SOP Title: RT-qPCR for Dengue, Chikungunya and Zika Viruses

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1. Purpose

To ensure a standardized and consistent approach to the PCR-based detection of **dengue, chikungunya and zika virus RNA in serum samples** collected as part of the ZAFI project.

2. Scope

This SOP applies to all laboratory personnel involved in the processing, analysis, and interpretation of samples for dengue, chikungunya and zika virus PCR testing under the ZAFI project across Kenya, Uganda, and Ethiopia.

3. Abbreviations

- **RT-qPCR:** Reverse Transcriptase Quantitative Polymerase Chain Reaction
- **DENV**: Dengue Virus
- **CHIKV**: Chikungunya Virus
- **ZIKV**: Zika Virus

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.
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

- Preparation of mastermix should be conducted in a “Clean room” or “Clean hood” that is separate to template RNA and any amplification procedures to prevent contamination
- Template (RNA) and samples should be stored separately to mastermix and assay reagents

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- When calculating the volume of mastermix to make, include enough volume for a single positive control per target and a negative control

6. Consumables and Reagents

Item	
Reagents	**Equipment**
TaqMan Fast Virus 1-Step Multiplex Master Mix	Microcentrifuge
Primers and Probes	Pipettes (10µl, 20 µl, 200 µl, 1000 µl)
Nuclease Free Water	Vortex
DENV positive RNA control material (gBlock)	**Tubes/Plates/other**
CHIKV positive RNA control material (gBlock)	Plastics consumables
ZIKV positive RNA control material (gBlock)	Pipette tips
	Disposable gloves

7. RT-qPCR Procedure

7.1 Preparing the PCR Reaction Mix

a) Retrieve the working (10uM) stocks of primers, probes and master mix for the DENV/CHIKV/ZIKV RT-qPCR and fully thaw reagents on ice.

b) When completely thawed vortex each tube briefly and spin in microcentrifuge to collect liquid at the bottom.

c) Calculate the number of reactions needed for the samples (one reaction per sample) **including a positive control for each target and a negative control**. Multiply the volume per reaction of each component by the total number of reactions.

d) Combine these volumes into one 1.5ml Eppendorf. ***This should be done in a separate room/hood to template addition.***

Table of Reactions			
Component	**Final concentration**	**Volume per Reaction** 96-well (0.1ml) plates	**Total volume to add to the 1.5ml Eppendorf assuming *n* reactions**
Reaction mix			
TaqMan Fast Virus 1-Step Multiplex Master Mix	1x	2.75	2.75 x *n*
DENV-Forward (10uM)	400nM	0.55	0.55 x *n*
DENV-Reverse1	400nM	0.55	0.55 x *n*
DENV-Reverse2	400nM	0.55	0.55 x *n*
DENV-Probe	200nM	0.275	0.275 x *n*
CHIKV-Forward	400nM	0.55	0.55 x *n*
CHIKV-Reverse	400nM	0.55	0.55 x *n*
CHIKV-Probe	200nM	0.275	0.275 x *n*
ZIKV-Forward	400nM	0.55	0.55 x *n*
ZIKV-Reverse	400nM	0.55	0.55 x *n*
ZIKV-Probe	200nM	0.28	0.28 x *n*
NFW	-	3.58	3.58 x *n*
Aliquot per well			

Reaction mix		10ul	
RNA/Template volume		2.5ul	
Total volume		**12.5ul**	

e) Vortex briefly to mix and spin to ensure all the liquid is at the bottom
f) Transfer 10µl of PCR Reaction Mix to each well of the plate
g) Retrieve RNA eluates immediately prior to template addition and thaw on ice
h) Transfer plate to template addition room/space and add 2.5µl RNA (extracted from sample), or nuclease-free water for Non-Template Control, to each well. Include Nuclease Free Water as a Non-Template Control and positive RNA as a positive control.
i) Seal the reaction plate with optical adhesive film or strip-caps and then centrifuge briefly to bring the PCR reaction mix to the bottom of the well.

7.2 Setting up the machine

a) Set up the following run profile on the qPCR machine (Table below).
b) Thermal profile

Thermal profile for DENV/CHIKV/ZIKV				
Step	**Temperature**	**Time**	**Cycles**	**Fluorescence acquisition (channel)**
Reverse Transcription	50	5:00	1	
RT-Inactivation/Initial Denaturation	95	0:20	1	
Denature	95	0:15	40	
Anneal/Extend	60	1:00		FAM/VIC/TEXASRED

7.3 Interpretation

a) The negative control must have no amplification present in any channel
b) The positive controls must have amplification in their respective channels
   - The DENV-std Positive control should amplify in the FAM channel
   - The CHIKV-std Positive control should amplify in the VIC channel
   - The ZIKV-std Positive control should amplify in the TEXRED channel
c) Results with a Ct below 38 in either FAM/VIC/TEXRED will indicate positive for the respective pathogen. E.g. amplification of a sample in the FAM channel would indicate positive for dengue if below Ct 38.

8. Record Keeping

- Enter results into the results sheet
- Enter results into the dedicated **KOBO form**

9. Primer Sequences

Primer/probe ID	**Sequence**
DENV-F	TAGTCTRCGTGGACCGACAAG
DENV-R1	CAGTTGACACRCGGTTTCTC
DENV-R2	GGGTTGATACGCGGTTTCTC
DENV-P	FAM-CGYCTWTCAATATGCTGAAACGCG-BHQ-1

CHIKV-F	ACCHTCRGTGTTCCATCTAAAR
CHIKV-R	GCCYGGRCTSATCGTTATT
CHIKV-P	VIC-ACAGTGGTTTCGTGYGARGGCTAY-BHQ1
ZIKV-F	CCGCTGCCCAACACAAG
ZIKV-R	CCACTAACGTTCTTTTGCAGACAT
ZIKV-P	TEXRED-AGCCTACCTTGACAAGCAGTCAGACACTCAA-BHQ1

10. Review and Approval

Approved By: Prof Siobhan Mor

Title: *Chief Investigator, ZAFI*

Date: *3 March 2026*