SOP Title: Micro-serum agglutination test (micro-SAT) for *Brucella* spp.

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Primary Author (s): Tom Edwards, Collins G.K. Atuheire, Caitlin Greenland-Bews
Secondary Author: Eric Fevre
Approver: Siobhan Mor

1. Purpose

To describe the procedure for detecting antibodies to *Brucella* using a micro-serum agglutination test (micro-SAT) in patient serum, adapted to the ZAFI project.

2. Scope

This SOP applies to all laboratory personnel responsible for diagnostic and research testing for *Brucella* antibodies at ZAFI-affiliated laboratories in Ethiopia, Uganda, and Kenya.

3. Abbreviations

- **SAT:** Serum Agglutination Test
- **MAT:** Microagglutination test
- **QC**: Quality Control, procedures to ensure test accuracy and reliability.
- **PPE**: Personal Protective Equipment, protective clothing and gear to reduce exposure to hazards.
- **NC**: Negative Control, a test sample that should produce a negative result to validate assay performance.
- **PC**: Positive Control, a test sample that should produce a positive result to validate assay performance.
- **NSB**: Non-Specific Binding, binding of assay reagents to unintended targets or surfaces, potentially leading to false results.

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.

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5. Safety & Precautions

Follow biosafety level 2 (BSL-2) practices when handling human serum or plasma samples. Wear PPE including gloves, lab coat, and eye protection. Avoid contact with skin and mucous membranes. Handle all specimens as potentially infectious. Dispose of waste according to the Biohazard Waste Disposal SOP.

6. Materials and Equipment

- SAT antigen
- PBS tablets, pH 7.3
- Powdered Safranin O dye
- Positive serum control
- Negative serum control
- V-well microtiter plates
- 37°C incubator
- Microtitre plate reading mirror
- Reagent troughs
- Plate sealers
- Balance (capable of weighing 1g)
- Pipette tips – 10ul, 200ul, 1ml, 5ml
- Bottles and tubes – 0.5:/1L durans, 50ml falcons tubes
- Measuring cylinders
- Calibrated micropipettes (single and multichannel) and tips
- Distilled or deionized water
- Waste container with disinfectant

7. Procedure

7.1 Calibration procedures

Positive and negative controls should be run with every test. If significant deviation is noted, the run must be repeated.

7.2 Pre-test preparations

a) Bring all reagents and samples for testing to room temperature (20-25°C) before use

b) Fill in test worksheet to designate controls and samples to appropriate wells (Annex 1)

7.3 Reagent Preparation

a) PBS buffer

- 1 tablet
- 100ml clean, good quality water
- *Store at +4°C for up to 1 month– Write date made on the lid*

b) 0.5% Safranin/PBS solution

- 1g Safranin powder
- 20ml PBS buffer
- *Store at +4°C for up to 1 month– Write date made on the lid*

c) 0.005% Safranin/PBS solution

- 0.25ml 0.5% aqueous safranin/PBS solution
- 24.75ml PBS buffer
- *Store at +4°C for up to 1 month– Write date made on the lid*

d) Working strength Safranin-stained Brucella abortus agglutination test antigen (1:50 dilution)

- 500µl SAT antigen
- 25ml 0.005% safranin/PBS solution
- *Store at +4°C for up to 14 days – Write date made on the lid*

7.4 Preparing Participant Samples for Analysis

a) Prepare a 1:20 dilution of test sera, positive and negative control sera in PBS in row H of a V-bottomed microtiter plate (add 5µL of sample to 95µl PBS) and mix thoroughly.

7.5 Assay Steps

a) In V bottomed microtiter plate, dispense 50µl PBS into wells G-A, rows 1-12
b) In Row 1 add 100µl of 1:20 dilution of positive control sera to well H, using 50µl volumes dilute control serum from 1:20 to 1:2560.
c) In Row 2 add 100µl of 1:20 dilution of negative control sera to well H, using 50µl volumes dilute control serum from 1:20 to 1:2560.
d) Add 100µl of appropriate test serum to row H according to test worksheet, using 50µl volumes dilute test sera from 1:20 to 1:2560.
e) Add 50µl working strength Safranin-stained Brucella abortus antigen to all wells.
f) Seal with plate sealer
g) Incubate at 37C for 18-24hours without disturbing
h) Read microtiter plate using plate reading mirror.

8. Data Analysis and Interpretation

8.1 Validation criteria

- The positive control serum should be +/-1 well from the expected titre defined by the datasheet
- If the positive control is outside this range, the assay is not valid.
- The negative control serum should be negative in all wells

8.2. Interpretation of results

a) **Negative results** = button of red-stained cells on the centre of the well, surrounded by a clear pink diluent.
b) **Positive results** = agglutination to produce a layer of stained cells covering the bottom of the well or by a diminished/absence of button of cells in the centre of the well surrounded by slightly opaque diluent.
    - **For positive results**: The titre is derived as the greatest dilution showing agglutination.

8.3. Reporting Procedure

a) Negative serum samples are reported as <20
b) Positive Samples
    - The titre of the test serum is derived as the greatest dilution showing agglutination. Positive results should be reported as the titre at which they were positive. E.g. 1:80 should be reported as 80.

8.4. Considerations

- Lipaemic, haemolysed and icteric samples should not be processed due to the increased risk of false positives.

9. Quality Control

- Positive and negative controls should be run with every test. If significant deviation is noted, the run must be repeated.

10. Documentation

- Record all results
- Enter results into dedicated KOBO form.
- Maintain QC logs for review.
- Archive raw data printouts and electronic files securely.

11: Review and Approval

Approved By: Prof Siobhan Mor

Title: *Chief Investigator, ZAFI*
Date: *23 July 2026*

Annex 1: Example worksheet and plate layout

Sample No.		H	G	F	E	D	C	B	A
	Dilution	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
Positive Control	1								
Negative Control	2								
	3								
	4								
	5								
	6								
	7								
	8								
	9								
	10								
	11								
	12								