Workshop proposal: Laboratory diagnosis of leptospirosis using standard MAT

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Leptospirosis is an infectious disease caused by Leptospira Interrogans serovars, that are transmitted from animals to humans. It's clinical manifestation is so variable that infection can not be proved reliably only on the basis of symptoms. Therefore, laboratory diagnosis is an essential tool for demonstration of the etiological agent. According to the WHO guidelines, Microscopic Agglutination Test (MAT) is the gold standard for diagnosis of leptospirosis. The main objective of the proposed workshop is to introduce and demonstrate the performing of standard MAT.

a. Preparation of EMJH base medium

EMJH base medium is used for serum diluent. To prepare one litre EMJH base medium, 2.3 g of Leptospira medium base EMJH is added into 1 l distilled water.

b. Preparation of antigen

A 5-7 days old culture of *Leptospira interrogans* in EMJH liquid medium is used as antigen. The density of leptospires is assessed using a counting chamber (Petroff-Hausser USA) and is adjusted to about 2 x 10^8 cells/Ml. The reference strains of leptspira can be provided by Leptospira Reference Laboratory, Amsterdam.

c. Preparation of microscope slides

Normal Microscope slides are washed in 100% ethanol then in distilled water and after they are dried each is divided into 8 squares using a black pentel pen.

d. Preparation of serum dilutions

The serum to be tested is serially diluted in a microtitre plate, starting from 1 in 12.5 dilution, using 2-fold dilution (1 in 25, 50, 100, 200, 400, 800, 1600, 3200 etc.).

A brief description of dark-field microscope and how to work and how to use it.
A brief description on how to use the live antigen of Leptospira and prepare it for the test.

e. MAT protocol

1) Using a P-20 Gillman pipette, 10 µL of the appropriate antigen is added to each square on the microscope slide.

2) 10 µL of each serum dilution is added beside the antigen on the slide, then the serum is mixed with the antigen. Care is taken to keep each dilution in its own square on the slide.

4) Three standard controls are included in the test; one positive standard serum, one negative standard serum and a dilution series with saline instead of serum as a control for autoagglutination of the antigen.

5) The slide is placed in a Petridish with moist paper to avoid evaporation, and incubated at 30°C for 90 minutes.

The slide is examined under a dark-field microscope, using a long working distance objective at x100 or x200 magnification. Agglutination is noted by observing clumps of leptospires. The lowest dilution that each serum is considered significant is 1:100. The end point titre is the highest titre in which 50% agglutination occurred.

Required equipment and material for the workshop:

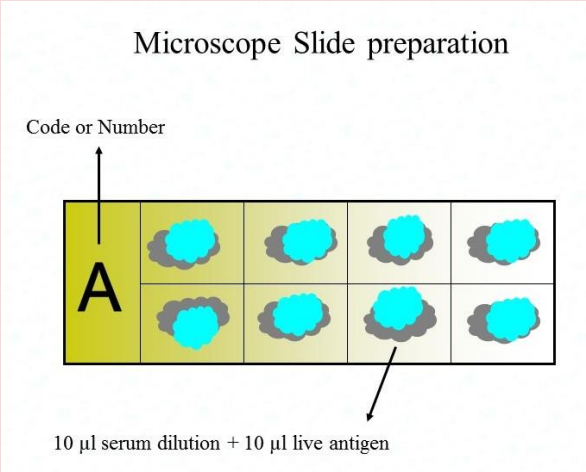
1-A dark-field microscope preferably equipped with digital camera and two dark-field condensers (dry and wet) i.e. Olympus BH2.
2-30ºC Incubator
3-Pipette P-10 , P-20, P-100, P-1000
4-Pipette tips
5-Microscope slide
6-Pentel pen
7-Petridish
8-Distilled water
9-PBS solution
10- Leptospira Live antigen (i.e. serovar Grippotyphosa)

11- **Standard antisera against serovar Grippotyphosa**
12- **Antiseptics**
13- **Lab desk**
14- **Safety Cabinet**
15- **Plastic gloves**

Here are some photos taken from different stages of the MAT protocol:



Leptospira under dark-field microscope



Adding and mixing serum and live antigen



Transferring microscope slide into a moisture place for 1.5 hour incubation at 30°C

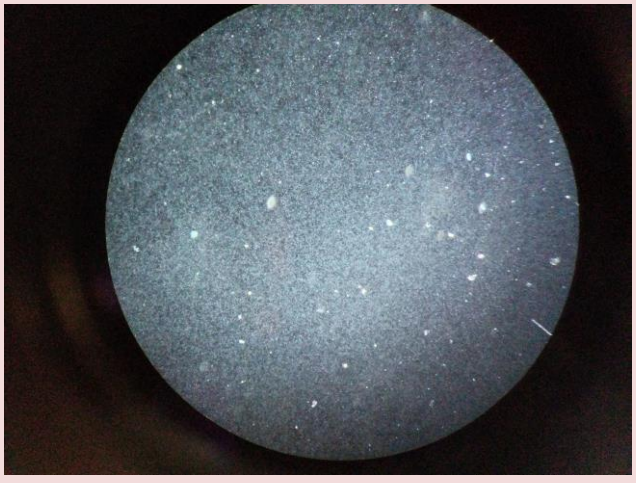
Incubation of the petridish at 30 for 1.5 hour



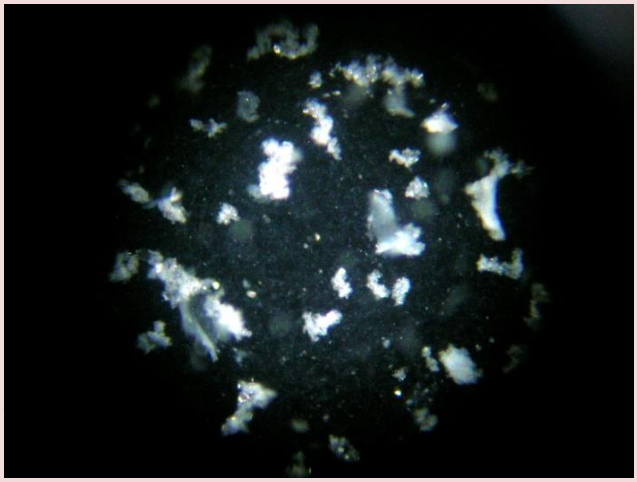
Dark-field Microscopic examination of the sample



Negative MAT sample



Positive MAT sample (3+)



Positive MAT sample (4+)

