disadvantages
Dark Field Microscopy (DFM)	Visualize Leptospirosis	Lack of sensitivity and specificity. 104 Leptospires/ml is necessary for one organism/field to be visible under DFM.
IgM ELISA	Most widely used	False positive, IgM cannot be detected in early stages of infection and canpersist in blood for years.
Microscopic Agglutination Test (MAT)	Gold Standard	Less sensitive in early phase of disease. Labor intensive and complicated procedure as there is a need to maintain Leptospira strain for preparing liveantigen.
Polymerase Chain Reaction (PCR)	Successful in detecting Leptospira DNA in serum and urine samples of patients	Expensive reagents, Requires large quantity of DNA. Cannot identify the infecting serovar.

[Table/Fig-1]: Advantages and disadvantages of diagnostic tests for the detection of Leptospirosis

Sensitive assay for the detection of Leptospira DNA that is based upon amplification of the Leptospirarrs (16S) gene have been developed [13]. The data suggest that the PCR assay can be used on biological samples such as CSF, urine, or blood as a diagnostic tool for cases of suspected leptospirosis. The use of this technique is precluded by the cost and technical factors in non-reference laboratories.

HISTORICAL ASPECTS

Leptospirosis is an emerging infectious disease caused by pathogenic species of the genus Leptospira that affects domestic and wild animals worldwide [14]. The classical description of leptospirosis is that of Weil's disease, a dramatic acute febrile and sometimes epidemic illness characterized by jaundice, splenomegaly and nephritis. This discovery by Weil in 1886, though not the first of its type, antedated the discovery of the infectious agent by about 30 years when it was discovered and described simultaneously in 1915 by Inada & Ido in Japan and Uhlenhuth & Fromme in Europe [15]. Stimson demonstrated by silver staining clumps of organisms with hooked ends and named them Spirochaetainterrogans for their resemblance to a question mark [16]. It affects more than 160 mammalian species, rodents being the most important reservoir, though other animals are also affected.

TAXONOMY AND CLASSIFICATION

The genus Leptospira belongs to the Leptospiraceae family of the order Spirochaetales. The nomenclature system used to organize leptospires has been revised, making review of the literature often confusing. The traditional system divided the genus into two species: the pathogenic Leptospirainterrogans and the nonpathogenic Leptospirabiflexa. These species were divided further into serogroups, serovars, and strains, based on shared antigens. L interrogans included more than 250 serovars that constitute 25 serogroups [17].

DISEASE ONSET AND PROGRESS

Leptospiraare excreted in the urine of the infected host and can survive in soil and infect a susceptible host by penetration through abraded skin, mucosa, conjunctiva, or intact skin after prolonged immersion in water, and ingestion through water or food, droplet infection [18]. Animals, including humans can be divided into maintenance host and accidental host. The disease is maintained in nature by chronic infection of the renal tubules of maintenance hosts [19]. Incubation period varies from 4-20 days though it usually manifests within 6-8 days. Occupation is a significant risk factor for humans [20]. Direct contact with infected animals accounts for most infection in farmers, veterinarians, abattoir workers, meat inspectors, pest control workers etc. Indirect contact is important for sewer workers, miners, soldiers, rice field workers etc [21].

PATHOPHYSIOLOGY

Leptospire invasion across the epithelium is followed by proliferation and widespread dissemination. Every major organ system may be affected, and leptospire antigens can be detected in affected tissues. Leptospire-mediated injury characterizes the initial phase of the disease. A host-immune response marks onset of the second phase of symptoms [22].

CLINICAL FEATURES OF LEPTOSPIROSIS

Symptoms

Symptom onset often occurs abruptly after the 2- to 20-day incubation period. Direct tissue injuries from leptospire invasion and toxins, which have been theorized yet never clearly elucidated, characterize the acute phase. Symptoms then abate with cessation of the systemic proliferation of leptospires.

The second or immune phase is characterized by increasing antibody titers and inflammatory infiltration of affected organ systems. Aseptic meningitis and renal dysfunction are hallmarks of the immune phase. Symptoms may persist for 6 days to more than four weeks, with a mean duration of 14 days.

Approximately 10% of patients diagnosed with leptospirosis develop signs of Weil disease. The classic definition of Weil disease is severe leptospirosis presenting with jaundice, renal failure, and pulmonary hemorrhage. Mortality rates among these patients is 10%, despite care in an Intensive Care Unit (ICU), and even higher in regions with less sophisticated care. Severe, fatal cases of leptospirosis may occur without associated jaundice.

In both children and adults, leptospirosis commonly presents with fever, myalgia, and headache. Lethargy, emesis, abdominal pain, photophobia, arthralgia, cough, diarrhea, or constipation also may occur. The differential diagnosis for these symptoms is confounding and ranges from benign viral syndromes of childhood to meningitis and sepsis [23].

Laboratory diagnosis of leptospirosis

Laboratory diagnosis of leptospirosis is mandatory because the clinical picture is not specific in either humans or animals, moreover, in endemic regions, existence of similar infections can cause confusion in the diagnosis.

The various diagnostic tools available for the detection of leptospirosis are enumerated hereunder.

General Clinical Laboratory Findings

A. Erythrocyte Sedimentation Rate is elevated, WBC counts range from below normal to moderately elevated.

B. Liver Functions Tests show an elevation in aminotransferases, bilirubin and alkaline phosphatase, hyperbilirubinaemia is out of proportion to jaundice in cases of icteric leptospirosis.

C. Renal Function Tests are usually impaired as indicated by raised plasma creatinine.

D. Urine Analysis demonstrates proteinuria, pyuria, microscopic haematuria, hyaline and granular casts.

E. Lumbar Puncture reveals an elevated CSF pressure, predominance of lymphocytes and polymorphs.

F. Peripheral Blood Smear shows peripheral leukocytosis with shift to left and thrombocytopenia.

DIRECT DIAGNOSTIC METHODS

Microscopy

Direct Microscopic observation is used to detect leptospires in body fluids, check culture growths etc. Dark Field Microscopy is the usual method, but immunostaining is useful in certain special circumstances

Darkfield and phase contrast: Leptospires are seen as thin, bright,

actively motile rods, moving with characteristic rapid spinning and jerking motility. Approximately, 10 leptospires/mL are necessary for one cell per field to be visible by darkfield microscopy. However, the positivity of darkfield microscopy decreases from 100% to 90.9% with increase in the duration of infection for greater than 1 week. Another disadvantage of this technique is that both false positive and false negative diagnosis can be easily made even in experienced hands [24].

Histochemical stains: A variety of histopathological stains have been used for the detection of leptospires in clinical specimens. The first to be used were the silver stains. The Wrthin-Starry stain is widely used now [25].

Immunostaining: It may be used to find leptospires where they are scarce, or where there is material that precludes the use of darkfield microscopy. But any immunosatin requires a primary antibody specific for the serovar being sought, on its own or in a pool or composite mixture of antibodies to different serovars. Too many varieties in a pool will dilute any one, so high titre antisera conjugates are required. In other words, it may be not be advantageous in early infections [26].

CULTURE

Fluid media are used for primary culture. Greater yields and faster growths are obtained in Tween (oleate)-albumin media such as EMJH (Ellinghausen, McCullough, Johnson, Harris) than media with rabbit serum (8-10% v/v). Media with rifampicin, neomycin, actidione are used for primary isolation from contaminated samples. The culture of these organisms takes almost 3 months and is thus, impractical for immediate diagnosis. The organism has a relatively long doubling time (6-8 hours or more). Additionally, they are highly infectious organisms requiring 'Biosafety level II' facilities [27].

MOLECULAR METHODS

Direct Polymerase Chain Reaction (PCR) on specimens enables rapid and direct diagnosis, at least in the early and convalescent stages of infection. The reaction detects leptospiral DNA in the specimen, down to extremely small amounts equivalent to the DNA content of about 10 leptospires or less. A limitation of PCR-based diagnosis of leptospirosis is the inability of most PCR assays to identify the infecting serovar [28].

A study on 103 patients of meningitis of unknown cause showed that 39.08% were positive by PCR, 3.88% by ELISA & 8.74% by MAT [29].

Nested PCR and PCR/RFLP for 16S ribosomal RNA gene amplification.

Leptospiral genomic DNA was extracted from suspected human serum samples. The DNA was air-dried, dissolved in TE buffer (10 mMTris-HCl, pH 8.0, 0.1 mM EDTA), and kept at –20°C until use.The DNA was quantified by agarose gel electrophoresis and spectrophotometrically by calculating the A 260 /A 280 ratios and the A 260 values to determine protein impurities and DNA concentrations. Leptospira DNA was amplified by using the primers.These primers amplified all pathogenic and non-pathogenic Leptospira species [30].

SEROLOGICAL AND OTHER INDIRECT METHODS

Most cases of leptospirosis are diagnosed by serology. Antibodies can become detectable by the 6th to 10th day of disease and reach peak levels within three to four weeks. Antibody levels may then gradually decline but remain detectable for years.

Microscopic Agglutination Test (MAT) [31]

The MAT is a sensitive assay, but because of the antigenic heterogeneity of Leptospira spp. requires a large number of serovars asantigens. In addition, it would not be useful at the early stages of the disease when the antibody to Leptospira spp. is not present or, if present, is at a low level in the CSF. Positive results are defined as a 4-fold rise in titer between acute and convalescent specimens. A single titer exceeding 1:200 or serial titers exceeding 1:100 suggest leptospirosis, but neither is diagnostic. Some patients have serological evidence of previous infection with a different leptospiralsero group. In these cases, serological diagnosis is complicated further by the "anamnestic response", in which the first rise in antibody titre is usually directed against the infecting serovar from the previous exposure.

Enzyme Linked Immunosorbent Assay (ELISA)

This test relies on the detection of IgM antibodies which appear in the blood a day or so earlier than those used in MAT. There is often poor correlation between MAT and ELISA results on sera of individuals. The reference standard is MAT, IgM antibodies become detectable during the first week of illness, allowing the diagnosis to be confirmed and treatment initiated while it is likely to be most effective though, antibody levels are generally low or absent during very early infection [32,33].

Though Microscopic agglutination test is considered to be the gold standard in the diagnosis of leptospirosis, its use as a routine diagnostic test in a clinical laboratory is limited. The test is both complex and tedious for routine use. Many studies have demonstrated Pan Bio ELISA to be more sensitive than MAT for detection of cases early in acute illness [34]. IgM antibodies start appearing during the first week of illness though antibody levels are low or not detectable very early on in the illness. Leptospirosis can be diagnosed on the basis of the presence of IgM antibodies by Pan Bio ELISA, in a single serum sample collected during the acute phase of the illness. A convalescent sample taken after two weeks is required to confirm the results. A limitation of using a single serum sample in the demonstration of IgM antibodies is the absence of antibodies very early on in the infection or the persistence of antibodies. IgM antibodies in leptospirosis persist for a long period with varying rates of decline [35]. A single serum sample taken during an acute febrile illness with symptoms of leptospirosis is presumptive evidence of infection, and therefore requires confirmation by further testing.

The bacterial concentration is less in serum than fresh blood. Studies comparing the PCR and IgM have demonstrated PCR alone to be less sensitive than serological tests over the course of the disease; it was the most sensitive method in those samples with no demonstrable antibodies collected during the very early stages of the disease [36,37]. Therefore use of PCR in combination with IgM ELISA would improve the sensitivity of the diagnosis of leptospirosis in the first phase of the disease.

Testing an in-house ELISA with formalin-treated and boiled bacteria from the intermediate species Leptospirafainei as an antigen to detect Leptospira-specific IgM antibodies. The samples, tested by a MAT as a reference test, were used to evaluate the ELISA. The kappa value was 0.92 (95% confidence interval 0.88–0.96), which indicated excellent agreement between the MAT and ELISA. The overall performance of this in-house ELISA suggests applicability as a rapid screening test for the diagnosis of leptospirosis in resource-limited settings and in hospitals and laboratories where a MAT is not available [38].

Indirect Haemagglutination Assay (IHA)

IHA testing is a rapid and easily performed method of diagnosis that is based on genus-specific antibodies. However, contrasting results have been obtained through various studies done to find the sensitivity and specificity of IHA in early infections. It has been shown to have a sensitivity of 92% and specificity of 95% compared with MAT. It can be concluded that IHA has a very limited scope in diagnosing Leptospirainfections before 8days [39].

Leptodipstick Assay

This is an assay that detects Leptospira-specific IgM antibodies in human sera [40].

CONCLUSION

When using a single sample collected during the early, acute phase of the disease, results of Pan Bio IgM ELISA can give us a presumptive diagnosis of leptospirosis. Very early on in the infection it may even fail to detect the presence of antibodies. PCR is a sensitive and specific technique which can detect the presence of DNA in the very early stage of the disease, so PCR together with IgM ELISA can be used to confirm the diagnosis, early on in the acute stage of the infection.

REFERENCES

[1] Wu W, BaoL, WuQ, LiS, Huang W. World Health Organization. Leptospirosis worldwide, *Wkly Epidemiol Rec.* 1999; 74: 237–242.647.
[2] Rebecca P, Deborrah D. Overview of the epidemiology, microbiology, and pathogenesis of Leptospira spp. in humans. *Microbes Infect.* 2000; 2: 1265-76
[3] Everard JD and Everard COR. Leptospirosis in the Caribbean. *Rev. Med. Microbiol.* 1993; 4: 114-22.
[4] John JT. Emerging and reemerging bacterial pathogens in India. *Indian J Med Res.* 1996; 103: 4-18.
[5] Roy S, Biswas D, Vijayachari P, Sugunan AP, Sehgal SC. A clone of Leptospirainterroganssensustricto is the major cause of leptospirosis in the Archipelago of the Andaman & Nicobar Islands, India. *Letters in Applied Microbiology.* 2005; 41; 179-85.
[6] Turner LH. Leptospirosis I. *Trans R. Soc Trop Med Hyg.* 1967; 61: 842–55.
[7] Chernukha YG, Shishkina ZS, Baryshev PM, Kokovin IL. The dynamics of IgM and IgG antibodies in leptospiral infection in man. *Zentralbl. Bakteriol.Mikrobiol. Hyg.* 1976; 236: 336–43.
[8] Silva MV, Camargo ED, Batista LA et al. Behaviour of specific IgM, IgG and IgA class antibodies in human leptospirosis during the acute phase of the disease and during convalescence. *J. Trop. Med. Hyg.* 1995; 98: 268–72.
[9] Cumberland PC, Everard COR, Wheeler JG, Levett PN. Persistence of anti-leptospirallg M, IgG and agglutinating antibodies in patients presenting with acute febrile illness in Barbados 1979–1989. *Eur J Epidemiol.* 2001; 17: 601–08.
[10] Brandão AP, Camargo ED, da Silva ED, Silva MV, Abrão RV, Macroscopic agglutination test for rapid diagnosis of human leptospirosis. *J ClinMicrobiol.* 1998; 36: 3138–42.
[11] Turner LH. Leptospirosis II. Serology. *Trans. R. Soc. Trop. Med.Hyg.* 1968; 62: 880–89.
[12] Pike RM. Laboratory-associated infections: summary and analysis of 3921 cases. *Health Lab. Sci.* 1976; 13: 105–14.
[13] Merièn P Amouriaux, Perolat P, Branton G, and Saint IG. Polymerase Chain Reaction for detection of Leptospira spp. In clinical samples. *Journal of Clinical Microbiology.* 1992; 30; 2219-24.
[14] World Health Organization. Leptospirosis worldwide. *Epidemiol. Rec.* 1999; 74: 237-42.
[15] Uhlenhuth P, Fromme W. Experimentelle Untersuchungenu¨ber die sogenannte Weilsche Krankheit (ansteckende Gelbsucht). *Med Klin.* 1915; 44: 1202–03.
[16] Stimson, AM. Note on an organism found in yellow-fever tissue. *Public Health Rep.* 1907; 22: 541-542a.
[17] Kmety E, and Dikken H. Classification of the species Leptospirainterrogans and history of its serovars. University Press Groningen, Groningen, The Netherlands. 1993.
[18] Diesch SL and McCulloch WF. Isolation of pathogenic leptospiresv from waters used for recreation. *Public Health Rep.* 1966; 81: 299–304.
[19] Babudieri, B. Animal reservoirs of leptospirosis. *Ann NY Acad Sci.* 1958; 70: 393–413.
[20] Waitkins, SA. Leptospirosis as an occupational disease. *Br J Ind Med.* 1986; 43: 721–25.
[21] Terry J, Trent M, Bartlett M. A cluster of leptospirosis among abattoir workers. *Commun Dis Intell.* 2000; 24: 158–60.
[22] Kelley PW. 1998. Leptospirosis, p. 1580–1587. In S. L. Gorbach, J. G.Bartlett, and N. R. Blacklow (ed.), Infectious diseases, 2nd ed. W. B.Saunders, Philadelphia, Pa.
[23] Edwards GA, Domm BM. Human leptospirosis. *Medicine.* 1960; 39: 117–56.
[24] Turner LH. Leptospirosis III. Maintenance, isolation and demonstration of leptospires. *Trans R Soc Trop Med Hyg.* 1970; 64: 623–46.
[25] Ellis WA, Brien JJO, Neill SD, Ferguson HW, Hanna J. Bovine leptospirosis: microbiological and serological findings in aborted fetuses. *Vet. Rec.* 1982; 110: 147–50.
[26] Uip DE, Amato Neto V and Duarte MS. Diagnosticoprecoce da leptospirosepordemonstracao de antigenosatrave´s de exameimuno-histoqui´minoemmu´sculo da panturrilha. *Rev Inst Med Trop Sao Paulo.* 1992; 34: 375–81.
[27] Johnson RC and Harris VG. Differentiation of pathogenic and saprophytic leptospires. 1. Growth at low temperatures. *J Bacteriol.* 1967; 94: 27– 31.
[28] Bal AE, Gravekamp C, Hartskeerl RA, MezaBrewster JD, Korver H, Terpstra WJ. Detection of leptospires in urine by PCR for early diagnosis of leptospires. *J Clin Microbiol.* 1994; 32: 1894-98.
[29] Romero EC, Billerbeck AEC, Lando VS, Camargo ED, Souza CC, Yasuda PH. Detection of leptospira DNA in patients with Aseptic meningitis by PCR. *J Clin Microbiol.* 1998; 36: 1453-55.
[30] Sedigheh Z, Neda S, Mandana A. Molecular epidemiology of leptospirosis in northern iran by nested polymerase chain reaction/restriction fragment length polymorphism and sequencing methods. *Am J Trop Med Hyg.* 2010; 82(5): 899–903.
[31] Galton MM, Powers DK, Hall AM and Cornell RG. A rapid microscopic-slide screening test for the serodiagnosis of leptospirosis. *Am J Vet Res.* 1958; 19: 505–12.
[32] Terpstra WJ. Ligthart GS, and Schoone GJ. ELISA for the detection of specific IgM and IgG in human leptospirosis. *J. Gen. Microbiol.* 1985; 131: 377–85.
[33] Terpstra WJ, GS Ligthart, GJ Schoone. 1980. Serodiagnosis of human leptospirosis by enzyme-linked-immunosorbent-assay (ELISA). *Zentbl. Bakteriol.* 247: 400–05.
[34] Levett PN, Branch SL, Whittington CU, Edwards CN, Paxton H. Two methods for rapid serological diagnosis of acute leptospirosis. *Clin Diagn Lab Immunol.* 2001; 8: 349–51.
[35] Ahmed SN, Shah S, Ahmed FMH. Laboratory diagnosis of leptospirosis. *J Postgrad Med.* 2005; 51: 195-200.
[36] Ooteman MC, Vago AR, Koury MC. Evaluation of MAT, IgM ELISA and PCR methods for the diagnosis of human leptospirosis. *Journal of Microbiological Methods.* 2005; 65 (2) 247-57.
[37] Fonseca de A C,Teixeira de Freitas VL. Polymerase chain reaction in comparison with serological tests for early diagnosis of human leptospirosis. *Trop Med Inst Health.* 2006; 11(11): 1699-707.
[38] Pascale B, Muriel V, Mathieu P. Evaluation of an in-house ELISA using the intermediate species Leptospirafainei for diagnosis of leptospirosis. *J Med Microbiol.* 2013; 62: Pt_6 822-27.
[39] Imamura S, Matsui H, Ashizawa Y. Indirect hemagglutination test for detection of leptospiral antibodies. *Jpn J Exp Med.* 1974; 44: 191–97.
[40] Gussenhoven GC, Menno AWG van Der Moorn, Marga GA, Goris WJ, Terpstra RA et al. LEPTO Dipstick, a dipstick assay for the detection of Leptospira-specific IgM antibodies in human sera. *Journal of Clinical Microbiology.* 1997; 35; 92-97.

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