SOP Title: Rift Valley Fever (RVF) IgM ELISA Using Abbexa Kit

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1. Purpose

To outline the standardized procedure for the **qualitative detection of Human Rift Valley Fever IgM antibodies in human serum,** using the Abbexa Human RVF IgM ELISA Kit, following the manufacturer's instructions as adapted to the ZAFI project.

2. Scope

This SOP applies to all laboratory staff involved in performing and interpreting the results of the Abbexa Human Rift Valley Fever IgM ELISA at ZAFI project laboratories in Uganda, Kenya, and Ethiopia.

3. Abbreviations

- **ELISA**: Enzyme-Linked Immunosorbent Assay.
- **QC**: Quality Control.
- **OD**: Optical Density.
- **IgM**: Immunoglobulin M, the first antibody produced during an initial immune response, indicating recent infection.
- **TMB**: 3,3',5,5'-Tetramethylbenzidine, a chromogenic substrate that produces a blue color when oxidized by HRP in the presence of hydrogen peroxide; turns yellow after acid stop solution is added.
- **HRP**: Horseradish Peroxidase, an enzyme conjugated to antibodies in ELISA that catalyzes the oxidation of TMB to produce a measurable color change.
- **QC**: Quality Control, procedures to ensure test accuracy and reliability.
- **OD**: Optical Density, a measure of light absorbance by the sample, read by a microplate reader.
- **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

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	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 and 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.

Manufacturer's safety notes: The Abbexa ELISA kit reagents are for in vitro diagnostic use only. Do not ingest. Avoid pipetting by mouth. Avoid microbial contamination of reagents. Dispose of chemical waste according to local regulations.

6. Materials and Equipment

- Abbexa Human RVF IgM ELISA Kit (Catalog No: abx055793)
- 37°C incubator
- Multi and single-channel pipettes with sterile tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled water
- Absorbent filter papers
- Graduated cylinders (100 ml and 1 L)
- Microplate reader (450 nm)
- ELISA shaker

7. Storage

- The test kit must be stored at temperatures between 2-8°C

8. Procedure

8.1 Reagent preparation

a) Dilute the concentrated Wash Buffer 30-fold with distilled water. Ensure crystals are dissolved.
E.g Add 20µl of concentrated wash buffer into 580ml of distilled water

8.2 Preparing Participant Samples for Analysis

a) When ready to analyse dilute samples 1:5 with Sample Diluent Buffer before use.
E.g. Add 10µl of sample to 40µl of sample diluent buffer

8.3 Assay Protocol

a) Equilibrate reagents and samples to room temperature.

b) Set 2 positive controls, 2 negative controls, one well per sample and one well for the control (zero) wells on the pre-coated plate respectively, and record their positions.
   i) Add the solution to the bottom of each well without touching the side walls. Pipette the standards and samples up and down to mix before adding to the wells. Avoid foaming/bubbles.
   ii) Aliquot 50µl of negative and 50µl of positive control into the set wells. Aliquot 50µl of sample diluent buffer in the control (zero) blank well.
   iii) Aliquot 50µl of diluted sample (see previous section on sample preparation) into the test sample wells. Gently tap the palte to mix, or use a microplate shaker.
   iv) Cover the plate and incubate for 30minutes at 37°C
c) Remove cover, discard the liquid and wash plate 5 times with 1x wash buffer.
   i) Fill well completely with 300µl wash buffer using a multichannel pipette or autowasher (1-2mins soaking period is recommended).
   ii) Complete removal of liquid at each step is essential for good performance.
   iii) After the final wash, remove any remaining wash buffer by aspirating or decanting.
   iv) Invert the plate and blot it against clean absorbent paper towels.
d) Aliquot 50µl of Detection reagent to each well (**except the blank well)**.
e) Cover the plate and incubate for 30minutes at 37°C.
f) Remove cover, discard the liquid and wash plate 5 times (As in step 3)
g) Aliquot **50µl of TMB Substrate A** and **50µl of TMB Substrate B** into each well.
h) Cover the plate, gently tap the plate to mix thoroughly. Incubate for 37°C for 10-15minutes
i) Aliquot 50µl of Stop Solution into each well. **NOTE:** It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactive the enzyme completely.
j) Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure to OD at 450nm immediately

9. Data Analysis and Interpretation

- Positive Control OD ≥ 1.00
- Negative Control OD ≤ 0.20
- Cut-off = Negative Control + 0.15
- OD < Cut-off → Negative
- OD ≥ Cut-off → Positive
- If controls out of range, repeat assay.

10. Quality Control

- Run kit-provided positive and negative controls in duplicate.
- The assay is valid only if control OD values meet manufacturer's specifications.
- Document all QC results in the ELISA QC Log.

11. Documentation

- Record all assay details in the ELISA Results sheet, including operator name, date, lot numbers, OD readings, and final interpretation (A template is available named: 'ELISA_PlatePlanResultsTemplate').
- Enter results into dedicated KOBO form.
- Maintain QC logs for review.
- Archive raw data printouts and electronic files securely.

12. Related Templates

- ELISA Plate Plan Results Template
- ELISA QC Log

13. Review and Approval

Approved By: Prof Siobhan Mor

Title: *Chief Investigator, ZAFI*
Date: *6 March 2026*