INTEGRATED BIOREPOSITORY OF H3AFRICA UGANDA

MAKERERE UNIVERSITY

COLLEGE OF HEALTH SCIENCES

STANDARD OPERATING PROCEDURE

TITLE: **Isolation of Bacterial Microbiome DNA From Mixed Samples Method**	**PAGE 1 of 9**

SOP #: **IBRH$_3$AU-SOP-BSP-018.1**	Effective Date: **01/08/2018**	Next Rev: **AUG 2021**
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VALIDATION AND RETIREMENT

	NAME	DATE
Validated by:		
Retired by:		

ACKNOWLEDGEMENT OF READING AND UNDERSTANDING

I have received and understood the training on this SOP. If I have not understood the training I have asked the trainer to retrain me to ensure that I completely understand all the requirements.

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1. PURPOSE

The purpose of this Standard Operating Procedure is to describe the procedure for an easy-to-use workflow for selective isolation of bacterial DNA from samples that are intrinsically rich in host DNA, such as bodily fluids or swabs. The method is specific for the identification of intact bacteria so it prevents false results due to nucleic acids from dead bacteria.

2. SCOPE

This document applies to all laboratory personnel intending to design sops, as well as quality documents in the molecular diagnostics laboratory.

3. PRINCIPLE

The QIAamp DNA Microbiome Kit efficiently depletes human and animal host DNA and yields enriched bacterial DNA. An optimized combination of mechanical and chemical lysis allows efficient disruption of bacterial cells. Target DNA is purified through adsorption to the silica membrane of QIAamp UCP Mini Columns, which have undergone proprietary DNA decontamination processes.

Proteinase K

Ready to use Proteinase K solution comes dissolved and stored at room temperature (15–25°C). To prolong the lifetime of Proteinase K, storage at 2-8°C is recommended.

4. RESPONSIBILITIES

In charge	Responsibility
Laboratory Personnel	Performing the prescribed activity as detailed in this procedure Abiding by all applicable Quality System requirements
Laboratory Management	Ensuring that all laboratory processes are appropriate for their intended use Ensuring that all personnel involved in this process are appropriately trained Ensuring that discrepancies, excursions etc, related to this process are investigated, documented and resolved in accordance with Quality system requirements.

5. ABBREVIATIONS

SOP- Standard Operating procedure

DNA-Deoxyribose Nucleic Acid

MDL-Molecular diagnostic Laboratory

6. EQUIPMENT

Micro centrifuge tubes (2 ml)

Overhead shaker

Shaker-incubator

Tissue Lyser

7. MATERIALS

Sterile pipet tips with aerosol barriers
Ethanol (96-100%)
Phosphate-buffered saline

8. PROCEDURES

8.1 Preparation of buffers

8.1.1 Buffer AHL

Thaw Buffer AHL at room temperature or at 2–8°C and mix well prior to use. To ensure optimal performance, do not freeze and thaw more than 3 times. In case less than a full bottle of Buffer AHL is needed, make sure to use sterile technique and/or work under laminar flow when removing the required amount of buffer to avoid contamination and microbial growth in the remaining buffer.

8.1.2 Buffer AW1

Add the appropriate amount of ethanol (96–100%) as indicated on the bottle and mix well.

8.1.3 Buffer AW2

Add the appropriate amount of ethanol (96–100%) as indicated on the bottle and mix well.

8.2 Procedure for 1 ml sample volume

8.2.1 Add 500 µl Buffer AHL to 1 ml of sample in a 2 ml tube (not provided)

8.2.2 Incubate for 30 min at room temperature with end over end rotation. For smaller sample volumes adjust the volume of Buffer AHL accordingly.

Optional: Instead of using end-over-end rotation, you can incubate non-viscous samples in a thermomixer at 600 rpm. Periodically check that the sample and Buffer AHL are mixing correctly.

8.2.3 Centrifuge the tube at 10,000xg for 10min and carefully remove the supernatant. Do not disturb the pellet as this will result in loss of bacterial material.

8.2.4 Add 190 µl Buffer RDD and 2.5µl Benzonase. Mix well and incubate at 37°C for 30min at 600rpm in a heating block or water bath.

8.2.5 Add 20µl Proteinase K and incubate at 56°C for 30min at 600rpm in a heating block or water bath.

8.2.6 Briefly spin the tube at slow speed to remove condensation. Add 200 µl Buffer ATL (containing Reagent DX). Mix well to avoid loss of sample material and transfer into a Pathogen Lysis Tube L.

Note: Careful mixing after the centrifugation step ensures efficient transfer of intact bacteria.

8.2.7 Lyse bacterial cells with Pathogen Lysis Tube L. The following three options have been verified as compatible with the QIAamp DNA Microbiome Kit.

A. Place the Pathogen Lysis Tube L in a TissueLyser LT for 10 min at 50 Hz or a TissueLyser II for 10 min at 30 Hz.

B. Place the Pathogen Lysis Tube L into a FastPrep-24 instrument. Apply a velocity of 6.5 m/s twice for 45 s each with a 5-min interval during which the samples should be stored on ice.

C. Place the Pathogen Lysis Tube L on a vortexer with a microtube foam insert and vortex for 10 min at maximum speed. Note: Avoid heating of samples during lysis.

8.2.8 Centrifuge the Pathogen Lysis Tube L at 10,000 x g for 1 min to reduce the amount of foam after lysis. Mix carefully and transfer the supernatant to a fresh micro centrifuge tube.

Note: Careful mixing after the centrifugation step will transfer possibly intact bacteria that are at the bottom of the tube. Do not transfer beads from the Pathogen Lysis Tube to subsequent reactions.

8.2.9 Add 40 µl Proteinase K, mix by vortexing and incubate at 56°C for 30 min at 600 rpm in a heating block or water bath.

8.2.10 Add 200 µl of Buffer APL2. Mix by pulse vortexing for 30 s.

8.2.11 Incubate at 70°C for 10 min and briefly spin the tube.

8.2.12 Add 200 µl ethanol to the lysate. Mix thoroughly by pulse vortexing for 15–30 s.

8.2.13 Carefully apply up to 700 µl of the mixture from step 8.2.11 to the QIAamp UCP Mini Column without wetting the rim. Close the cap and centrifuge at 6,000 x g for 1 min.

8.2.14 Discard the flow-through. Put the column back into the collection tube to repeat step 8.2.12 with any remaining mixture from step 8.1.11.

Note: Flow-through containing Buffer APL2 or Buffer AW1 is not compatible with bleach.

8.2.15 Transfer the QIAamp UCP Mini Column to a fresh collection tube. Carefully open the cap and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6,000 x g for 1 min. Place the QIAamp UCP Mini Column into a fresh 2 ml collection tube and discard the filtrate.

Note: Flow-through containing Buffer APL2 or Buffer AW1 is not compatible with bleach.

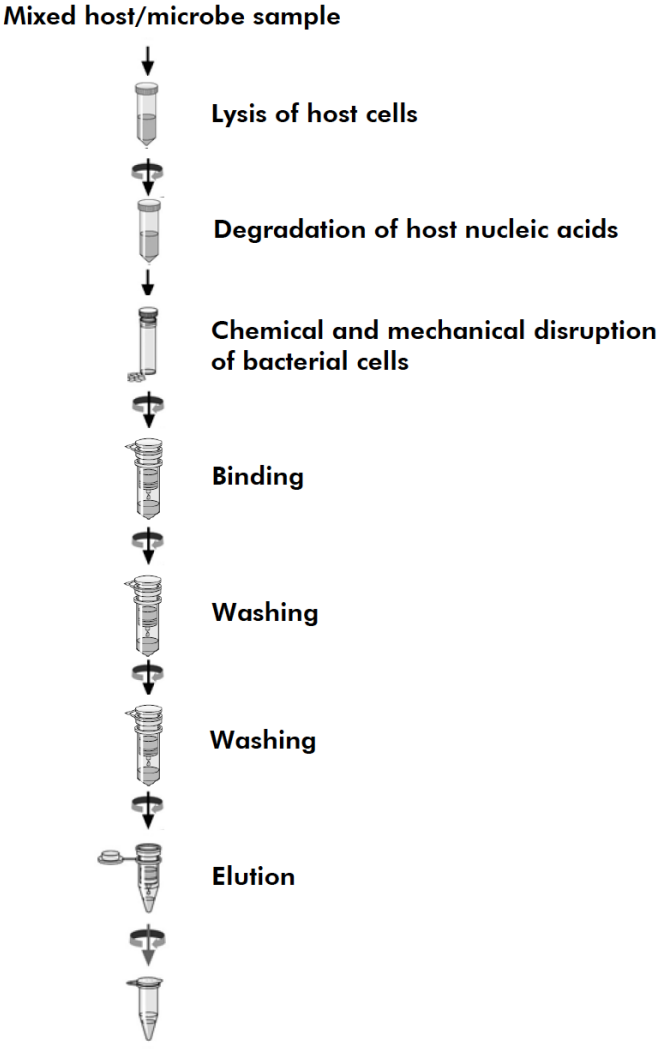
8.2.16 Carefully open the QIAamp UCP Mini Column and add 500 µl Buffer AW2 without wetting the rim. Centrifuge at full speed (20,000 x g) for 3 min.

8.2.17 Place the QIAamp UCP Mini Column into a fresh 2 ml collection tube. Discard the filtrate. Centrifuge at full speed (20,000 x g) for 1 min.

8.2.18 Place the QIAamp UCP Mini Column into a fresh 1.5 ml tube and apply 50 µl Buffer AVE directly onto the center of the membrane. Close the lid and incubate at room temperature for 5 min.

Optional: Depending on the downstream application, water or Buffer EB may be used instead. Centrifuge at 6,000 x g for 1 min to elute the DNA

QIAamp DNA Microbiome Kit procedure:



9. REFERENCE

QIAamp® DNA Microbiome Hand book, May 2014

10. REVISION HISTORY

Revision No	Effective Date	Description of Changes Made from Preceding Revision	Approved by/ Date

ANNEX 1: DOCUMENTATION OF SUGGESTED CHANGES TO THIS SOP

CLAUSE	SUGGESTION	BY	DATE