

#### PROCEDURE

BU-SOP-04-1

Version: 1.0

Effective date: March 2020

## Standard Operating Protocol 4: QPCR preparation with Internal Positive Control

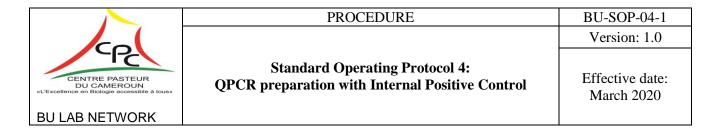
Author (s)	Reviewer	Authorizer
Numfor Hycenth	BU Lab network members	
Estelle Marion		Advisory Board
Sara Eyangoh		·

#### **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**

l.	PURPOSE	2
II.	APPLICATION DOMAIN	2
III.	ASSOCIATED DOCUMENTS	2
IV.	TYPE OF SAMPLES	2
٧.	REAGENTS AND CONSUMABLES	2
VI.	EQUIPMENT	2
VII.	PROCEDURE	2
VIII.	INTERNAL QUALITY CONTROL (IQC)	4
IX.	SAFETY PRECAUTIONS	4
X.	REFERENCE	4
XI.	READING AND UNDERSTANDING LIST	.5



#### **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

	PROCEDURE	BU-SOP-04-1
		Version: 1.0
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- 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  $90\mu l$  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,



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Standard Operating Protocol 4: QPCR preparation with Internal Positive Control	Effective date: March 2020

#### 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 <a href="https://apps.who.int/iris/handle/10665/111738">https://apps.who.int/iris/handle/10665/111738</a>; accessed on 28-11-19



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## XI. READING AND UNDERSTANDING LIST

NAME OF PERSONNEL	DATE	SIGNATURE