Department	Micro Laboratory		Document no	MICLAB 160	
Title	Handling of Media Diluents and Reagents in the Microbiology Laboratory				
Prepared by:		Date:		Supersedes:	
Checked by:		Date:		Date Issued:	
Approved by:		Date:		Review Date:	

1.0 **DOCUMENT OWNER**
Laboratory/Quality Manager

2.0 **PURPOSE**

This document describes the general procedure for the receival, use and storage of all media, diluents and reagents in the Microbiology Laboratory at a GMP site.

3.0 **SCOPE**

All Microbiological media, reagents and diluents must comply with specified documentation, preparation, storage and quality control requirements before permitting their use in the laboratory. All batches of agar prepared in the Microbiology Laboratory must be examined for their ability to support the formulation of colonies by organisms that they are designed to grow.

4.0 **RESPONSIBILITY \ BUSINESS RULES**

All microbiology staffs.

5.0 **PROCEDURE**

5.1 Receival of Dehydrated Media (Including Supplements)

5.1.1 Upon receival of media check the invoice against the purchase order. Record the date of receival on the container.

5.1.2 Check expiry date and storage conditions.

5.1.3 Store according to manufacturer's instructions and in a manner which allows oldest media to be used first.

5.2 Preparation of Media

5.2.1 Media preparation should take place in a clean environment relatively free of draughts and moisture.

5.3 Media Volume Checks

5.3.1 Media dispensed into volumes requiring final volumes of 9.9mL or 9.0mL require volume checks after autoclaving.

5.3.2 After autoclaving randomly select thirteen bottles.

5.3.3 Place an appropriate beaker onto a balance and tare.

5.3.4 Pour media from one bottle into a beaker and record value on "Media Quality Control Report" (Appendix 1).

5.3.5 Repeat steps 5.3.3 – 5.3.4 for the remaining 12 bottles.

5.4 Quality Control of Prepared Media - Sterility

5.4.1 To check sterility of broths, incubate at 30°C±1°C for 72 hours. For agars, melt down, pour plates and incubate for 48 hours at 30°C±1°C.

5.4.2 Plain agar, water, saline, hard water and all diluents do no require a fertility test.

5.5 Quality Control of Prepared Media - Fertility

5.5.1 Fertility of Broth Media

5.5.1.1 To prepare a bacterial culture, inoculate 10 mLs of Tryptone Soya Broth (TSB) with the required organism for the media being tested (table 5.5.1.8). Incubate for 24 hour at 30 ± 1°C.

5.5.1.2 Prepare yeast suspensions in the same manner with TSB substituted for Sabouraud Liquid Medium.

NOTE: Z.rouxii requires 25 ± 1°C incubation.

5.5.1.3 Inoculate media using a 1 microlitre plastic disposable loop.

5.5.1.4 Immerse only the loop itself and not the stem.

5.5.1.5 Use a fresh loop after each media inoculation.

5.5.1.6 The incubation time and temperature will depend on the test organism and media tested.

5.5.1.7 Only media which have growth (turbidity) pass the fertility test.

NOTE:Due to the medias turbidity the fertility bottles for Letheen Broth + 2% Lecithin + 4% Tween 80 must be streaked onto a Tryptone Soya Agar Plate and incubated at 30℃±1°C for 24 hours to check growth

5.5.1.8 Control Microorganisms Used For Fertility Testing of Broth Media

MEDIUM	INCUBATION CONDITIONS	CONTROL ORGANISMS POSITIVE	CONTROL ORGANISMS NEGATIVE	ACCEPTABLE RESULTS
Sabouraud Liquid Medium	Aerobic 48 hrs, 25°C	C. albicans (ATCC 10231) Z. rouxii (NCYC 381)	N/A	Growth Growth
Synthetic Broth	Aerobic 24 hrs, 37°C	P. aeruginosa (ATCC 9027) E. coli (NCTC 9001) S. aureus (NCTC 6571)	N/A	Growth Growth Growth
Thioglycollate Media + 0.5% Tween 80	Aerobic 24-48 hrs, 30°C	Cl. perfringens (NCTC 8237)	P. aeruginosa (ATCC 9027) E. coli (NCTC 9001)	Growth Growth Growth
Tryptone Soya Broth	Aerobic 48 hrs, 30°C	P. aeruginosa (ATCC 9027) S. aureus (NCTC 6571) E. coli (NCTC 9001)	N/A	Growth Growth Growth
Tryptone Soya Broth + 4% Tween 80	Aerobic 48 hrs, 30°C	P. aeruginosa (ATCC 9027) S. aureus (NCTC 6571) E. coli (NCTC 9001)	N/A	Growth Growth Growth
Tryptone Water	Aerobic 48 hrs, 37°C	E. coli (NCTC 9001)	E.aerogenes (NCTC 10006)	Growth Growth

5.5.2 Fertility of Agar Media - Ecometric Quality Control Evaluation

5.5.2.1 *Materials Required List*

- Sterile Petri Dishes
- Ecometric Quality Control Evaluation stamp and black stamp pad
- Test cultures
- 1 microlitre plastic disposable loop
- Vortex
- Tryptone Soya Broth (TSB) - 10mL in MacCartney bottles
- Biohazard safety cabinet

5.5.2.2 *Work Instruction*

This procedure involves the inoculation of standardised cultures on agar plates with a carefully standardised sequential streaking technique.

Based on the principal of an ever decreasing number of colony forming units per surface area, this is a quantitative evaluation of the ability of media to support the growth of certain micro-organisms.

5.5.2.3 *Culture Preparation*

Determine cultures required to perform fertility testing by referring to Table 5.5.2.4.

Follow steps 5.5.1.1 - 5.5.1.2 for culture preparation (follow table 5.5.2.4 for agars).

5.5.2.4 Control Microorganisms for Ecometric Quality Control Evaluation of Media

MEDIUM	INCUBATION CONDITIONS	CONTROL ORGANISMS POSITIVE	CONTROL ORGANISMS NEGATIVE	ACCEPTABLE RESULTS
Reinforced Clostridial Agar	Anaerobic 48 hrs, 30°C	C. perfringens (NCTC 8237) C. sphenoides (ATCC 19403)	N/A	Growth index ≥ 3
Sabouraud Dextrose Agar	Aerobic 5 days, 25°C	C. albicans (ATCC 10231)	N/A	Growth index ≥ 3
Simmons Citrate Agar	Aerobic 24-48 hrs, 37°C	K.edwardsii var altantae (NCTC 10896)	E. coli (NCTC 9001)	Positive Control Growth index ≥ 3 Negative Control – No Growth
Sporulation Agar	Aerobic 24 hrs, 37°C	B. subtilis (A.TCC 11714)	N/A	Growth index ≥ 3
Tryptone Soya Agar	Aerobic 48 hrs, 30°C	S. aureus (NCTC 6571) P. aeruginosa (ATCC 9027) E. coli (NCTC 9001)	N/A	Growth index ≥ 3
Tryptone Soya Agar + 4% Tween	Aerobic 48 hrs, 30°C	S. aureus (NCTC 6571) P. aeruginosa (ATCC 9027) E. coli (NCTC 9001)	N/A	Growth index ≥ 3
Tryptose Sulphite Cycloserine Agar	Anaerobic 24 hrs, 37°C	C. perfringens (NCTC 8237)	E. coli (NCTC 9001)	Growth index ≥ 3
Violet Red Bile Glucose Agar	Aerobic 24 hrs, 37°C	E. coli (NCTC 9001)	S. aureus (NCTC 6571)	Positive Control -Growth index ≥ 3 Negative Control – No Growth
Violet Red Bile Lactose Agar	Aerobic 24 hrs, 37°C	E. coli (NCTC 9001)	S. aureus (NCTC 6571)	Positive Control -Growth index ≥ 3 Negative Control - No Growth
X L D	Aerobic 48 hrs, 37°C	S. salford (IMVS 1710)	C. Freundii (NCTC 9750)	Growth index ≥ 3

5.5.2.5 Plate Preparation

Pour sterile petri dishes with agar retained for the purpose of Ecometric Quality Control Evaluation (EQCE).

When set, stamp the base of agar plates with the EQCE stamp.

5.5.2.6 Inoculation/Streaking Technique

5.5.2.6.1 Turn the UV lamp on in the Biohazard Safety Cabinet for at least 15 minutes prior to use.

5.5.2.6.2 Open cabinet and decontaminate work bench with Viraclean prior to use.

5.5.2.6.3 Immerse only the loop itself and not the stem in the test culture. Begin each plate inoculation with a fresh loop.

5.5.2.9 Media undergoing QC assessment must be kept separately and not used until the QC tests are completed and passed.

5.6 Storage – General

5.6.1 Store reagents and dehydrated media in cool, dry, dark conditions.
5.6.2 Reagents that require refrigeration are to be kept in a designated refrigerator.
5.6.3 Pay particular attention to agars and broths with special storage requirements and short shelf lives.
5.6.4 Regular stock checks should be made of dehydrated and laboratory prepared media regarding quantity and expiry date. Discard any expired media as per 5.2.5.

5.7 Storage of Bottled Agars and Broths

5.7.1 All prepared bottled agars, diluents and broths must be tightly sealed and stored at room temperature in a dry cupboard.
5.7.2 Label all stored bottles as in 5.2.14.
5.7.3 Store Mannitol Selenite Cysteine broth (MSC) and Rappaport - Vassiliadis Enrichment Broth (RV) in capped test tubes at 2 - 8°C.

5.8 Storage of Poured Plates

5.8.1 Pour plates under laminar flow to ensure an aseptic environment.
5.8.2 Label plates on the underside with name of media, date poured, batch number of bottled agar, expiry date and store inverted in a designated refrigerator with the newest plates placed towards the back.
5.8.3 Ready-made plates should be checked for contamination before refrigeration.
5.8.4 Bismuth Sulphite Agar (BSA) and Eosin Methylene Blue (EMB) agars should be wrapped in aluminium foil when storing in the fridge.

5.9 Determination of expiry date for microbiological media

5.9.1 If a new supplier or formulation is used, quality control of microbiological culture media and determination of expiry date needs to be performed.
5.9.2 Follow steps 5.2.1 – 5.2.16 for media preparation.
5.9.3 In required intervals perform quality control of prepared media (eg. fertility and Ecometric Quality Control Evaluation) following steps 5.5.1 – 5.5.2.7.
5.9.4 Once the expiry date is determined update the relevant media procedure to include the expiry date.

6.0 DEFINITIONS / ACRONYMS

EQCE - Ecometric Quality Control Evaluation

8.0 SUMMARY OF CHANGES

Version #	Revision History
MicLab-160	New

