# SOP Title: qPCR for *Rickettsia* spp. (Typhus and Spotted Fever Group)

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## 1. Purpose

To ensure a standardized and consistent approach to the PCR-based detection of ***Rickettsia* spp. DNA in whole blood 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 *Rickettsia spp.* PCR testing under the ZAFI project across Kenya, Uganda, and Ethiopia.

## 3. Abbreviations

- **qPCR:** Quantitative Polymerase Chain Reaction

## 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 DNA and any amplification procedures to prevent contamination
- Template (DNA) 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 Advanced Master Mix | Microcentrifuge |
| Primers and Probes | Pipettes (10µl, 20 µl, 200 µl, 1000 µl) |
| Nuclease Free Water | Vortex |
| Rickettsia Standard Positive Control (gBlock) | **Tubes/Plates/other** |
| | Plastics consumables |
| | Pipette tips |
| | Disposable gloves |

## 7. qPCR Procedure

### *7.1 Preparing the PCR Reaction Mix*

a) Retrieve the working (10uM) stocks of primers, probes and master mix for the Rickettsia qPCR and fully thaw reagents on ice. (Details of preparation of primers is detailed in **SOP 325 Preparation of primers and probes for qPCR**).

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 a 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 Advanced Master Mix (2x) | 1x | 5.50 | 5.5 x *n* |
| Forward Primer (10uM) | 200nM | 0.28 | 0.28 x *n* |
| Reverse Primer (10uM) | 200nM | 0.28 | 0.28 x *n* |
| Probe (10uM) | 200nM | 0.28 | 0.28 x *n* |
| NFW | - | 4.68 | 4.68 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 DNA eluates immediately prior to template addition and thaw on ice

h) Transfer plate to template addition room/space and add 2.5µl DNA (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 DNA/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 each well is selected to collect data in the FAM channel

| **Thermal Profile for Rickettsia qPCR** | | | | |
|---|---|---|---|---|
| **Step** | Temperature | Time | Cycles | **Fluorescence acquisition (channel)** |
| **Hold** | 50 | 2:00 | 1 | |
| **Hold** | 95 | 0:20 | 1 | |
| **Denature** | 95 | 0:03 | 40 | |
| **Anneal/Extend** | 60 | 0:30 | | 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)
c) Results with a Ct value below 38 can be considered positive.

## 8. Record Keeping

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

## 9. Primer Sequences and Further Details

| **Primer/probe ID** | **Sequence** |
|---|---|
| Rickettsia-F | TCG CAA ATG TTC ACG GTA CTT T |
| Rickettsia-R | TCG TGC ATT TCT TTC CAT TGT G |
| Rickettsia_CS-P | 6-FAM-TGC AAT AGC AAG AAC CGT AGG CTG GAT G-BHQ-1-3 |

## 10. Review and Approval

Approved By: Prof Siobhan Mor

Title: *Chief Investigator, ZAFI*
Date: *3 March 2026*