SOP Title: IgM ELISA for Zika Virus

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1. Purpose

To describe the procedure for the quantitative detection of **Zika Virus IgM in human serum and plasma using the Abbexa Human ZIKV IgM ELISA Kit** (Catalog No: abx055830), following the manufacturer's instructions and adapted to the ZAFI project.

2. Scope

This SOP applies to all laboratory personnel involved in the diagnosis of Zika Virus IgM in human serum and plasma samples at ZAFI project laboratories in Uganda, Kenya, and Ethiopia.

3. Abbreviations

- **ZIKV IgM**: Immunoglobulin M specific to Zika Virus.
  - **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

Kit Components:

- Pre-coated 96-Well Microplate: 12 x 8
- Standard: 0.3 ml x 6 tubes (20, 10, 5, 2.5, 1.25, 0 ng/ml)
- Wash Buffer (20X): 25 ml
- Sample Diluent Buffer: 6 ml
- Detection Reagent: 10 ml
- TMB Substrate A: 6 ml
- TMB Substrate B: 6 ml
- Stop Solution: 6 ml
- Plate Sealer: 3
- Hermetic Bag: 1

Additional Materials Required but Not Provided:

- 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
- 100 ml and 1 liter graduated cylinders
- Microplate reader (450 nm)
- ELISA shaker

7. Storage

- Store at 2-8°C

8. Procedure

8.1 Reagent Preparation

a) Prepare standards as per kit instructions (20 to 0 ng/ml). Please refer to the table below. Six (6) tubes of the standard are provided

Tube	0	1	2	3	4	5
Concentration (ng/ml)	20	10	5	2.5	1.25	0

b) Dilute the concentrated Wash Buffer 1:20 ((i.e. add 20 ml of concentrated wash buffer into 380 ml) with distilled water).

8.2 Sample Preparation

a) Dilute samples 1:5 (add 10 µL of sample to 40 µL of sample diluent buffer) with Sample Diluent Buffer before analysis.

8.3 Assay Protocol

a) Equilibrate all reagents and samples to room temperature.
b) Set up standard, sample, and control (zero) wells on the pre-coated plate and record their positions.
   - 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 or bubbles.
c) Add 50 µl of the standards (20ng/ml, 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml) to standard wells.
d) Add 50 µl diluted samples to test sample wells.
   - Gently tap the plate to mix or use a microplate shaker.
e) Cover with plate sealer, incubate 30 min at 37°C.
f) Remove the cover and discard the solution. Wash plate 5 times with 1X Wash Buffer (Details below).
   - Fill each well completely with Wash buffer (350µl) using a multi-channel pipette or autowasher (1-2mins soaking period is recommended).
   - Complete removal of liquid at each step is essential for good performance.
   - After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
g) Add 100 µl Detection Reagent to each well, incubate 60 min at 37°C.
h) Remove the cover, discard the solution and wash plate 5 times (As described above).
i) Add 50 µl TMB Substrate A + 50 µl TMB Substrate B into each well. Cover the plate, gently tap to mix thoroughly and incubate 15 min at 37°C (Avoid exposure to light).
j) Add 50 µl Stop Solution to each well.
   - **Note**: It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
   - **Note**: before measuring the OD (next step) 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.
k) Read OD at 450 nm immediately.

l) For calculation of OD 450, average the OD450 readings for each reference standard, and each sample and then subtract the average control (zero) OD reading. (See section 7. Data Analysis).

9. Data Analysis and Interpretation

a) Acceptable CV: Intra-assay <10%, Inter-assay <12%
b) QC Acceptance Criteria:
   Mean Positive Control OD ≥ 1.00
   Mean Negative Control OD ≤ 0.20
   Cut-off = Negative Control OD + 0.15
c) A sample OD higher than the cut-off can be considered positive

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*