

## SOP Title: Rose Bengal Test (RBT) for *Brucella* spp.

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### 1. Purpose

To describe the standardized procedure for conducting the **Rose Bengal Test (RBT) for detection of anti-*Brucella* antibodies in human serum samples** collected by the ZAFI project.

### 2. Scope

This SOP applies to all laboratory personnel performing and interpreting the RBT at ZAFI project laboratories in Uganda, Kenya, and Ethiopia.

### 3. Definitions and Abbreviations

- **RBT:** Rose Bengal Test
- **Agglutination:** Clumping of particles due to antibody-antigen reaction

### 4. Responsibilities

Role	Responsibility
<b>Laboratory technician</b>	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.
<b>Laboratory lead/supervisor</b>	Oversee laboratory testing; ensure staff competency and adherence to quality and biosafety standards; review and validate results; escalate issues as appropriate.
<b>Site PI</b>	Ensure relevant staff are trained on this SOP.

### 5. Safety and Precautions

- Handle all samples and reagents in Biosafety Level 2 conditions.
- Decontaminate all surfaces and discard materials in biohazard waste bins.
- Spills must be reported and managed per **SOP 401 on Biohazard Waste Disposal and Safety**

## 6. Materials and Equipment

- Rose Bengal reagent (RBT vials)
- Agglutination tile
- 5–100 µL pipette + tips
- Applicator sticks
- Timer or stopwatch
- 1% hypochlorite solution (for decontamination)
- PPE: lab coat, gloves, eye protection
- RBT Results Form / Run Log

## 7. Pre-Testing Preparation

- Ensure all reagents and samples are at room temperature (20–25°C).
- Label the agglutination tile with sample and control IDs.
- Prepare no more than 5 samples per tile run.
- Fill in RBT Run Log Form including Project ID, Sample IDs, and technician name.

## 8. Procedure

- a) Pipette 25µL of RBT reagent onto each labelled circle on the tile.
- b) Pipette 25µL of each serum sample/control next to its respective reagent drop.
- c) Use a clean applicator stick to mix each sample-reagent pair gently.
- d) Immediately start timer and rock the tile manually for 4 minutes.
- e) Observe for visible agglutination at 4 minutes.
- f) Discard used tips, tiles and applicators in 1% hypochlorite.

## 9. Quality Control Criteria

- Valid Run:
  - Positive control shows clear agglutination
  - Negative control shows no agglutination
- If controls fail, invalidate run and repeat test.

## 10. Interpretation of Results

Result	Interpretation (see Image below)
Agglutination present	Positive for anti-Brucella antibodies
No agglutination	Negative
Controls invalid	Repeat test

negative



positive

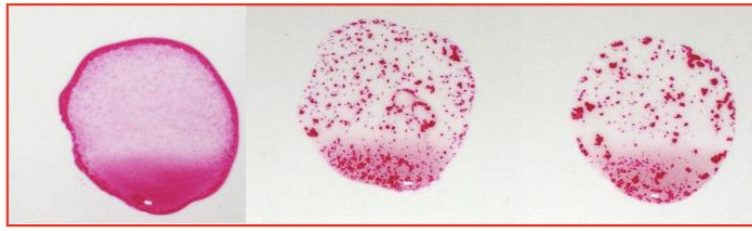


Image courtesy of University of Navarra RBT protocol

#### 11. Documentation

- Enter results into **RBT Run Log**
- Enter validated results into dedicated **KOBO form**
- Archive result forms and logs securely

#### 12. Related templates

- RBT Run Log

#### 13. Review and Approval

Approved By: *Prof Siobhan Mor*

Title: *Chief Investigator, ZAFI*

Date: *3 March 2026*