Standard Operating Procedure

Title: Media Preparation in Microbiology Laboratory

1. General Preparation

On receipt of dehydrated media into the Microbiology Laboratory, **record the date received** clearly on the outside of the container

Distilled Water must be used in the preparation of **all** media and must be collected on the day of use.

Upon initially opening a container of media the **date must be written clearly** on the container.

Preparation details are to be recorded in log book.

If the batch is prepared in different vessels, e.g. 20L in two 10L buckets, then each 10 L is **considered to be a separate batch.** This is identified in the log bookby the use of **Lot No.** in the batch record. **It is important that each batch remains separate as individual pH, positive control and negative control results will be required.** When labelling, ensure that each lot is identified, as it will be necessary to select the correct lot when entering test details in log book.

Mixing is an important requirement for all media preparation. When preparing media with Tween additional mixing is essential. **All media containing Tween will need to be heated as this will decrease the amount of time required for mixing.**

All agar preparations must be soaked in cold distilled water for a minimum of 15 minutes prior to heating. This allows the agar to swell and absorb the water. Agars need to be brought to the boil to allow the agar to be dispersed. Burning of agar reduces the effectiveness of the agar ie SDA, clarity reduced.

The media is to be re-constituted according to the manufacturers' directions on the container (with the exception of those below), have the pH determined and bought to within specification (as outlined in section 2) and volumes sufficient for current testing methods are to be distributed into suitable containers. These volumes and containers are best determined by observing those specified in individual product and raw material control methods and by referring to Appendices 1 and 2.

1.1. **Peptone Water: Diluent prepared from Peptic Digest of Animal Tissue.**
Prepare a 0.1% w/v solution.

Peptone Water + 5% v/v Tween 80
Prepare 0.1% w/v solution to which 5% v/v Tween 80 is added. Certain control methods specify the addition of glass beads to particular volumes of this solution.

Peptone Water + 1% v/v Tween 80
Prepare 0.1% w/v solution to which 1% v/v Tween 80 is added. Certain control methods specify the addition of glass beads to particular volumes of this solution. The temperature of the water on the time of collection has an effect on colour of peptone water from batches to batches.

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1.4.5. Remove 100ml bottles from boiling water and place carefully into approximately 5cm of cold water in a plastic tray. Screw down caps tightly. Take care when removing bottles from hot water, use heatproof gloves. **Bottles may crack when placed into cold water – these should be discarded.**

1.4.6. pH sample after sterilising.

1.4.7. Once bottles are cool, label and incubate as outlined in **point 5** below.

1.5. **Tryptone Soy Agar + Supplement**
TSA+L+P (TSA + 0.1% w/v Lecithin + 0.7% w/v Polysorbate) or add 5 mL Amyl Supplement SP430 to 100 mL.

1.6. **Reinforced Clostridial Agar + Supplement**
RCA+L+P (RCA + 0.1% w/v Lecithin + 0.7% w/v Polysorbate) or add 5 mL Amyl Supplement SP430 to 100 mL.

1.7. **LA + SP430 Supplement**
50ml of SP430 Supplement per 1L of Distilled Water. Bring to the boil. After autoclaving, gently swirl the bottles to disperse the supplement.

2. pH

Calibrate the pH meter before proceeding any further.

Check the pH of liquid Media and Peptone waters prior to dispensing into bottles. Use a small sample from the bulk media. Ensure that the media is cooled to approximately 25°C before testing. **Agars do not require a pH measurement**. Adjust if necessary using either 1M NaOH (↑) or 1M HCl (↓) to ensure that the pH lies within + 0.2 units of the manufacturer's stated pH. The pH ranges for media can be found on the bottles of media. This result is then entered in the log book.

NOTE: If batches are being put in different autoclave loads and or batches are being made in 2 lots (e.g. 20L as 2 x 10L), ensure that each load has a ph measurement and both 10L buckets has a pH measurement prior to dispensing.

Media Type	pH Range		Media Type	pH Range
Peptone Water	6.9 ± 0.2		NB	7.4 ± 0.2
TSB	7.3 ± 0.2		LB	7.0 ± 0.2
FTM	7.1 ± 0.2		Lactose Broth	7.0 ± 0.2
Ringers	7.0 ± 0.2		EE medium	7.2 ± 0.2
RCM	6.8 ± 0.2		MacConkeys Broth	7.4 ± 0.2

A designated 100ml bottle, (use a 20ml bottle for batches being bottled in 20ml McCartney bottles), for all batches should be labelled as the "pH sample" and autoclaved with the batch. After autoclaving, this pH sample should be allowed to cool and re-tested to check that pH results are still within specification.

Media outside the specified pH range should not be used in testing. An OOS investigation is to be conducted under a DR if the media outside of specification needs to be used, this is with approval from the Microbiology Manager or senior Technician. Comments are to be recorded in the 'Comments' section in log book.

3. Pre-Autoclaving Procedure

The 20ml and 100ml bottles are placed in wire trays. Each tray is labelled with a piece of autoclave tape stating the type of media and expiry date. Caps are left loose.

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11.1. **NOTE**: Care must be taken that the 14 day incubation period is completed and all stasis work has been conducted prior to signing media ready for use.

11.2. **The same person cannot prepare the Media or Peptone Water and then sign for use**. This information is recorded in log book.

11.3. If a QC check does not meet the specifications, the Microbiology Manager or senior Technician is to be consulted and the batch of **Media or Peptone Water is not to be used without their authorisation.**

12. Storage of Media

Media is stored in the either Storeroom and the temperature of the area is to be monitored daily with a designated thermometer and recorded on to **Form 660**.

Media is not to be used until it has completed all QC testing and meets the requirements, until then when stored on shelves in either storeroom there should be a Quarantine sign, stating in process QC testing.

13. Checklist for Media Preparation

13.1. **Each morning**

Have you:

- Checked the hot room for media that has completed the **5** day incubation period?
- Checked the **Media Preparation Diary** for media that has completed the **14** day incubation period?
- Entered these end incubation dates into log book?
- Signed the “ready to use” field, providing that **all other details including stasis results have been entered**?

13.2. **Media Preparation**

Have you:

- Labelled wire trays?
- Measured and adjusted the pH prior to filling (for liquid media)?
- Labelled the required number of bottles for stasis?
- Labelled a bottle for pH testing after autoclaving (for liquid media)?
- Labelled 2 bottles from each autoclave load for a 14 day incubation and entered the projected finish date into the **Media Preparation Diary**?
- If required - attached autoclave tape to lids or over-wrapped lids with Kraft paper?
- Entered all details into log book; including the number of bottles filled?

13.3. **After Autoclaving**

Have you:

- Tightened all lids and labelled all bottles?
- Measured the pH and entered the result into log book?
- Incubated the batch at the required temperature?

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15. Appendix 2 - Media Preparation Table: AGARS

AGAR Medium	Supplier	Vol./bottle	Bottle size/type	Batch size	Stasis	Min. level	General Comments
TSA		50ml	100ml Flat	3L	100ml	10 - 15	
		20ml	20ml McCartney	600ml	100ml	6	
NA		200ml	250ml Schott	2 x 5L	100ml	10 - 15	If no stock of prep.
		20ml	20ml McCartney	600ml	100ml	6	
NA + 3% T80		200ml	250ml Schott	2 x 5L	100ml	10 - 15	5L = 150 ml T80
RCA		20ml	20ml McCartney	600ml	100ml	6	
LA		400ml	500ml Schott	10L or 20L	100ml	5	10L = 500ml SP430
		20ml	20ml McCartney	600ml	100ml	As needed	
R2A		200ml	250ml Schott	4L	100ml	As needed	
SDA		50ml	100ml Flat	3L	100ml	10 - 15	
TSA + L + P		20ml	20ml McCartney	400ml	100ml	As needed	
RCA + L + P		20ml	20ml McCartney	400ml	100ml	As needed	
MCA		100ml	100ml Flat	1L	100ml	As needed	
MSA		100ml	100ml Flat	2L	100ml	As needed	

16. Summary of Changes

Version #	Revision History
MICLAB 030	New

End of Procedure

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