SOP Title: IgM ELISA for West Nile Virus

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1. Purpose

To describe the procedure for **detecting IgM antibodies against West Nile Virus using the Abbexa Human West Nile Virus-IgM ELISA Kit**, following the manufacturer's instructions and as adapted for the ZAFI project.

2. Scope

This SOP applies to all laboratory personnel involved in performing and interpreting the results of the CCHF IgM ELISA Kit at ZAFI project laboratories in Uganda, Kenya and Ethiopia.

3. Definitions

- **WNF**: West Nile fever, caused by west Nile virus that is transmitted by mosquitoes.
- **ELISA**: Enzyme-Linked Immunosorbent Assay, a plate-based immunoassay technique used to detect and quantify soluble substances such as antibodies, antigens, proteins, and hormones.
- **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 procedures; document results accurately in logbooks and KOBO tools; report deviations or issues to the laboratory lead/supervisor.

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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

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

All components listed in the kit including

- Abbexa Human West Nile Virus-IgM ELISA Kit (Catalogue No: **abx055291**)
- Calibrated micropipettes (single and multichannel) and tips
- Microplate reader (450 nm)
- Wash bottle or automated plate washer
- Incubator (37°C)
- Distilled or deionized water
- Waste container with disinfectant

7. Storage

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

8. Procedure

8.1 Reagent Preparation

a) Bring all reagents and samples to room temperature (20–25°C) before use.
b) Wash buffer: Dilute the concentrated Wash Buffer 1:30 (i.e. add 20 ml of concentrated wash buffer into 580 ml) with distilled water).
c) Prepare the required number of wells according to the plate layout.

8.2 Preparing Participant Samples for Analysis

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 Steps

a) Set 2 positive and 2 negative control, test sample and control (zero) wells on the pre-coated plate respectively, and record their positions.

Note: 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.

b) Add 50µl of negative and 50µl positive control into the set wells. Aliquot 50µl of sample diluent buffer in the control (zero) blank well.
c) Aliquot 50µl of appropriately diluted sample (diluted 5-fold, please see above) into the test sample wells.
   - Gently tap the plate to mix, or use a microplate shaker.
d) Cover the plate and incubate at 37°C for 30 minutes.
e) Remove the cover and discard the liquid. Wash the plate 5 times with 1X wash buffer (Details below).
   - Fill each well completely with Wash buffer (300µl) 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.
f) Aliquot 50µl of detection reagent to each well (except the blank well). Cover the plate and incubate for 30mins at 37°C.
g) Remove the cover, discard the liquid, and repeat the wash process as described above, 5 times.
h) Aliquot 50ul if TMB Substrate A and 50ul of TMB Substrate B into each well. Cover the plate and gently tap the plate to mix thoroughly. Incubate at 37°C for 10-15mins. Avoiding exposure to light.
i) Aliquot 50ul of Stop Solution into each well. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
j) Ensure there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the OD at 450nm immediately.

9. Data Analysis and Interpretation

- Mean OD of the Positive Control should be ≥1.00
- Mean OD of the Negative Control should be ≤ 0.20
- CUT OFF Value = Negative control + 0.15

If the positive control value is ≥1.00 and the Negative control value is ≤ 0.20, the test is valid, otherwise, the test is invalid.

If OD of Samples <CUT OFF, the test samples are considered negative.

If OD of Samples ≥ CUT OFF, the test samples are 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*

Annex 1: Result Interpretation Reference Table

Cut-off value calculation and interpretation should follow the Abbexa HWNV IgM ELISA kit instructions.

Result	**Criteria**
Positive	Sample OD ≥ Cut-off Value
Negative	Sample OD < Cut-off Value
Equivocal	Sample OD within ±10% of Cut-off Value; repeat test