SOP Title: IgM ELISA for Chikungunya Virus

SOP ID: 327, Version: 1.0

Effective Date: 6 March, 2026
Review Date: 6 March, 2027
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1. Purpose

To describes the procedure for detecting **IgM antibodies against Chikungunya virus using the EUROIMMUN Anti-Chikungunya Virus ELISA (IgM)** following the manufacturer's instructions and as adapted to the ZAFI project.

2. Scope

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

3. Abbreviations

- **CHIKV:** Chikungunya virus, a virus 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

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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 & 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 EUROIMMUN 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

- EUROIMMUN Anti-Chikungunya Virus ELISA (IgM) Test (Catalogue: EI 293a-9601 M)
- 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 8.1 Reagent Preparation

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

b) Prepare 1^{x} wash buffer from the 10x concentrate provided by diluting concentrated buffer with distilled water (1 in 10) *E.g. 1 part reagent plus 9 parts distilled water*

NOTE: If crystallisation has occurred in the concentrate warm to ~37°C and mix well before diluting
NOTE: 1x wash buffer should be prepared fresh for each ELISA run.

c) Prepare the required number of wells according to the plate layout.

8.2 Preparing Participant Samples for Analysis

a) Patient samples are diluted 1:101 with the green-coloured sample buffer. *E.g 10ul sample to 1.0ml sample buffer*

b) Mix well by vortexing (Sample pipettes are not suitable for mixing)
c) Incubate mixture for at least 10minutes at room temperature (18-25 °C)
d) Sample can then be pipetted into the microplate wells according to the pipetting protocol.

8.3 Sample Incubation

a) Add 100µl of controls (positive and negative), calibrators, and diluted samples into designated wells:
   i) One well for calibrator
   ii) One well for positive control
   iii) One well for negative
   iv) One well per sample
b) Cover with protective foil and Incubate for 60minutes at 37°C +/- 1°C)

8.4 Washing

a) MANUAL: Empty the wells and subsequently wash 3times using 300µl of working-strength wash buffer for each wash
b) AUTOMATIC: Wash the reagent wells 3 times with 450 µl working-strength wash buffer -For washing details check insert or Local guidelines.
c) Leave the wash buffer in each well for 30-60seconds per washing cycle and then empty the wells
d) After washing (Manual and automated) thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

NOTE: Residual liquid (>10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false readings

NOTE: Insufficient washing (less than 3 wash cycles/too small wash buffer volumes/too short residence times) can lead to false high readings.

NOTE: Free/empty positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

8.5 Conjugate Incubation

a) Pipette 100µl conjugate reagent (Peroxidase-labelled anti-human IgM) to each well
b) Incubate for 30minutes at room temperature (18-25 °C)

8.6 Washing

a) MANUAL: Empty the wells and subsequently wash 3times using 300µl of working-strength wash buffer for each wash
b) AUTOMATIC: Wash the reagent wells 3 times with 450 µl working-strength wash buffer (Program setting e.g. TECAN Columbus washer “OverFlow mode”)
c) Leave the wash buffer in each well for 30-60seconds per washing cycle and then empty the wells
d) After washing (Manual and automated) thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

8.7 Substrate Incubation

a) Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for 15minutes at room temperature protecting from light (18-25 °C)

8.8 Stopping

a) Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

8.9 Measurement

a) Prior to measuring, slightly shake the microplate to ensure a homogenous distribution of the solution
b) Photometric measurement should be made at a wavelength of 450nm and a reference wavelength of 620nM **within 30 minutes of adding the stop solution**.

9. Data Analysis and Interpretation

a) The extinction value/OD of the calibrator defines the upper limit of the reference range of non-infected persons (**cut off**) recommended by the manufacturer (EUROIMMUN).
b) Values above the indicated cut off are to be considered as positive, those below as negative.
c) Use the following formula to calculate the ratio, where extinction value is OD:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator}} = \text{Ratio}$$

NOTE: The manufacturer (EUROIMMUN) recommends interpretation of results as follows:

Ratio	Interpretation
<0.8	Negative
≥0.8 to <1.1	Borderline
≥1.1	Positive

NOTE: For duplicate determinations, the mean of the two values should be taken. If the two values deviate substantially from one another, the manufacturer (EUROIMMUN) recommends retesting the samples.

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_PlatePlan_ResultsTemplate).
- 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*