

<b>Department</b>	<b>Micro Laboratory</b>	<b>Document no</b>	<b>MICLAB – METHOD 016</b>		
<b>Title</b>	<b>Maintenance of Culture Collection for Aseptic Practices in the Laboratory</b>				
Prepared by:		Date:		Supersedes:	
Checked by:		Date:		Date Issued:	
Approved by:		Date:		Review Date:	

## 1.0 **SUMMARY OF CHANGES**

<b>Version #</b>	<b>Revision History</b>
<b>MICLAB – METHOD 016</b>	New

## 2.0 **PURPOSE**

This document deals with the correct means of maintaining a culture collection with an emphasis on aseptic practices in the laboratory.

## 3.0 **SCOPE**

All laboratory staff must follow aseptic handling instructions to ensure continued purity of the culture collection.

## 4.0 **RESPONSIBILITY \ BUSINESS RULES**

All microbiology staff appointed to maintenance of the culture collection at the GMP site.

## 5.0 **PROCEDURE**

### **5.1 Materials and Reagents required**

- 5.1.1 Tryptone Soya Agar (TSA) slopes
- 5.1.2 Sabouraud Dextrose Agar (SDA) slopes
- 5.1.3 White adhesive labels 3.5 cm<sup>2</sup>
- 5.1.4 White, green, blue and yellow adhesive dots
- 5.1.5 Diagnostic media plates
- 5.1.6 Storage baskets
- 5.1.7 TSA & SDA plates
- 5.1.8 Metal file/ diamond pen
- 5.1.9 10ml Tryptone Soya Broth (TSB) in test tubes / MacCartney bottles

Department	Micro Laboratory	Document no	MICLAB – METHOD 016
Title	Maintenance of Culture Collection for Aseptic Practices in the Laboratory		

**SAFETY NOTE: All microbiological cultures used in the microbiology laboratory are classified as either Risk Group 1 (low individual & community risk) or Risk Group 2 (moderate individual risk, limited community risk) micro-organisms as defined by AS/NZS 2243.3 (2002).**

## **5.2 Subculturing**

**NOTE: All work related to opening of freeze- dried ampoules and subculturing of cultures must be performed in the biohazard safety cabinet.**

All cultures used in the laboratory must be obtained from an approved source, e.g. TGAL, Queensland University.

Culture collection is subcultured on a bi-monthly basis onto a fresh set of agar slopes. Subcultures can be streaked from master cultures up to five times after which a new freeze-dried ampoule must be opened.

- 5.2.1 Establish viability and purity of all master cultures by streaking onto Tryptone Soya Agar (TSA) plates for bacteria and Sabouraud Dextrose Agar (SDA) plates for fungi. Incubate bacterial plates for 24-48 hours and fungal plates for 5-7 days at a temperature specified for each organism.
- 5.2.2 If culture is impure proceed as in 5.3.
- 5.2.3 If culture is pure, streak onto 2 slopes of TSA or SDA (as applicable) and inoculate 1 MacCartney bottle of Tryptone Soya Broth (TSB) if required. Ensure the new white Master slope is inoculated first, followed by the new routine slope and broth.
- 5.2.4 Label all slopes and broths as in section 5.5.
- 5.2.5 Incubate slopes and broth as in 5.2.1.
- 5.2.6 When cultures have grown, check purity of white Master slope by streaking onto TSA or SDA plates. Incubate plates at a temperature recommended for each organism. Check culture performance by streaking (inoculating) diagnostic media.
- 5.2.7 If culture is impure, proceed as in 5.3.
- 5.2.8 If culture is pure, document and store as detailed in SF150101.
- 5.2.9 For the first and fifth subculture obtain a biochemical profile for bacterial cultures via an API identification system (if possible).

Department	Micro Laboratory	Document no	MICLAB – METHOD 016
Title	Maintenance of Culture Collection for Aseptic Practices in the Laboratory		

### **5.3 Isolation of Pure Culture**

- 5.3.1 Pick a single isolated colony and streak onto TSA/SDA plate.
- 5.3.2 Incubate for 24-48 hours at a required temperature.
- 5.3.3 Remove plate and check for purity and colony morphology.
- 5.3.4 Check Gram reaction and other biochemical tests as required.
- 5.3.5 Set up an API system.
- 5.3.6 If results obtained are satisfactory, return culture into culture collection.
- 5.3.7 If results are not satisfactory, open new freeze- dried ampoule (see section 5.4).

### **5.4 Opening of Freeze Dried Ampoules**

- 5.4.1 Decontaminate ampoules by swabbing with 70% alcohol or Viraclean.
- 5.4.2 Place the ampoule on a bench previously decontaminated with approved sanitising solution and score the glass above the cotton plug. Cut the glass all the way around the ampoule starting from the score mark, using a file or diamond pencil
- 5.4.3 Break the ampoule, applying gentle pressure away from the body and keeping fingers away from file marks.
- 5.4.4 Using a sterile 1 ml pipette remove a few drops of TSB from a MacCartney bottle/test tube and add to the ampoule. Use TSB for bacterial and fungal cultures.
- 5.4.5 Dissolve the pellet by gently washing with TSB.
- 5.4.6 Aseptically transfer all the TSB into the MacCartney bottle/test tube containing the remainder of the TSB. Label the bottle with culture name, number and date.
- 5.4.7 Incubate broths for 24-48 hours at a temperature specified for each organism.
- 5.4.8 Proceed as in 5.2 onwards.

Department	Micro Laboratory	Document no	MICLAB – METHOD 016
Title	Maintenance of Culture Collection for Aseptic Practices in the Laboratory		

### **5.5 Documentation and Labelling of Culture Collection**

- 5.5.1 On arrival, freeze-dried ampoules and other stock cultures must be entered onto SF150117 – Freeze Dried Culture Log.
- 5.5.2 Enter details of sub cultures onto “Culture Collection Worksheet”.
- 5.5.3 Each culture must be labelled with the bacteria/fungus name, reference number, subculture number and date of subculture. All details can be found in the worksheet.
- 5.5.4 Each culture is given a laboratory number written in black on a self-adhesive white, yellow and blue dot which is placed on the top of the lid.
- 5.5.5 Slopes with white dots are called “master cultures” and must not be used for routine work.
- 5.5.6 Slopes with yellow dots are called “working cultures” and are used for routine work.
- 5.5.7 Broth cultures receive a blue dot, are also called “working cultures” and are used for routine work.

### **5.6 Environmental Culture Collection**

- 5.6.1 A collection is kept of cultures isolated from environmental samples.
- 5.6.2 Due to their short life span, each environmental isolate should be replaced with the most recently isolated sample of the same species.
- 5.6.3 Environmental isolates are recorded as per “Environmental Culture Collection Worksheet”.
- 5.6.4 Environmental cultures receive a green dot with culture number written in black.

## **6.0 DEFINITIONS / ACRONYMS**

NA