Author (s)	Reviewer	Authorizer
Numfor Hycenth Estelle Marion Sara Eyangoh	BU Lab network members	Advisory Board

ABREVIATIONS

BU	Buruli Ulcer
PCR	Polymerase Chain Reaction
QPCR	Real-time Polymerase Chain Reaction
IPC	Internal Positive Control
Ct	Cycle Threshold
WHO	World Health Organization

Table of Contents

I. PURPOSE

This Standard Operating Protocol (SOP) aims at presenting the different steps involved in preparing QPCR for *Mycobacterium ulcerans* using the internal positive control.

II. APPLICATION DOMAIN

To be applied to all laboratory members of the BU LAB Network for the PCR diagnosis of Buruli ulcer

III. ASSOCIATED DOCUMENTS

Worksheet: QPCR mix calcul and plate plan.

IV. TYPE OF SAMPLES

► Swabs are used for sampling of opened undermined lesions.

► Fine needle aspiration (FNA) is used for sampling of closed lesions or opened but not undermined.

► Biopsy is not recommended for case confirmation of Buruli ulcer.

V. REAGENTS AND CONSUMABLES

See list in annex1

VI. EQUIPMENT

See list in annex 2

VII. PROCEDURE

7.1 Prepare the experiment

1. Complete the plate plan of the worksheet for each new amplification with samples
2. Count the number of amplifications needed to be prepared. Important, count always 3 supplemental reactions to have enough mix at the end.
3. Calculate and fill in the table of PCR mix preparation
4. Switch on the thermocycler and fill in the plate plan

7.2 PCR mix preparation

Under a clean PCR hood

1. Wear gloves and a disposable blouse dedicated to this space.
2. Defrost the following reagents: QPCR master mix / primers and probe (IS2404) / IPC Primers and probe CY5/ an aliquot of sterile water
3. Probe (IS2404) dilution: 10 fold dilution before using
   - centrifuge the defrosted tube
   - in a new screw cap tube, add 18µl of water
   - add 2µl of probe

- vortex slowly and centrifuge for a few seconds

4. Primers (IS2404) dilution :
   - centrifuge the two aliquots of primer tubes
   - add directly 95µl of water into each tube
   - vortex slowly and centrifuge for a few seconds

7.3 PCR mix preparation:

1. follow the quantity of water, master mix, probe and primers calculated in the worksheet
2. prepare a new screw cap tube and mix the master mix by reversal
3. always start by pipetting water, then the primers/probe (IPC), the 2 primers and the probe (IS2404). Finish by adding the master mix.
4. mix the tube by reversal and centrifuge it few seconds
5. prepare a rack with 8 strip tubes
6. transfer 20µl of the mix in each tube
7. do not close the tubes

7.4 PCR mix and samples

1. On a dedicated bench, bring the rack with the PCR strip, the patient samples et plasmid :
2. Add 5µl of patient samples following the plate plan
3. Close the patient strips
4. Prepare the standard range of plasmid DNA in screw cap tubes

   - Defrost an aliquot of 10µl tube at 1E8 bact/ml.
   - Add 90µl of water directly in the tube = first point of the standard curve= 1E7 bact/ml.
   - Add 45µl of water in 5 new screw cap tubes.
   - Perform cascade dilution by pipetting 5µl of the first tube and mix it to the 45µl tube.
   1E7 bact/ml, 1E6 bact/ml, 1E5 bact/ml, 1E4 bact/ml, 1E3 bact/ml, 1E2 bact/ml,
   - Add 5µl of plasmid DNA in strip tubes following the plate plan.
   - close the strips.

7.5 Amplification

1. Centrifuge the strips and place it into the thermocycler.
2. Run the program of amplification

7.6 PCR Analysis

1. check the two negative controls: extraction and mix preparation
2. check the standard curve: ensure that Ct values and R2 are OK
3. check the internal control (IPC Diagenode CY5) : 27<Ct<35
4. limit of detection for human: <35 cycles.
5. Calculate the number of bacilli/ml for positive samples.
6. write the result in the manual registration book and in the worksheet for the clinician.
7. disseminate the results following the procedure validating in your country: email, whatsapp,

VIII. INTERNAL QUALITY CONTROL (IQC)

Internal positive control (IPC)

IX. SAFETY PRECAUTIONS

Always consider all used materials as infectious and discard appropriately.

X. REFERENCE

1. Laboratory diagnosis of Buruli ulcer: A WHO Manual for Health-care providers (edited by: Françoise Portaels) 2014. Available at *https://apps.who.int/iris/handle/10665/111738*; accessed on 28-11-19

XI. READING AND UNDERSTANDING LIST

NAME OF PERSONNEL	DATE	SIGNATURE