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.

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. Leptospiros 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 several 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, bacteruria 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;
PATHOPHYSIOLOGY
Leptospirosis 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, hyperbilirubinemia 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].

Histological 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 Wthrn-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 leptospiiral 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 as antigens. 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 Leptospirafaminei 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 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