



Basics of Diagnostic Laboratory Tests for Leptospirosis

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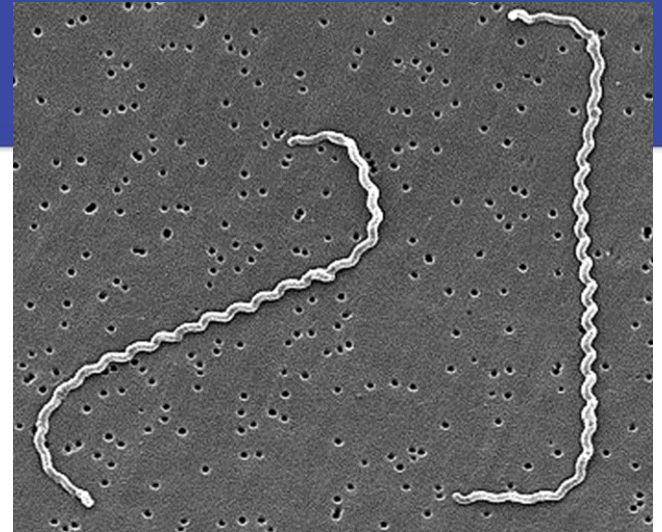
Internship oriented by: Maria Cristina Schneider, D.V.M., M.Sc., Sc.D.

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Background

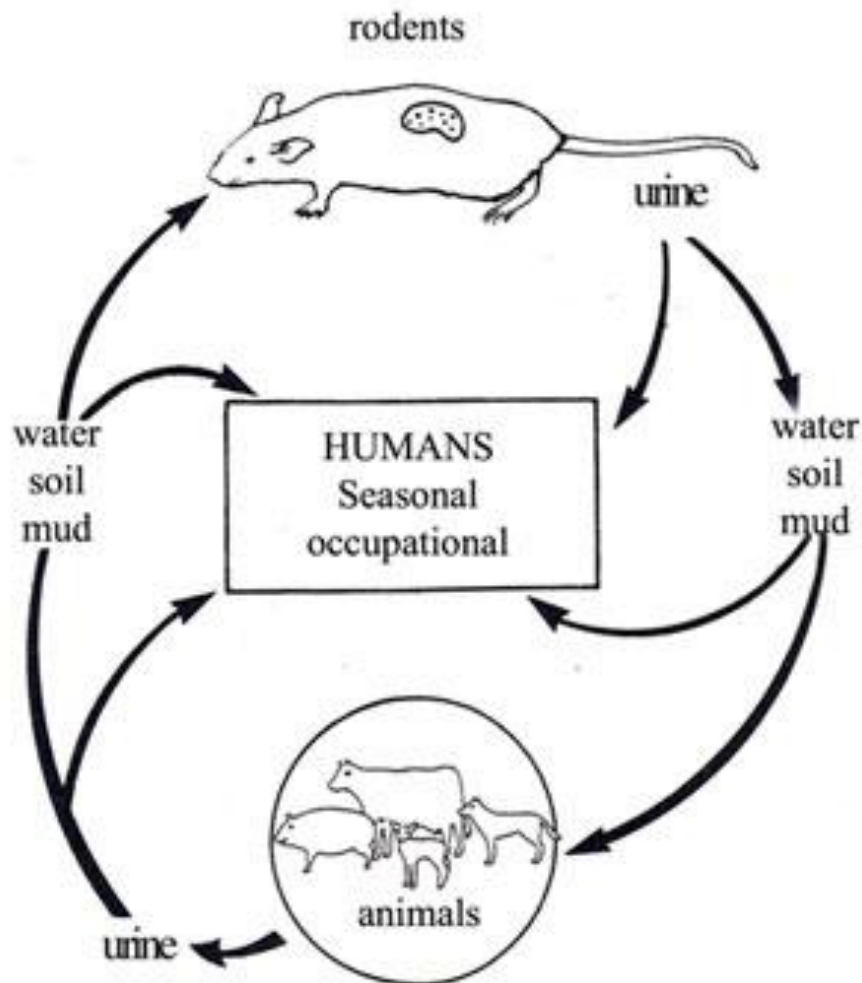
- Genus *Leptospira*
- Free-living & saprophytic
 - *L. biflexa*
- Pathogenic
 - *L. interrogans*
- >200 serovars divided into 25 serogroups
- All *Leptospira* species appear identical
 - Morphology-helical rods, 0.1µm in diameter, 6-12µm in length



Leptospira interrogans. Photo credit: Janice Haney Carr. CDC Public Health Image Library ID# 1220 (1)



Transmission



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

Source: Faine, et al. *Leptospira and Leptospirosis: 2nd Edition*. 1999 (3).



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 6-12th day

■ Mortality of 5-30% (WHO)

Sources:

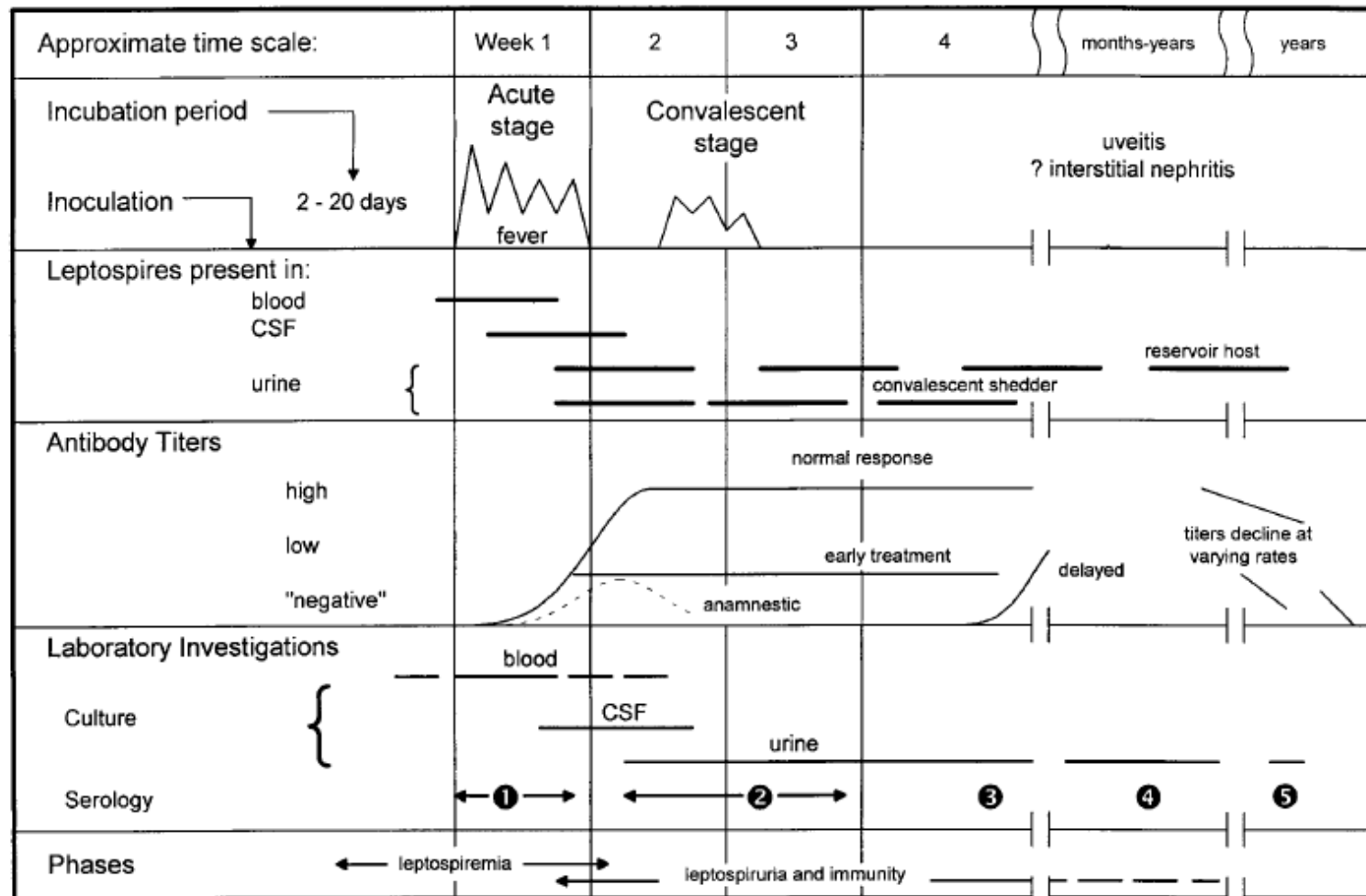
I. WHO 2003 (2).

II. Levett, P. N. “Leptospirosis” Clin Microbiol Rev. 2001(4).

III. Heymann, DL. American Public Health Association. 2004 (5).



Clinical Symptoms



Source: Levett, P.N., "Leptospirosis." *Clin Microbiol Rev.* Apr. 2001 p. 296-326 (4)



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 Tests

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

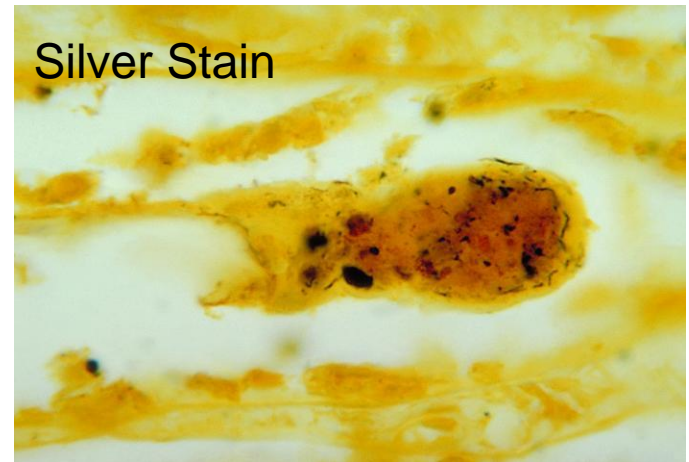


Photo credit: Dr. Martin Hicklin. CDC Public Health Image Library ID# 2769-Silver Stain of Kidney (6).

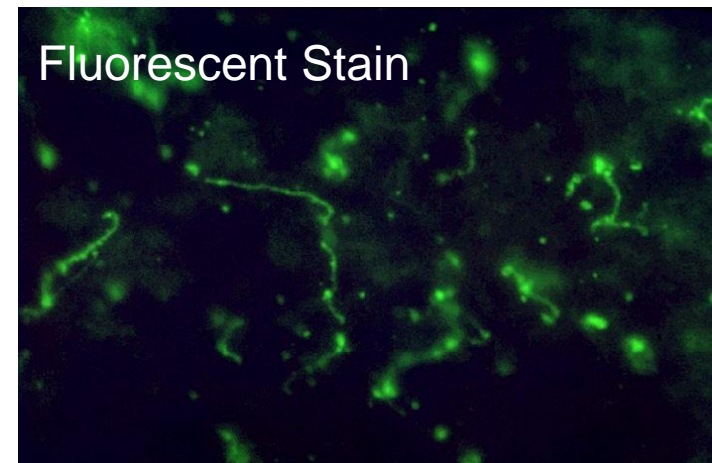


Photo credit: Mildred Galton. CDC Public Health Image Library ID# 1346-Fluorescent Stain of Liver Smear (7).



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”



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

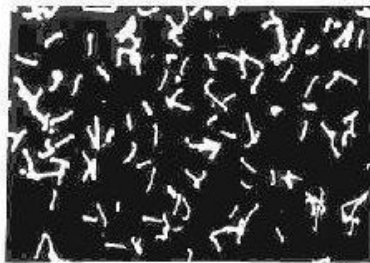


Indirect Laboratory Tests

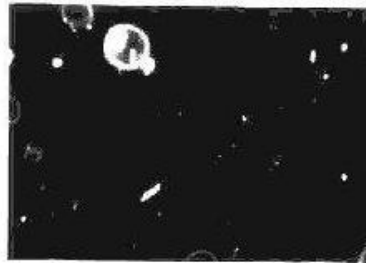
■ Microscopic Agglutination Test (MAT)

- 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

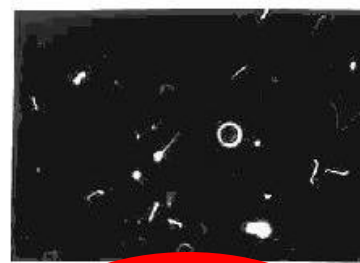
Microscopic Agglutination Test (MAT)



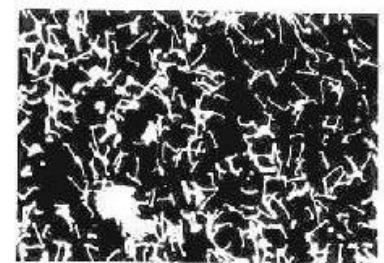
Negative control



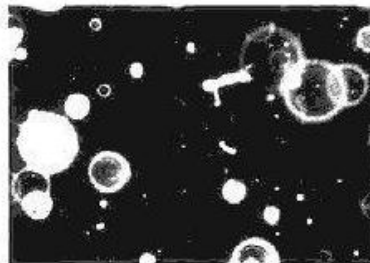
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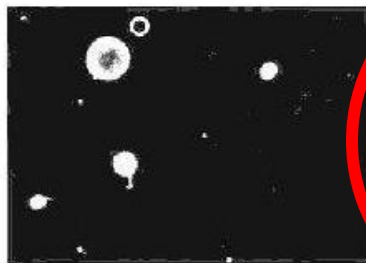
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1:20



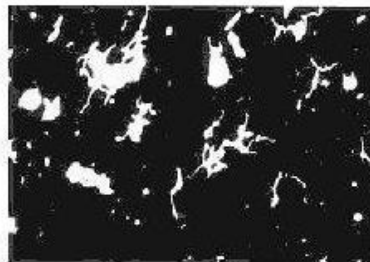
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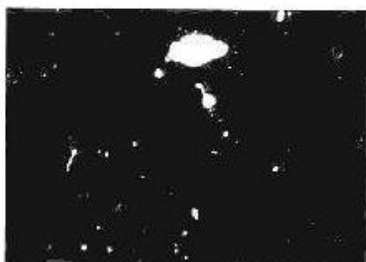
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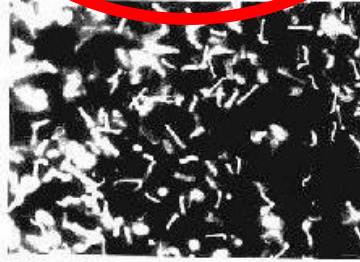
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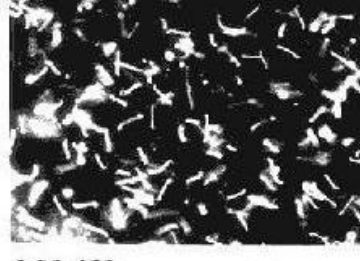
1:10 240



1:80



1:1280



1:20 480

- 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



Indirect Laboratory Tests

ELISA: Enzyme-Linked Immunosorbent Assay

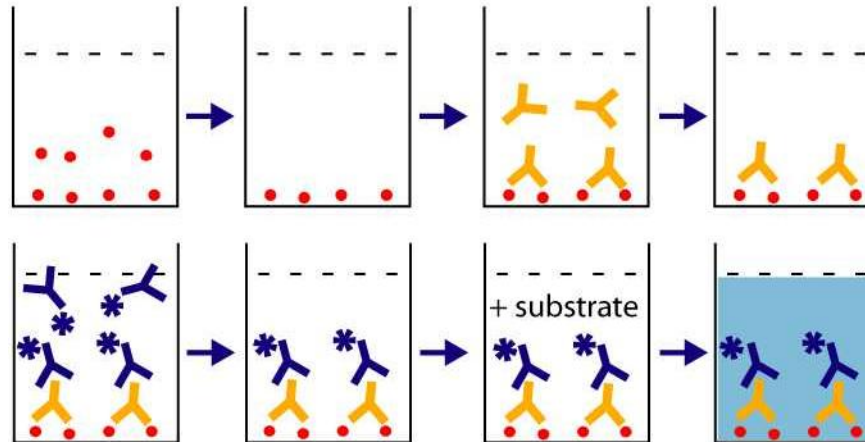
- Broadly reactive antigen is used for detection of *Leptospira* specific antibodies
- The antibody complex is visualized by a colormetric change that is measured by a spectrophotometer
- Assays for either IgM or IgG and both IgM/IgG can be performed on patient serum



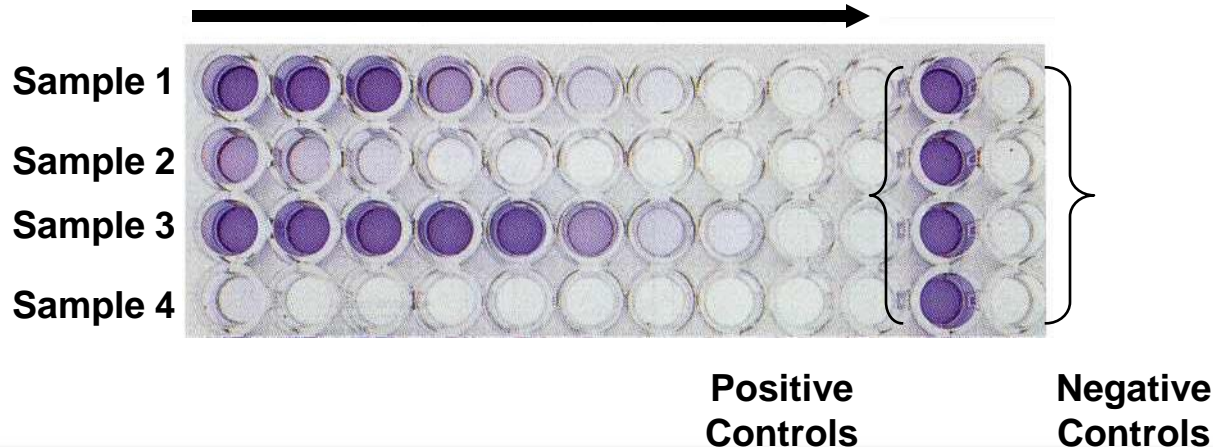
Indirect Laboratory Tests

ELISA

Source: Paul, W. E. (ed.) Fundamental Immunology, 6th ed. 2008 (9).



Serum Dilution Series





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



Laboratory Tests

Laboratory Diagnostic Methods for Leptospirosis

Direct

Detection of *Leptospira*
pathogen

Microscopy

Culture

PCR

Indirect

Detection of *Leptospira*
specific antibodies



MAT

Gold
standard

ELISA

IgM
IgG
Both

RDTs

Lateral Flow
Flow Through
Agglutination



Specimen Collection & Transport

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



Specimen Collection & Transport

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





Specimen Collection & Transport

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

LEPTOSPIRA REFERENCE UNIT Public Health Laboratory, County Hospital, Hereford HR1 2ER Telephone: 01432 277707; Fax: 01432 351396			
PLEASE USE THIS FORM FOR ALL REQUESTS TO THE LEPTOSPIRA REFERENCE UNIT The following information is required to aid the diagnosis and epidemiology of leptospirosis. Please complete ALL sections:			
SURNAME _____		FORENAME(S) _____ SEX M / F AGE _____ (yrs)	
DOB ____ / ____ / ____		POSTCODE _____	
Clinical details <input type="checkbox"/> Flu-like illness <input type="checkbox"/> Headache <input type="checkbox"/> Myalgia <input type="checkbox"/> Pyrexia <input type="checkbox"/> Lethargy <input type="checkbox"/> Malaise <input type="checkbox"/> Vomiting <input type="checkbox"/> Diarrhoea <input type="checkbox"/> Conjunctivitis <input type="checkbox"/> Abnormal LFTs <input type="checkbox"/> Jaundice <input type="checkbox"/> Hepatic failure <input type="checkbox"/> Renal failure <input type="checkbox"/> Meningitis <input type="checkbox"/> No symptoms <input type="checkbox"/> Died <input type="checkbox"/> Medical screen <input type="checkbox"/> Other (specify) _____	Occupation <input type="checkbox"/> Farmer - arable <input type="checkbox"/> - livestock <input type="checkbox"/> Farm worker - arable <input type="checkbox"/> - livestock <input type="checkbox"/> Outdoor - manual <input type="checkbox"/> - with animals <input type="checkbox"/> Fish - farmer <input type="checkbox"/> - worker <input type="checkbox"/> - filleter <input type="checkbox"/> Abattoir - worker <input type="checkbox"/> - butcher <input type="checkbox"/> Indoor - manual <input type="checkbox"/> - office <input type="checkbox"/> - domestic <input type="checkbox"/> Water worker - sewage <input type="checkbox"/> - plumber <input type="checkbox"/> Veterinarian <input type="checkbox"/> Medical <input type="checkbox"/> Military <input type="checkbox"/> Teacher <input type="checkbox"/> Student <input type="checkbox"/> Housewife <input type="checkbox"/> Retired <input type="checkbox"/> Unemployed <input type="checkbox"/> Other (specify) _____	Water contact <input type="checkbox"/> Water sport <input type="checkbox"/> - swimming <input type="checkbox"/> - rowing <input type="checkbox"/> - windsurfing <input type="checkbox"/> - canoeing <input type="checkbox"/> - white water canoe <input type="checkbox"/> - surfing <input type="checkbox"/> Fishing <input type="checkbox"/> River <input type="checkbox"/> Canal <input type="checkbox"/> Lake <input type="checkbox"/> Pond <input type="checkbox"/> Ditch <input type="checkbox"/> Sewage <input type="checkbox"/> No known contact <input type="checkbox"/> Other (specify) _____	Animal contact <input type="checkbox"/> Farm livestock <input type="checkbox"/> - cattle <input type="checkbox"/> - sheep <input type="checkbox"/> Dogs <input type="checkbox"/> Rats <input type="checkbox"/> Mice <input type="checkbox"/> Other animals (specify in additional info. box) <input type="checkbox"/> No known contact Type of contact <input type="checkbox"/> Occupational <input type="checkbox"/> Recreational (specify in additional info. box) <input type="checkbox"/> Wound / abrasion <input type="checkbox"/> Immersion <input type="checkbox"/> Bite <input type="checkbox"/> Other (specify) _____
Recent travel abroad <input type="checkbox"/> Yes <input type="checkbox"/> No If YES, please give details (when / where) in additional info. box		Leisure activities Please specify below	
Date of onset of symptoms : _____ Date of antibiotic treatment: _____ Antibiotic treatment: _____ Specimen type: _____ Date collected: _____ Reference No. _____ _____ _____		Additional information:	
Requesting laboratories test: <input type="checkbox"/> CFT <input type="checkbox"/> ELISA <input type="checkbox"/> Other (specify): _____ Results: _____ Previous samples sent: <input type="checkbox"/> Yes <input type="checkbox"/> No Date: _____ If Yes, LRU number and date of sample: _____ Primary source laboratory if not the requesting laboratory: _____ Requesting laboratory: _____ Address: _____ Contact Doctor: _____ Date: ____ / ____ / ____		LRU use only IgM ELISA MAT Infecting serogroup _____ Epidemiology Serology Requested _____ Completed _____ Lab no: _____	

Source: WHO Publication. Human Leptospirosis: Guidance for Diagnosis, Surveillance and Control. 2003. (2)



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*.
2. World Health Organization (WHO). Human Leptospirosis: Guidance for Diagnosis, Surveillance and Control. Geneva. 2003.
3. Source: Faine, et al. Leptospira and Leptospirosis: 2nd Edition. CRC Press. Boca Raton. 1999
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6. Photo Credit: Dr. Martin Hicklin. CDC Public Health Image Library ID# 2769. Silver Stain of Kidney tissue.
7. Photo Credit: Mildred Galton. CDC Public Health Image Library ID# 1346. Leptospira bacteria in liver impression smear. FA stain.
8. Royal Tropical Institute/WHO Collaborating Center for Reference and Research on Leptospirosis. "Leptospirosis reference and diagnostic services." Accessed online: 16 Nov 2012.
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<http://www.mdconsult.com.proxygw.wrlc.org/das/pdxmd/body/382400869-8/0?type=med&eid=9-u1.0-_1_mt_1014555#2378586>. Posted: 9 Aug 2011. Accessed online: 16 Nov 2012.
12. Photo Credit: Janice Haney Carr. CDC Public Health Image Library. ID#: 138: Scanning electron micrograph of *Leptospira* sp.



Thank you!

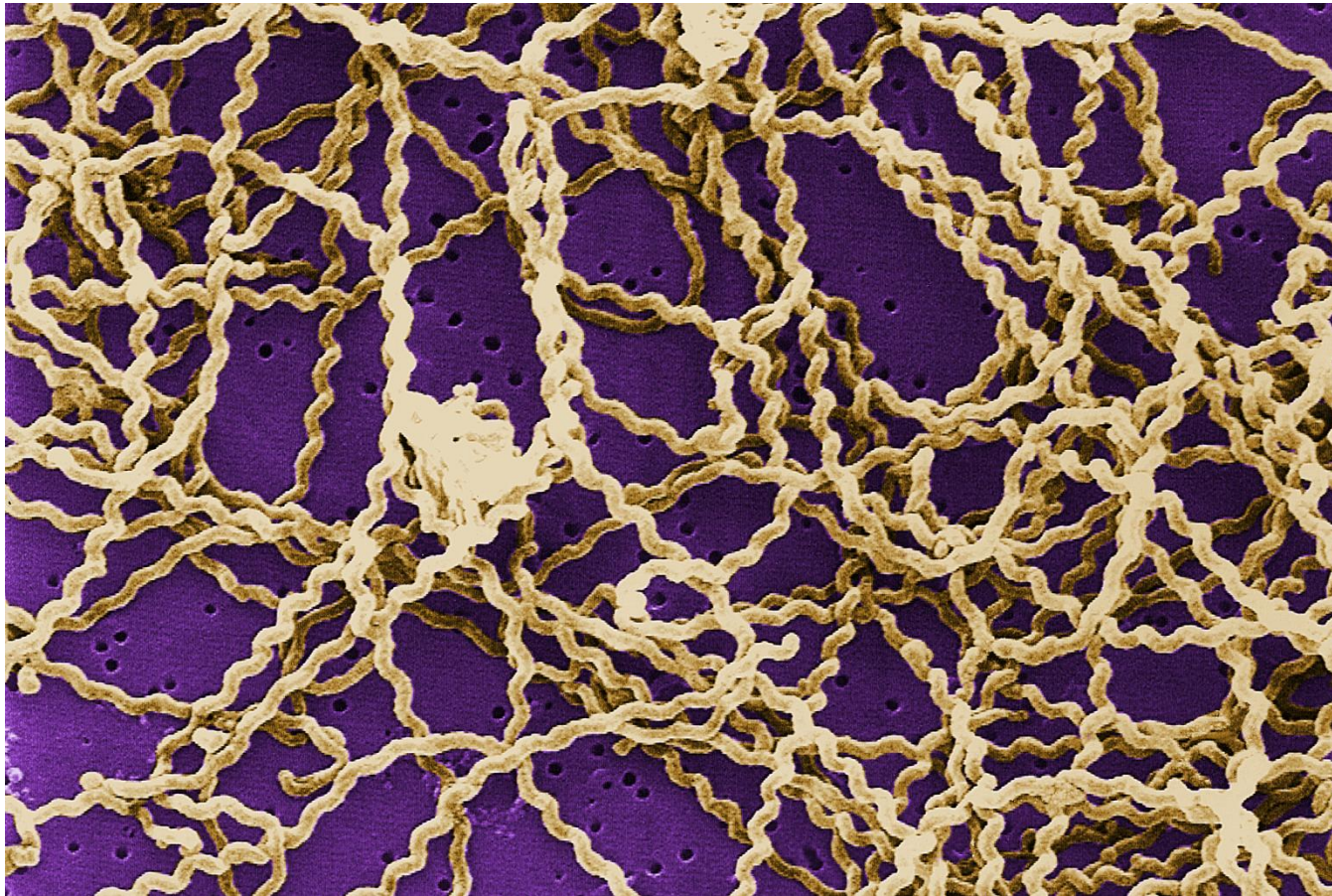


Photo Credit: Janice Haney Carr. CDC Public Health Image Library. ID#: 138: Scanning electron micrograph of *Leptospira* sp. (12).