SOP Title: qPCR for *Brucella* spp.

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1. Purpose

To ensure a standardized and consistent approach to the PCR-based detection of ***Brucella* 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 *Brucella* 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

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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
Bcsp31_std positive control material (gBlock)	**Tubes/Plates/other**
IS711_std positive control material (gBlock)	Plastics consumables
	Pipette tips
	Disposable gloves

8. qPCR Procedure

7.1 Preparing the PCR Reaction Mix

a) Retrieve the working (10uM) stocks of primers, probes and Master mix for the *Brucella* qPCR (Table 1) **including the 0.3uM stocks of RNAseP primer and probe**.

b) When the reagents are 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 Advanced Master Mix (2x)	1X	5.50	5.50 x *n*
bcsp31_1163_F (10uM)	300nM	0.42	0.42 x *n*
bcsp31_1163_R (10uM)	300nM	0.42	0.42 x *n*
bcsp31_Tq_probe-FAM (10uM)	200nM	0.28	0.28 x *n*
IS711_421_F (10uM)	300nM	0.42	0.42 x *n*
IS711_Tq_R_ (10uM)	300nM	0.42	0.42 x *n*
IS711 probe-VIC (10uM)	50nM	0.28	0.28 x *n*
RNAse P primer mix (0.3uM)		0.42	0.42 x *n*
RNAse P probe mix (TEXASRED (0.3uM))		0.17	0.17 x *n*
NFW	-	2.7	2.7 x *n*
Aliquot per well			
Reaction mix		10ul	
DNA		2.5ul	
Total volume		**12.5ul**	

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

f) Centrifuge in a microcentrifuge to bring reaction mix to the bottom of the tube.

g) Ailquot 10µl volume of PCR Reaction Mix to each well of the plate

h) Retrieve DNA eluates immediately prior to template addition and thaw on ice
i) Transfer plate to template addition room/space and add 2.5µl DNA, or nuclease-free water for Non-Template Control, or positive control to separate wells.
j) 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.

8.2. 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, VIC and TexasRed channels

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, VIC and 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
   - Bcsp standard (positive control) should amplify in the FAM channel
   - IS711 standard (positive control) should amplify in the VIC Channel
c) Positive amplification should be observed in the TexasRed channel for all human samples
d) Results with a Ct below 38 in either the FAM or VIC channel 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
bcsp31_1163_F	TCTTTGTGGGCGGCTATCC
bcsp31_1199_R	CCGTTCGAGATGGCCAGTT
bcsp31_Tq (probe)	FAM-ACGGGCGCAATCT-MGB-NFQ
IS711_421_F	CGCTCGCGCGGTGGAT
IS_Tq_R	CTTGAAGCTTGCGGACAGTCACC
IS711_probe	VIC-ACGACCAAGCTGCATGCTGTTGTCGATG-BHQ1
RNaseP_F	CCAAGTGTGAGGGCTGAAAAG
RNaseP_R	TGTTGTGGCTGATGAACTATAAAAGG
RNaseP_probe	TEXASRED–CCCCAGTCTCTGTCAGCACTCCCTTC–BHQ

11. Review and Approval

Approved By: Prof Siobhan Mor

Title: *Chief Investigator, ZAFI*

Date: *28 February 2026*