CPC
CENTRE PASTEUR DU CAMEROUN «L'Excellence en Biologie accessible à tous»

# Standard Operating Protocol 3: Extraction and Purification of DNA from *M. ulcerans* (without Internal Positive Control)

PROCEDURE

BU-SOP-03-2

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Effective date: March 2020

BU LAB NETWORK

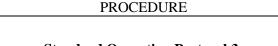
Authors	Reviewers	Authorizer
Numfor Hycenth Estelle Marion Sara Eyangoh	BU Lab network members	Advisory Board

#### ABREVIATIONS

BU	Buruli Ulcer
PCR	Polymerase Chain Reaction
VHO World Health Organization	

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Standard Operating Protocol 3: Extraction and Purification of DNA from *M. ulcerans* (without Internal Positive Control)

## I. PURPOSE

This Standard Operating Protocol (SOP) aims to present the different steps allowing DNA extraction and purification of samples for PCR targeting *Mycobacterium ulcerans*.

## **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

None

## 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 DNA EXTRACTION

DNA extraction is performed by bacterial lysis through Alkaline lysis using the Genolyse kit (Ref

51610, Hain LifeScience)

- 1.For each sample, use 400µl of specimen suspension prepared previously and placed in a microtube with screw cap (see in SOP2).
- 2. Centrifuge the tube at 12000g for 15min at RT
- 3. Discard delicately the supernatant using a P1000 filter tip. A pellet can be visible or not.
- 4. Resuspend the pellet with 400µl of water and centrifuge at 12000g for 15min at RT
- 5. Discard delicately the supernatant
- 6.Resuspend the pellet in 50µl of A-LYS buffer
- 7.Incubate 10min at 95°C
- 8. Centrifuge tube for 10 sec to pellet the suspension



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- 9.Add 50µl of Buffer A-NB to neutralize
- 10. keep tube at 4°C if use in the day for PCR amplification or store at -20°C
- NB: For swabs and FNA samples, no need to perform purification step

#### 7.2 DNA PURIFICATION FOR BIOPSY SAMPLES

DNA purification is recommended in cases of biopsy samples to remove traces of inhibitors.

Use the "QIAquick purification kit" (ref 28106, QIAGEN) as following :

- 1. Add 300µl of PB buffer to each sample and 10µl of Na Acetate 3M
- 2. Load the column with the sample and centrifuge 1min, 5000g, RT
- 3. Wash the column with  $750\mu l$  of PE buffer and centrifuge 1min 5000g RT
- 4. Repeat a second centrifugation without adding a buffer in order to remove any trace of ethanol
- 5. Eluate DNA by adding 100µl of EB buffer and centrifuge 1min 5000g RT
- 6. Store at 4°C for short time storage or at -20°C for long time storage.

#### VIII. INTERNAL QUALITY CONTROL (IQC)

Not applicable

#### **IX. SAFETY PRECAUTIONS**

Always consider all used materials as infectious and discard appropriately.

#### X. REFERENCE

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

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## XI. READING AND UNDERSTANDING LIST

NAME OF PERSONNEL	DATE	SIGNATURE