

 OSPEDALE SAN RAFFAELE	<b>MAINTENANCE OF MYCOBACTERIAL STRAINS</b>	<b>IOS EBP-DMA 008</b>	
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**Destinatari: Personale del Settore Micobatteri (SM)**

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<b>Compilazione</b> Settore DMA <i>P. Mantegani</i>	<b>Sviluppo</b> CSDMA <i>E. Borroni</i>	<b>Verifica</b> RfQ-AQ <i>D. Cirillo, L. Boldrini</i>	<b>Approvazione</b> CU <i>D. Cirillo</i>
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## 1. SCOPE

The storage and maintenance of mycobacterial reference strains and clinical isolates is an important part of good laboratory practice in a mycobacteria laboratory. This IOS describes optimal storage conditions for mycobacterial strains (clinical and reference strain) to ensure their viability and retention of biological characteristics over time.

## 2. APPLICATION

This IOS describes optimal storage conditions for mycobacterial strains and it is applicable to the whole mycobacteria area.

## 3. DEFINITIONS AND ABBREVIATIONS

BSC: Biological Safety Cabinet  
LJ: Löwenstein–Jensen  
MGIT: Mycobacterial Growth Indicator Tube  
ADC: Albumin, Dextrose, Catalase

## 4. RESPONSIBILITIES

The supervision and the correct application of the following instruction is a responsibility of the area coordinator. The execution of the procedure is responsibility of area technicians, master students and coordinator.

## 5. EQUIPMENT AND MATERIALS

- Liquid cultures (MGIT tubes or ADC-enriched Middlebrook 7H9 broth)
- Solid cultures (Löwenstein–Jensen)
- Autoclave
- Biological Safety Cabinet, Class I or II, annually certified,
- Incubator
- Freezer, -80°C
- Sterile loops, calibrated to 10 µl, and/or short, sterile graduated pipette or single-channel microlitre Pipettes with sterile tips, for delivery of 1-ml volumes
- Separate waste containers (autoclavable) for pipettes and disposables
- Cryovials with screw-caps
- BBL Trypticase Soy Broth (**Cat N. 221093 Becton and Dickinson**)
- Glycerol (**Cat N. G5516-500ml Sigma-Aldrich**)
- BBL Middlebrook ADC Enrichment (**Cat. N. 211887 Becton and Dickinson**)

## 6. PROCEDURES

It is possible to maintain mycobacterial cultures by reculturing on solid medium, like Löwenstein-Jensen medium. There are, however, many examples of marked genetic re-arrangements in mycobacterial strains during *in vitro* culturing. It is therefore of the utmost importance to preserve reference strains and clinical isolates under circumstances with the lowest metabolic activity. Label each mycobacterial isolate that arrives at the laboratory with a unique strain number.

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Mycobacterial strains must be stored in conditions that preserve their viability and protect specific strain characteristics.

### **6.1 Storing solid mycobacteria cultures**

- Select a well-grown (non-contaminated) solid culture of the mycobacterial strains that should be stored.
- Label cryovial tubes with the strain number of the respective strain.
- Prepare BBL Trypticase Soy Broth plus 20% glycerol, autoclave and store the freezing medium at 4°C for no longer than six months.
- Scrape as many colonies as possible from an egg-based medium slant (LJ) or from agar medium. Suspend colonies in a 1,5 ml cryovial containing 1 ml sterile BBL Trypticase Soy Broth plus 20% glycerol.
- Store at -80°C for decades

### **6.2 Storing liquid mycobacteria cultures**

- Select a well-grown (non-contaminated) liquid culture (MGIT tube or ADC-enriched Middlebrook 7H9 broth) of each the mycobacterial strains that should be stored.
- Label cryovial tubes with the strain numbers of the respective strains.
- Add 20% sterile glycerol to the tube.
- Pipette 1 mL suspension to the labelled cryovial tube.
- Store at -80°C for decades.

## **7. RECORDING AND REPORTING**

Register the storage of the cultures on an appropriate form or in the strain collection database

## **8. RELATED DOCUMENTS**

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