

SOP Title: qPCR for *Leptospira* spp.

SOP ID: 310, Version: 1.0

Effective Date: 3 March, 2026

Review Date: 3 March, 2027

Primary Author (s): Tom Edwards, Caitlin Greenland-Bews

Secondary Author: Eric Fevre

Approver: Siobhan Mor

1. Purpose

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

3. Abbreviations

- **qPCR:** Quantitative Polymerase Chain Reaction

4. Responsibilities

5. 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.

6. 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

7. 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
Leptospira positive control material (gBlock/Standard)	Tubes/Plates/other
	Plastics consumables
	Pipette tips
	Disposable gloves

8. qPCR Procedure

7.1 Preparing the PCR Reaction Mix

- Retrieve the working (10uM) stocks of primers, probes and the master mix for the Leptospira qPCR and fully thaw reagents on ice.
- When completely thawed vortex each tube briefly and spin in microcentrifuge to collect liquid at the bottom.
- 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
- 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	Total volume to add to the 1.5ml Eppendorf assuming n reactions
		96-well (0.1ml) plates	
Reaction mix			
TaqMan Fast Advanced Master Mix (2x)	1x	5.50	$5.50 \times n$
Lepto Forward Primer (10uM)	500nM	0.69	$0.69 \times n$
Lepto Reverse Primer (10uM)	500nM	0.69	$0.69 \times n$
Lepto Probe (10uM)	125nM	0.18	$0.18 \times n$
NFW	-	3.94	$3.94 \times n$
Aliquot per well			
Reaction mix		10ul	
DNA/Template volume		2.5ul	
Total volume		12.5ul	

- Vortex briefly to mix and spin to ensure all the liquid is at the bottom.
- Aliquot 10µl of PCR Reaction Mix to each well of the plate
- Retrieve DNA eluates immediately prior to template addition and thaw on ice
- Transfer the plate to the template addition space/room and add 2.5 µl DNA (extracted from sample), or nuclease-free water for Non-Template Control, or positive control to separate wells.
- 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 Leptospira qPCR				
Step	Temperature	Time	Cycles	Fluorescence acquisition (channel)
Hold	50	2:00	1	
Hold 2	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 channel
 - Lepto_standard (positive control) should amplify in the FAM channel
- c) Results with a Ct below 38 can be considered as positive.

9. Record keeping

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

10. Primer Sequences

Primer/probe ID	Sequence
Lepto_For_Primer	GGATCTGTGATCAACTATTACGGATA
Lepto_Rev_Primer	CCCATTTCAGCGATTAC
Lepto_Probe	FAM-TAAAGCCAGGACAAGCGCCG-BHQ1

11. Review and Approval

Approved By: *Prof Siobhan Mor*

Title: *Chief Investigator, ZAFI*

Date: *3 March 2026*