Basics of Diagnostic Laboratory Tests for Leptospiroxirosis

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Disclaimer: The content of this presentation is for information purposes only and is not an official PAHO/WHO guideline document.
Background

- Genus *Leptospira*
- Free-living & saprophytic
  - *L. biflexa*
- Pathogenic
  - *L. interrogans*
- >200 serovars divided into 25 serogroups
- All *Leptospira* species appear identical
  - Morphology-helical rods, 0.1µm in diameter, 6-12µm in length

Zoonotic Transmission Cycle

- *Leptospira* spp. is maintained in reservoir mammalian hosts that include rodents, livestock, wild & domestic animals
- Pathogenic *Leptospira* are excreted into water & soil
- Infection can occur after bacteria penetrate a break in skin barrier or
- Infect through mucosal membranes in nose, eyes, & mouth
- Humans are incidental hosts

Leptospirosis: Clinical Symptoms

- **Phase I: Leptospiremic**
  - Anicteric form: 90% of cases
  - **Symptoms:** Sudden onset of fever, intense myalgia (calves & thighs), conjunctival suffusion, and severe headache
  - Lasts for 4-9 days

- Brief afebrile period

- **Phase II: Immune Phase or “Weil’s Disease”**
  - Icteric Form: 5-10% of cases progress to serious disease
  - **Symptoms:** Hepatic & renal dysfunction, jaundice, circulatory collapse (shock)
  - From 6-12\(^{th}\) day

- Mortality of 5-30% (WHO)

**Sources:**
I. WHO 2003 (2).
### Clinical Symptoms

<table>
<thead>
<tr>
<th>Approximate time scale:</th>
<th>Week 1</th>
<th>2</th>
<th>3</th>
<th>4 (months-years)</th>
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<td>leptospiruria and immunity</td>
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Differential Diagnosis

- Illnesses with symptoms that are similar to leptospirosis:
  - Dengue
  - Yellow Fever
  - Influenza

- Broad spectrum of symptoms presents a challenge for healthcare workers
Clinical Symptoms

- Diagnosis is based on the entire clinical picture
  - Clinical Symptoms
  - Comprehensive Patient History:
    - occupation
    - recreation activities
    - lifestyle
    - seasonal fluctuations in rainfall
    - recent climate/disaster event (flooding or hurricane)
  - Laboratory confirmation
    - variety of available methods
Laboratory Tests

- **Direct Assays**
  - Detection of *Leptospira* pathogen
  - Acute leptospiremic stage first 10 days of illness

- **Indirect Assays**
  - Detection of antibodies produced in response to *Leptospira* infection
  - After 5th day of illness and can last for years
Laboratory Diagnostic Methods for Leptospirosis

**Direct**
- Detection of *Leptospira* pathogen
  - Microscopy
  - Culture
  - PCR

**Indirect**
- Detection of *Leptospira* specific antibodies
  - MAT
  - ELISA
  - RDTs
Direct Laboratory Tests

- Microscopy
  - Dark field & phase-contrast
  - Silver & Fluorescence staining

- Pros
  - Early detection
  - Variety of patient specimens

- Cons
  - Artifact
  - Low sensitivity & specificity
  - Requires sophisticated microscopes

Photo credit: Dr. Martin Hicklin. CDC Public Health Image Library ID# 2769-Silver Stain of Kidney (6).

Photo credit: Mildred Galton. CDC Public Health Image Library ID# 1346-Fluorescent Stain of Liver Smear (7).
Direct Laboratory Tests

- **Culture**
  - Incubation at 28-30°C for 4-6 weeks
  - Semi-solid and liquid culture media

- **Pro**
  - Definitive ID of infecting serovar

- **Cons**
  - Delayed results due to slow growth rate of *Leptospira*
  - Cumbersome to maintain cultures for extended time periods
  - Culture is **not** warranted for acute clinical diagnosis

Direct Laboratory Tests

Culture Specimens

- **Blood Samples**
  Collect within 10 days of illness onset
  Transport in tube with heparin at room temp
  (refrigeration or freezing is detrimental to pathogenic leptospires)

- **CSF Samples**
  Collect between 5-10 days after onset of symptoms

- **Urine Samples**
  Collect between 10-30 days of illness onset
  Limited survival of leptospires in urine & must be processed within 2 hours to avoid loss of viability

- **Post Mortem Samples**
  Collect tissue aseptically and as soon as possible after death
  Transport in sterile container at + 4°C to prevent autolysis of cells

Direct Laboratory Tests

- **Polymerase Chain Reaction-PCR**
  
  Use of nucleic acid amplification of *Leptospira* specific target to detect pathogen from patient serum sample

- **Pro**
  - Rapid results - presence of leptospires can be detected before development of antibodies

- **Cons**
  - Not extensively evaluated in clinical applications and should only be performed on an experimental basis
  - According to the Royal Tropical Institute (WHOCC) in Amsterdam “A Real-time PCR has been developed and is in the process of validation”

Sources: WHO 2003 (2) & KIT 2012 (8)
Laboratory Tests

Laboratory Diagnostic Methods for Leptospirosis

Direct
Detection of *Leptospira* pathogen
- Microscopy
- Culture
- PCR

Indirect
Detection of *Leptospira* specific antibodies
- MAT
- ELISA
- RDTs
Indirect Laboratory Tests

- **Serology**
  - Detection of an antibody (either IgM or IgG) in blood after seroconversion has occurred
    - IgM-biomarker of current or recent infection
    - IgG-biomarker of past infection
  - Detectable titers of antibodies appear in the blood approx. 6–10 days after the onset of disease
  - All rapid diagnostic tests (RDTs) utilize serological principles to detect antibodies

Indirect Laboratory Tests

- Microscopic Agglutination Test (MAT)
  - Panel of live cell suspensions mixed with diluted patient sample to test for serum antibodies
  - Examine agglutination reactions for the presence of clumps
  - Positive Result = Four-fold rise in titer between acute and convalescent phase sera run in parallel
  - Gold standard used in reference laboratories

Microscopic Agglutination Test (MAT)

- View of agglutination reactions under dark-field microscope
- Magnification 200x
- The endpoint/titer is the highest dilution where at least 50% of leptospires are agglutinated
- End titer for this sample is 1:5,120

Indirect Laboratory Tests

- Microscopic Agglutination Test (MAT)
  - Pros
    - High specificity and sensitivity
  - Cons
    - Diagnosis is relative when acute and convalescent serum samples are collected in intervals less than 10 days
    - Labor intensive-maintenance of living cultures including reference and local strains

ELISA: Enzyme-Linked Immunosorbent Assay

- Broadly reactive antigen is used for detection of *Leptospira* specific antibodies
- The antibody complex is visualized by a colorimetric change that is measured by a spectrophotometer
- Assays for either IgM or IgG and both IgM/IgG can be performed on patient serum
Indirect Laboratory Tests

ELISA

Indirect Laboratory Tests

ELISA

■ Pros
  ■ Only one serum specimen is required for diagnosis
  ■ Automated process that yields results within a few hours
  ■ Antigen coated plates are stable at room temperature
  ■ Earlier detection of *Leptospira* specific antibody—as early as 6-8 days after illness onset

■ Cons
  ■ Requires local standardization
  ■ Genus specific antigens commonly indicated for screening prior to complementary tests

Indirect Laboratory Tests

Rapid Diagnostic Tests (RDTs)

- Results are available within minutes, or at most 2 hours
- Samples for RDTs require little or no processing
- Result interpretation is straightforward
- Simple to use and require minimal facilities, equipment, & training
- Stable reagents may be stored under extreme conditions

Indirect Laboratory Tests

**RDTs**
- Variety of different RDT technologies
  - **Lateral-flow**: tests where the user adds the specimen directly onto the strip and reads the results after a specified amount of time has elapsed
  - **Flow through**: kits of individual cassettes with extraction, wash buffers and a “reveal” reagent to obtain results
  - **Agglutination**: tests based on agglutination of particles in a sample after the addition of antigenic reagent; agglutination reaction can be visualized with the naked eye

**Pros**
- Potential for point-of-care diagnostics for quick results
- Easy to use in low-resource settings and in the field

**Cons**
- Cross-reactive antibodies also have been described in patients with syphilis, relapsing fever, Lyme disease, and legionellosis
- Patients that are immunocompromised, malnourished, or have immune system defects may yield false negative results
- Values for % Sensitivity and % Specificity vary between different RDTs

Sources: USAID 2008 (10) & MD Consult 2011 (11).
Laboratory Tests

Laboratory Diagnostic Methods for Leptospirosis

Direct
- Detection of *Leptospira* pathogen
  - Microscopy
  - Culture
  - PCR

Indirect
- Detection of *Leptospira* specific antibodies
  - MAT
  - ELISA
  - RDTs
    - Gold standard
    - IgM
    - IgG
    - Both
    - Lateral Flow
    - Flow Through
    - Agglutination
Microscopic Agglutination Test (MAT)

- **Sample**: Minimum of two clotted blood or serum samples
- **Container**: Sterile tube
- **When to obtain MAT specimens**:
  - First sample: at the first clinical care
  - Second sample: about 10 days after the first sample
- **Storage and transportation of MAT specimens**:
  - Separation of serum from whole blood must be conducted before dispensing serum into a sterile plastic freezing vial.
  - Serum must be transported between 0°C to 4°C
  - Serum should be stored at 4°C for short term or at -20°C if samples are stored for long time periods

Enzyme Linked Immunosorbent Assay (ELISA)

- **Sample**: Clotted blood or serum sample
- **Container**: Sterile tube
- **When to obtain an ELISA specimens**: approximately 6-8 days after the onset of clinical symptoms

**Storage and transportation of ELISA specimens**:

- Separation of serum from whole blood must be conducted before dispensing serum into a sterile plastic freezing vial.
- Serum must be transported between 0°C to 4°C
- Serum should be stored at 4°C for short term or at -20°C if samples are stored for long time periods

Specimen Collection & Transport

Culture Specimens Revisited

- **Blood Samples**
  Collect within 10 days of illness onset
  Transport in tube with heparin at room temp
  (refrigeration or freezing is detrimental to pathogenic leptospires)

- **CSF Samples**
  Collect between 5-10 days after onset of symptoms

- **Urine Samples**
  Collect within 10-30 days of illness onset
  Survival of leptospires is limited and must be processed within 2 hours of voiding

- **Post Mortem Samples**
  Collect tissue aseptically and as soon as possible after death
  Transport in sterile container at +4°C to prevent autolysis of cells

The following data must be recorded and accompany any specimen sent for lab tests:

- Date of sample collection
- Specimen type
- Date of illness onset
- Date of Antibiotic treatment (if any)
- Type of Antibiotic treatment (if any)
Example: Form Requesting Laboratory Testing for Leptospirosis

Conclusions

- Phase of illness determines the appropriate lab test for successful diagnosis of Leptospirosis
  - Leptospiremic Phase in the first week – direct lab methods
  - Immune Phase after first week – indirect lab methods
- Every laboratory test has both advantages and limitations
- A negative RDT result does **not** rule out leptospirosis and must be confirmed using the gold standard of MAT
- Proper specimen collection and transport is essential to yielding accurate laboratory results
References

1. Photo Credit: Janice Haney Carr. CDC Public Health Image Library. ID#:1220: Scanning electron micrograph of *Leptospira interrogans*.
6. Photo Credit: Dr. Martin Hicklin. CDC Public Health Image Library ID# 2769. Silver Stain of Kidney tissue.
Thank you!

Photo Credit: Janice Haney Carr. CDC Public Health Image Library. ID#: 138: Scanning electron micrograph of *Leptospira* sp. (12).