SOP Title: RT-qPCR for West Nile Virus

SOP ID: 316, Version 1.0

Effective Date: 3 March, 2026
Review Date: 3 March, 2027
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1. Purpose

To ensure a standardized and consistent approach to the PCR-based detection of **West Nile 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 West Nile virus PCR testing under the ZAFI project across Kenya, Uganda, and Ethiopia.

3. Abbreviations

- **RT-qPCR:** Reverse Transcriptase Quantitative Polymerase Chain Reaction
- **WNV**: West Nile 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
- When calculating the volume of mastermix to make, include enough volume for a single positive control per target and a negative control

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6. Consumables and Reagents

Item	
Reagents	**Equipment**
TaqMan Fast Virus 1-Step Master Mix	Microcentrifuge
Primers and Probes	Pipettes (10µl, 20 µl, 200 µl, 1000 µl)
Nuclease Free Water	Vortex
WNV gBlock (Standard) positive control material	**Tubes/Plates/other**
	Plastics consumables
	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 WNV 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-wel (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 (No Rox)	1x	2.75	2.75 x *n*
WNV Forward Primer (10uM)	400nM	0.55	0.55 x *n*
WNV Reverse Primer (10uM)	400nM	0.55	0.55 x *n*
WNV Probe (10uM)	200nM	0.28	0.28 x *n*
Nuclease Free Water	-	6.88	6.88 x *n*
Aliquot per well			
Reaction mix		10ul	
DNA/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), nuclease-free water for Non-Template Control, or positive 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.

b) Ensure that each well is set to collect data in the FAM channel

Thermal profile for WNV qPCR				
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

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 (FAM for WNV)

c) Results with a Ct below 38 in the FAM channel can be considered as positive.

8. Record keeping

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

9. Primer Sequences

Primer/probe ID	**Sequence**
WestNileV Forward	CAGACCACGCTACGGCG
WestNileV Reverse	CTAGGGCCGCGTGGG
WestNileV Probe	FAM - TCTGCGGAGAGTGCAGTCTGCGAT - TAMRA

10. Review and Approval

Approved By: Prof Siobhan Mor

Title: *Chief Investigator, ZAFI*

Date: *3 March 2026*