SOP Title: qPCR for *Salmonella* Typhi (typhoid) and pan-*Salmonellae*

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1. Purpose

To ensure a standardized and consistent approach to the PCR-based detection of ***Salmonella* DNA in positive blood culture 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 *Salmonella* 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 (2x)	Microcentrifuge
Primers and Probes	Pipettes (10µl, 20 µl, 200 µl, 1000 µl)
Nuclease Free Water	Vortex
Salmonella_ttr gBlock (Standard) positive control material	**Tubes/Plates/other**
Salmonella_staG gBlock (Standard) positive control material	Plastics consumables
	Pipette tips
	Disposable gloves

7. qPCR Procedure

7.1 Preparing the PCR Reaction Mix

a) Retrieve working (10uM) stocks of primers, probes and master mix for the Salmonellae 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 Advanced Master Mix (2x)	1x	5.50	5.50 x *n*
Salmonella_ttr_F Primer	200nM	0.28	0.28 x *n*
Salmonella_ttr_R Primer	200nM	0.28	0.28 x *n*
Salmonella_ttr Probe	100nM	0.14	0.14 x *n*
Salmonella_staG_F Primer	400nM	0.55	0.55 x *n*
Salmonella_staG_R Primer	400nM	0.55	0.55 x *n*
Salmonella_staG Probe	200nM	0.28	0.28 x *n*
NFW	-	3.44	3.44 x *n*
Aliquot per well			
Reaction mix		10ul	
DNA/Template volume		2.5ul	
Total volume		**12.5ul**	

e) Vortex and spin briefly to ensure all the liquid is at the bottom.

f) Aliquot 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) Take the plate into the template addition space and add 2.5µl DNA (extracted from sample), nuclease-free water for Non-Template Control, or positive control to each well.

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 and TexasRed channel.

Thermal profile for Salmonella qPCR				
Step	**Temperature**	**Time**	**Cycles**	**Fluorescence acquisition (channel)**
Hold 1	50	2:00	1	
Hold 2	95	0:20	1	
Denature	95	0:03	40	
Anneal/Extend	60	0:30		FAM/TexasRed

7.3 Interpretation

a) The negative control must have no amplification present in any channel

b) The positive control must have amplification in their respective channels

- Salmonella_ttr standard (positive control) should amplify in the FAM channel
- Salmonella_staG standard (positive control) should amplify in the TexasRed Channel

c) Results with a Ct below 38 can be considered as positive.

- Positive in the FAM channel indicates positive for *Salmonella* species
- Positive in the TexasRed Channel indicates positive for *S.* Typhi

8. Record keeping

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

9. Primer Sequences

Primer/probe ID	**Sequence**
Salmonella_ttr_forward	CTCACCAGGAGATTACAACATGG
Salmonella_ttr_Reverse	AGCTCAGACCAAAAGTGACCATC
Salmonella_ttr_Probe	FAM-CACCGACGGCGAGACCGACTTT-BHQ1
Salmonella_staG_forward	CGCGAAGTCAGAGTCGACATAG
Salmonella_staG_reverse	AAGACCTCAACGCCGATCAC
Salmonella_staG_Probe	TexasRed-CATTTGTTCTGGAGCAGGCTGACGG-BHQ2

10. Review and Approval

Approved By: Prof Siobhan Mor

Title: *Chief Investigator, ZAFI*

Date: *3 March 2026*