Destinatari: **Coordinatore, Tecnici e Studenti del Settore Genotipizzazione Micobatteri - EBP**

CONTENT

Rev.	Descrizione modifiche	Data
0	Prima emissione	01/10/2010
1	Cambio ragione sociale	07/01/2013
2	Cambio Titolo	19/05/2014

Compilazione	Sviluppo	Verifica	Approvazione
Settore TM *E.Borroni*	*Coordinatore di Settore* *E. Borroni, P. Miotto*	*RfQ-AQ* *D. Cirillo, L. Boldrini*	*CU* *D. Cirillo*

1. SCOPE

The present instruction describes procedures to inactivate viable bacteria belonging *to M. tuberculosis complex* and mycobacteria other than tuberculosis (MOTT) in order to extract DNA for typing procedures.

2. APPLICATION

The present instruction is applicable within the mycobacterial typing area (TM) and within the mycobacterial research area. The whole procedure is held in Biosafety Level 3 laboratory (BSL 3), while the extracted DNA are stored in a -20 °C outside the BSL 3 laboratory.

3. DEFINITIONS AND ABBREVIATIONS

DNA	Deoxyribonucleic Acid
MOTT	Mycobacteria Other Than Tuberculosis
BSL3	Biosafety Level 3
MGIT	Mycobacterial Growth Indicator Tube
LJ	Loewenstein-Jensen

4. RESPONSIBILITIES

The supervision and the correct application of the following instruction is a responsibility of the area coordinators.

5. EQUIPMENT AND MATERIALS

- Pasteur pipettes
- 1,5 ml screw cap Eppendorf tubes
- Pipettes 100 µl; 200 µl; 1000 µl
- Sterile water
- Thermomixer
- Ultrasonic water bath
- Centrifuge
- Adsorbent paper

6. PROCEDURES

6.1 DNA extraction from positive MGIT tubes

- Take 1 – 1.5 ml from a positive MGIT tubes and put it in a 1.5-2,0 labelled screw cap Eppendorf tube
- Insert the tube in a Thermomixer at the temperature of 95 °C for 30 minutes.
- Place the Eppendorf tube containing inactivated bacteria into a ultrasonic bath for 12 minutes
- Centrifuge 10 minutes at 13000 rpm (4° C)
- Transfer the DNA solution (supernatant) in a 1.5 ml eppendorf tube in order to eliminate cellular debris
- Store the DNA solution in a -20 °C freezer

6.2 DNA extraction from positive Löwenstein-Jensen tubes

- Put 1 ml of sterile water in a 1.5-2 ml screw cap eppendorf tube.
- Take 2 or 3 loops of bacterial culture and put them into the screw cap eppendorf tube.
- Insert the tube in a Thermomixer at the temperature of 95 °C for 30 minutes.
- Place the Eppendorf tube containing inactivated bacteria into a ultrasonic bath for 12 minutes
- Centrifuge 10 minutes at 13000 rpm (4° C)
- Transfer the DNA solution (supernatant) in a 1.5 ml eppendorf tube in order to eliminate cellular debris
- Store the DNA solution in a -20 °C freezer

6.3 DNA extraction from sediments

- From decontaminated sediments, take 500 µL and put it into a 1.5 ml eppendorf tube with screwed cap
- Centrifuge the tube 15 minutes at 13000 rpm (4°C)
- Discard the surfactant and resuspend the sediment in 75 – 100 µL
- Insert the tube in a Thermomixer at the temperature of 95 °C for 30 minutes.
- Place the Eppendorf tube containing inactivated bacteria into a ultrasonic bath for 12 minutes
- Centrifuge 10 minutes at 13000 rpm (4° C)
- Transfer the DNA solution (surfactant) in a 1.5 ml eppendorf tube in order to eliminate cellular debris
- Store the DNA solution in a -20 °C freezer

6.4 Chemical DNA extraction

This procedure is carried out using the GenoLyse® DNA Extraction Kit Ver 1.0 (Hain Lifescience GmbH) from sediments:

- Transfer 500 µL of decontaminated sample material into a labelled 1.5 ml screw cap tube.
- Centrifuge 15 minutes at 10000g in a standard table top centrifuge
- Discard the supernatant and resuspend pellet in 100 µL Lysis Buffer (A-LYS) by vortexing.
- Incubate sample for 5 minute at 95 °C in a thermo m ixer. Briefly spin down.
- Add 100 µL Neutralization Buffer (A-NB) to lysate and vortex sample for 5 seconds.
- Spin down for 5 minutes at full speed (13000 rpm).
- Transfer the supernatant into a new tube for storage at -20 °C.

Notes:

1. When DNA is extracted from positive cultures, the ultrasonic water bath step could be skipped

7. RECORDING AND REPORTING

All DNA solutions must be labelled with an internal serial number and fill into the general database along with all information available on this sample and into specific Country databases.

8. RELATED DOCUMENTS

- GenoLyse® DNA Extraction Kit Ver 1.0 Package Insert
- Bemer-Melchior P, Drugeon HB “Inactivation of *Mycobacterium tuberculosis* for Typing Analysis” JCM 1999;2350