



Information for Clinical and Laboratory Support for Diagnosis, Management and Treatment of Leptospirosis in the Aftermath of a Disaster

What is the risk of leptospirosis outbreaks after a disaster?

Outbreaks of leptospirosis are caused by exposure to water contaminated with the urine of infected animals, including rodents, cattle, pigs, horses, dogs, and wild animals; animals may be infected without apparent symptoms. Flooding can increase the risk of disease in animals and humans. Humans become infected through contact with water, food, or soil contaminated with urine from infected animals, or contact with urine or tissues of infected animals. *Leptospira*, the causative agents of leptospirosis, enter the body through cut or abraded skin and mucous membranes, such as the eyes or nose. Drinking or eating contaminated water or food may also cause infection. The disease is not known to be spread from person to person.

Human infection can occur following contact with contaminated waters and persons boating, wading or immersed in the floodwaters may be exposed, including both rescuers and evacuees. Many of these persons have cuts and abrasions which increases the risk for infection.

What are the clinical presentations associated with leptospirosis?

The incubation period is usually 5-14 days but ranges from 2-30 days. The illness lasts from a few days to 3 weeks or more.

Illness usually begins abruptly with fever and other symptoms. Leptospirosis may present with a wide variety of clinical manifestations, ranging from a mild flu-like illness to a serious and sometimes fatal disease. It may also mimic many other diseases, such as dengue fever and other viral hemorrhagic diseases. Icterus (jaundice) is a common symptom of leptospirosis but is also found in many other diseases such as hepatitis, and many of the symptoms of leptospirosis can be mistaken for other diseases, including influenza and other common viral infections and tropical diseases such as dengue fever, malaria, and typhoid.

Leptospirosis may occur in two phases. Symptoms during the first phase include fever, chills, severe headache, muscle aches, vomiting, or diarrhea. They may also include jaundice, conjunctival suffusion, abdominal pain, or a rash. The patient may recover for a time but become ill again. If the second phase occurs, the disease is more severe. If left untreated, the patient could develop kidney damage, meningitis or meningoencephalitis, liver failure, and pulmonary hemorrhage with respiratory failure. Finally, leptospirosis may present as a combination of symptoms known as Weil's syndrome, characterized by jaundice, renal failure, hemorrhage and myocarditis with arrhythmias. Case fatality ratios are usually low but may reach 20% in patients with severe illness.

Persons such as those exposed to the floodwaters following a hurricane who might be at increased risk for leptospirosis and who have a fever should see a doctor. Leptospirosis should be considered as a possible cause of acute febrile illness.

How is leptospirosis diagnosed?

Clinical diagnosis is difficult because of the varied and non-specific presentation. The diagnosis of leptospirosis should be considered in any patient presenting with an acute febrile illness with fever, chills,

conjunctival suffusion, headache, myalgias and/or jaundice. The diagnosis is more difficult when patients present with symptoms of cough, dyspnea, nausea, vomiting, abdominal pain, diarrhea, arthralgias and a skin rash. Conjunctival suffusion and muscle tenderness, most notable in the calf and lumbar areas, are the most distinguishing physical findings.

Any patient meeting the following clinical criteria with exposure to flood waters should be tested.

Clinical criteria for leptospirosis:

1. Fever > 38.0°C (100.4°F)
2. AND at least TWO from COLUMN A or ONE from COLUMN B

COLUMN A
Headache
Myalgias
Jaundice
Chills/Rigors
Skin rash
Conjunctival suffusion without purulent discharge

COLUMN B
Aseptic meningitis
Acute renal failure
Hemorrhagic pneumonitis
Cardiac arrhythmias, EKG abnormalities
Liver failure
Jaundice with acute renal failure (Weil's disease)

What laboratory tests are used to diagnose leptospirosis?

Serologic testing should be requested for patients in whom there is a high suspicion for leptospirosis, based on signs and symptoms and on assessment of exposure to animals or environments contaminated with animal urine, such as exposure to floodwaters following a hurricane.

Instructions for collecting and storing serum specimens and on shipping serum samples to CDC, are included in Appendix 1.

Presumptive Laboratory Testing:

Leptospirosis is presumptively diagnosed by laboratory testing of a serum specimen. Rapid and reliable serologic tests are simple to do and commercially available. CDC can provide *Leptospira* Dip-S-Tick (DST) IgM dot-ELISA test kits (PanBio Integrated Diagnostics) to local laboratories for serological testing of suspected leptospirosis cases in support of the response to a hurricane. The DST is suitable for in-the-field testing of suspected patients; confirmatory testing is available at CDC.

Performance characteristics

In a recent study comparing this and three other rapid diagnostic tests with microscopic agglutination testing, the standard reference test for leptospirosis, the DST had the following characteristics¹:

- The highest reported sensitivity (95.5%) and specificity (98.8%)
- Sensitivity of 50% when testing acute phase sera alone (obtained within 14 days of illness onset)
- Ability to detect antibodies in some cases as early as 3 days following symptom onset
- The least cross-reactivity resulting from other infectious and autoimmune diseases

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- Good correlation with MAT testing results
- Easy to perform, yielding results in one hour.
(¹Bajani *et al. J Clin Micro* 2003:803-809.)

Requirements for Testing

The package insert outlines the test characteristics and instructions for performing them. They are easy to do, provided pipettes and a water bath/heating block are available.

1. Water bath or heating block at 50°C
2. Pipettes (10ul, 2ml)
3. Tube rack (for cuvettes if using water bath)
4. Thermometer (reaction temperature must be monitored)
5. Beaker or vessel for rinsing sticks
6. Deionized or distilled water (for rinsing sticks).

Test Procedure and Interpretation

A detailed protocol is included with each kit. Also see the attached bench protocol (Appendix 2).

A visible dot with circumscribed edges is considered a positive reaction.

No dot or a difficult to see dot is a negative reaction.

Two dots or more are considered presumptive evidence of leptospirosis. The assay detects IgM antibody however, *Leptospira* specific IgM antibodies have been shown to persist more than a year after infection.

Confirmatory Laboratory Testing:

Specimens testing positive by the rapid DST assay should be sent to CDC for confirmation testing using the microscopic agglutination test (MAT). In an outbreak setting, an MAT titer of 400 or greater in a person with a compatible illness is highly suggestive of acute leptospirosis.

How should leptospirosis be treated?

Treatment with effective antimicrobial agents (e.g., penicillin, amoxicillin, or doxycycline) should be initiated early in the course of the disease, as soon as the diagnosis of leptospirosis is suspected and preferably before the fifth day after the onset of illness. An infectious disease or tropical medicine specialist should be consulted. Clinicians should not wait for laboratory test results before starting treatment with antibiotics because serological tests do not become positive until about a week after the onset of illness.

Treatment is recommended for patients with a high index of suspicion for leptospirosis, based either on signs and symptoms or on potential exposure to leptospirosis, and with at least a presumptive positive result for leptospirosis on a serological assay.

Intravenous penicillin G (1.5 million U IV q6h or ceftriaxone 1.0 gm q 24h for 7 days) is the treatment of choice for severe cases or patients requiring hospitalization. Intravenous Penicillin G decreases duration of symptoms and may prevent leptospiruria, but providers should be aware of a low risk for a Jarisch-Herxheimer reaction (acute febrile reaction with headache, myalgias and aggravated clinical picture less than 24 hours duration) to occur with IV penicillin therapy.

Treatment of patients with mild disease oral doxycycline can shorten the course of illness and decrease the occurrence of leptospiruria. For patients with mild disease, oral doxycycline (100 mg PO BID or IV for

7 days) is effective; doxycycline should not be used in patients younger than 8 years of age or in pregnant women.

How can leptospirosis be prevented?

Persons at an increased risk for disease should wear protective clothing and footwear and minimize contact with potentially contaminated water. There is no vaccine available.

If an outbreak of leptospirosis is identified, antibiotic chemoprophylaxis may be useful in persons with exposure to the presumptive source of the outbreak (e.g., floodwaters) to prevent disease. Effective antibiotics include doxycycline (200 mg taken orally, once weekly), begun 1 to 2 days before exposure and continuing through the period of exposure.

How can I get more information about leptospirosis?

More information about leptospirosis, including precautions, is provided on the CDC website:
http://www.cdc.gov/ncidod/dbmd/diseaseinfo/leptospirosis_g.htm

Questions can be directed to the CDC Information Line at 1-800-CDC-INFO, (Email: cdcinfo@cdc.gov) or through the CDC Directors Emergency Operations at 770-488-7100.

Appendix 1 - Collection of serum specimens for laboratory testing for leptospirosis:

An 8 to 10 ml volume of blood should be collected aseptically from a peripheral vein using appropriate gauge needles and a Vacutainer™ serum separator tube (SST). Separation of serum from the blood cells must be done within 60 minutes of sample collection to prevent hemolysis. Serum separation should be completed before testing, storing or shipping the serum. Approximately 5 ml of serum will be obtained from a 10 ml blood volume (laboratories may be testing for multiple potential etiologies).

All serum separation must be done aseptically to avoid contamination. To separate serum from cells or the clot the Vacutainer™-drawn blood must be stored at room temperature for at least 30 minutes for complete clot formation to minimize the potential of trauma-induced hemolysis. The serum must be separated from the clotted blood by centrifugation, and then decanted using sterile technique into PLASTIC freezing vials with leak-proof screw caps. The plastic vials must be labeled appropriately, with patient name and / or identification number. After obtaining a serum aliquot for testing for leptospirosis using the Dip-S-Tick IgM assay, the remaining serum aliquots should be stored cold (0 - 4°C) or at -20 °C.*

For confirmatory testing at CDC, a 0.5 ml aliquot of the serum specimen should be shipped in plastic vials, appropriately labeled with patient identifiers (identification number/code or other identifiers). Specimens should be shipped overnight on cold packs to CDC using the following address:

Centers for Disease Control and Prevention
Data and Specimen Handling Activity (DASH)
BLDG 4, RM B35—G12
1600 Clifton Road NE
Atlanta, GA 30333
ATTN: Mary Ari, Leptospirosis Laboratory

***NOTE: As a precaution, CDC recommends shipping only part of the serum sample, and retaining an aliquot that should remain frozen at the shipping site. This provides a backup sample in case CDC sample shipment is lost or arrives in unacceptable condition.**

**Appendix 2: Dip-S-Tick leptospirosis IgM Dot ELISA "Bench Protocol"
Provided by Meningitis and Bacterial Zoonoses Branch**

1. Set water bath or heating block to 50^oC (insert thermometer to monitor temperature for the duration of testing).
2. Allow kit to warm up to room temperature.
3. Arrange cuvettes in a rack (assign 4 cuvettes for each specimen).
4. Dispense 2ml sample diluent in the 1st of 4 cuvettes assigned for each specimen (RC #1).
5. Dispense 2ml enhancer in the 2nd of 4 cuvettes assigned for each specimen (RC #2).
6. Dispense 2ml conjugate in the 3rd of 4 cuvettes assigned for each specimen (RC #3).
7. Dispense 2ml substrate (developer) in the 4th cuvette assigned for each specimen (RC #4).
8. Allow reagents to heat up in bath for 10 mins before commencing.
9. During this time add 10 microliters of specimen to be tested in 1st cuvette containing 2ml sample diluent.
10. Mark assay strip with specimen ID (upper white portion).
11. Pre-wet assay strip by immersing in clarifier (distilled or deionized water) vessel for not more than 4 mins.
12. Stir Strip (10-15 quick up & down motions) in cuvette RC #1 (2 ml sample diluent + 10 microliters specimen).
13. Allow strip to stand in cuvette RC #1 for 5 minutes.
14. Remove and rinse in clarifier (by swift back and forth motion for 6-10 seconds) and change clarifier.
15. Stir strip (6-10 quick up and down motions) in RC #2 (2ml enhancer) and allow to stand in cuvette for 5mins.
16. Remove and rinse as described above in #13.
17. Stir strip in RC #3 (2ml conjugate), allow to stand in cuvette for 15 mins (check kit master label to verify time).
18. Remove and rinse as previously described in #13, let stand in clarifier for 5 mins.
19. Stir strip in RC #4 (2ml Developer), let stand in cuvette for 5mins.
20. Rinse in clarifier and blot excess moisture.
21. Allow strip to dry.
22. Read assay strips:
 - Pos = a dot with distinct border in center of window
 - Neg = no dot or dot difficult to see
 - Positive results = 2 or more dots are considered presumptive positive for leptospirosis, specimen should be submitted for confirmation.

For more information, visit www.bt.cdc.gov/disasters,
or call CDC at 800-CDC-INFO (English and Spanish) or 888-232-6348 (TTY).