SOP Title: MAT for *Leptospira* spp.

SOP ID: 321, Version: 1.0

Effective Date: 3 March, 2026
Review Date: 3 March, 2027
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1. Purpose

To provide a standardized procedure for the detection of *Leptospira* antibodies in serum samples using the Microscopic Agglutination Test (MAT), as adapted to the ZAFI project.

2. Scope

This SOP applies to all laboratory personnel performing and interpreting the results of the MAT at ZAFI project laboratories in Uganda.

3. Definitions and Abbreviations

- **MAT:** Microscopic Agglutination Test
- **EMJH**: Ellinghausen-McCullough-Johnson-Harris medium
- **Antigens**: 7-day-old live Leptospira cultures grown in commercially enriched EMJH medium
- **Panel**: List of reference strains of 12 pathogenic serovars/serogroups

4. Responsibilities

Role	Responsibility
Laboratory technician	Perform laboratory testing according to this SOP; handle and process samples appropriately; adhere to biosafety and quality control procedures; document results accurately in logbooks and KOBO tools; report deviations or issues to the laboratory lead/supervisor.
Laboratory lead/supervisor	Oversee laboratory testing; ensure staff competency and adherence to quality and biosafety standards; review and validate results; escalate issues as appropriate.
Site PI	Ensure relevant staff are trained on this SOP.

5. Safety Precautions

- Treat all samples as potentially infectious
- Use gloves, lab coats, and eye protection
- Decontaminate spills immediately and dispose waste per the SOP on Biohazard Waste Disposal

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6. Equipment, supplies and reagents

Item	
Incubator (set at 29.5°C), stereo microscope, micropipettes	Table-top waste bag
Microplate shaker	Autoclave waste bag
96-well microtitre plates, normal saline	Result templates
MAT Screening and Titration Worksheets (A001 F1 & F2)	5 mL or 10 mL pipette tips
10 mL screw cap tubes	50 mL, 10 mL, and 2 mL racks
200 µL and 1000 µL pipette tips	70% alcohol (for disinfection)
Reagent reservoirs	Normal saline (for sample dilution)
Flat-bottom microtitre plates	Live *Leptospira* antigens (as per panel)
Plate covers	Positive control sera
Tissue paper	Negative control sera

7. Procedure

A. Screening

7.1 Prepare Plate Plan

a) Determine the number of serum samples and the panel of serovars.
b) Design the screening plate plan accordingly.
c) Reserve the first two rows of the first plate for control sera:
   - Row A: Positive control
   - Row B: Negative control
d) Repeat for every 10 plates prepared.

7.2 Dilute Serum Samples

a) Prepare 1:50 dilution by mixing 100 µL of test serum with 4.9 mL of normal saline.
b) Use 10 mL screw cap tubes for dilution.
c) Label all tubes accurately.

7.3 Add Control Sera to Plate

a) Add 100 µL of positive control serum to designated wells in Row A.
b) Add 100 µL of negative control serum to designated wells in Row B.
c) Follow the predefined plate plan.

7.4 Add Diluted Test Serum to Plate

a) Dispense 100 µL of each diluted test serum into respective wells.
b) Adhere strictly to the screening plate layout.

7.5 Add Serovars (Antigen)

a) Dispense 100 µL of the corresponding Leptospira serovar into each designated well.
b) Use fresh pipette tips for each dispense to prevent contamination.

7.6 Shake Plates

a) Cover plates with appropriate covers.
b) Shake plates at 400 rpm for 30 seconds using a microplate shaker.

7.7 Incubate

a) Incubate plates at 29.5 °C for 2.5 hours.
b) Use a stopwatch to ensure accurate incubation time.

7.8 Post-Incubation Handling

a) Gently blot moisture from plates using tissue paper.
b) Handle carefully to avoid disturbing well contents.

7.9 Microscopic Examination

a) Examine wells using a stereo microscope under high light intensity.
b) Observe for agglutination.

7.10 Record Results

a) Identify wells showing ≥50% agglutination for any serovar.
b) Mark these as positive reactions in the result templates.

B. Titration

7.11 Identify Positive Samples

a) Review results from the screening procedure.
b) Record all serum samples that tested positive.
c) Note the specific Leptospira serovar(s) that each sample reacted to.

7.12 Retrieve Serum Dilutions

a) Locate the 1:50 diluted serum samples previously prepared.
b) Ensure proper labeling and traceability for each sample.

7.13 Prepare Titration Plate Layout

a) Design a plate map based on positive samples and serovars.
b) Reserve the first two rows in the first plate for:
    - Row A: Positive control sera
    - Row B: Negative control sera

7.14 Add Normal Saline for Serial Dilution

a) In the first plate, add 100 µL of normal saline to:
    - Rows D–H (4th to 8th rows)
b) In subsequent plates, add 100 µL of normal saline to:
    - Rows B–H (2nd to 8th rows)

7.15 Load Test Samples

a) Add 100 µL of each 1:50 diluted serum to:
    - Row C and D of the first plate
    - Row A and B of subsequent plates
b) Follow the layout defined in the titration plan.

7.16 Perform Serial Dilution

a) Use a multi-channel pipette to perform two-fold dilutions.
b) Start from the second row with the serum sample (Row D for the first plate or B in the subsequent plates).
c) Work downward through the column.
d) Use new pipette tips for each sample to prevent cross-contamination.

7.17 Add Serovars

a) Dispense 100 µL of the relevant Leptospira serovar into each well in a column-wise manner.
b) Follow the titration plate plan precisely.

7.18 Shake and Incubate

a) Cover the plates with appropriate covers.
b) Shake plates at 400 rpm for 30 seconds using a microtitre plate shaker.
c) Incubate at 29.5 °C for 2.5 hours.
d) Use a stopwatch or label each set with an end-time reminder.

7.19 Post-Incubation Handling

a) After incubation, gently blot the underside of plates with tissue paper.
b) Avoid disturbing the contents of the wells.

7.20 Microscopic Examination

a) Use a stereo microscope with high light intensity.
b) Examine each well for agglutination patterns.

7.21 Interpretation and Reporting

a) Identify the highest dilution showing ≥50% agglutination.
b) Record this as the final titre for each sample.
c) Use the **MAT Titration Worksheet** (A001 F2) to document results.

8. Data Analysis and Interpretation

- **Positive**: Agglutination in ≥50% leptospires at 1:100 dilution or higher.
- **Negative**: No agglutination at 1:100.
- Report titres as the reciprocal of the highest reactive dilution

9. Quality Control

- Include positive/negative controls on the first plate of every 10 tested.
- Use fresh controls if new serovar batches are opened.
- Read and confirm controls before evaluating samples.
- Invalidate results for any serovar that fails control criteria.
- Require dual-review of test results.

10. Documentation and Reporting

- Use approved forms (A001 F1 & F2) and Plate Layout Sheets.

- Enter final results into KOBO
- Store completed forms securely for audit.

11. Related Templates

- A001 F1 MAT Screening Worksheet
- A001 F2 MAT Titration Worksheet1
- A001 F3 MAT Titration Worksheet2

12. References

Goris MGA, Hartskeerl RA (2013). Leptospirosis serodiagnosis by the microscopic agglutination test. Curr Protoc Microbiol.

13. Review and Approval

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Date: *3 March 2026*