

Department	Micro Laboratory	Document no	MICLAB – METHOD 023		
Title	Preparation of Lethen Broth + 2% Lecithin and 4% Tween				
Prepared by:		Date:		Supersedes:	
Checked by:		Date:		Date Issued:	
Approved by:		Date:		Review Date:	

1.0 SUMMARY OF CHANGES

Version #	Revision History
MICLAB – METHOD 023	New

2.0 PURPOSE

This procedure describes the method for preparation of Lethen Broth + 2% Lecithin and 4% Tween (AMYL AM89, DIFCO 268110).

3.0 SCOPE

This procedure describes the methods for preparation of Lethen Broth + 2% Lecithin and 4% Tween, a highly nutritious and general use medium for the growth of bacteria and fungi.

4.0 RESPONSIBILITY \ BUSINESS RULES

All microbiology staffs.

5.0 PROCEDURE

5.1 Materials and Reagents Required

- 5.1.1 Glass stirrer
- 5.1.2 Three Beakers (microwaveable)
- 5.1.3 Measuring Cylinder
- 5.1.4 1N NaOH and 1N HCl
- 5.1.5 Plastic pipettes
- 5.1.6 RO water
- 5.1.7 Automatic dispenser
- 5.1.8 Lethen Broth (Amyl Media AM 89) or Difco Broth (268110)
- 5.1.9 Tween 80 (Polysorbate 80BP item 119A012)
- 5.1.10 Lecithin Bacteriological (Amyl media RM299)
- 5.1.11 Glassware
- 5.1.12 Amyl Supplement SP430

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5.2 Method

5.2.1 PREPARATION OF COMPLETE MEDIUM USING AMYL MEDIA (AM 89)

- 5.2.1.1 Weigh out 9g per litre of Lethen Broth powder.
- 5.2.1.2 Add 500mL of RO water and mix well to dissolve.
- 5.2.1.3 Weigh out 40g per litre of Tween 80 into a beaker. Add approximately 200mL of RO water and dissolve in microwave with frequent mixing.
- 5.2.1.4 Weigh out 20g per litre of Lecithin Bacteriological into a beaker. Add approximately 200mL of RO water and dissolve in microwave with frequent mixing.
- 5.2.1.5 Add 50mL Supplement per litre.
- 5.2.1.6 Combine all dissolved ingredients and make up to required volume with RO water.
- 5.2.1.7 Adjust pH to 7.0.
- 5.2.1.8 Dispense required volume into clean, dry glassware.
- 5.2.1.9 Sterilise by autoclaving at 121°C for 15 minutes.
- 5.2.1.10 pH after sterilisation should be 7.0 ± 0.2 .

5.2.2 PREPARATION OF COMPLETE MEDIUM USING DIFCO MEDIA (268110)

- 5.2.2.1 Weigh out 25.7g per litre of Difco Broth Powder into a beaker.
- 5.2.2.2 Add 500mL of RO water and mix well to dissolve.
- 5.2.2.3 Weigh out 35g per litre of Tween 80 into a beaker. Add approximately 200mL of RO water and dissolve in microwave with frequent mixing.
- 5.2.2.4 Weigh out 19.3g per litre of Lecithin Bacteriological into a beaker. Add approximately 200mL of RO water and dissolve in microwave with frequent mixing.
- 5.2.2.5 Combine all dissolved ingredients and make up to required volume with RO water.
- 5.2.2.6 Adjust pH to 7.0.
- 5.2.2.7 Dispense required volume into clean, dry glassware.
- 5.2.2.8 Sterilise by autoclaving at 121°C for 15 minutes.
- 5.2.2.9 pH after sterilisation should be 7.0 ± 0.2 .

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5.3 Quality Control Requirements

QUALITY CONTROL REQUIREMENTS
STORAGE: 6 months at room temperature in cupboard.
CONTROL ORGANISM: <u>P.aeruginosa</u> or <u>E.coli</u> A.T.C.C 9027 N.C.T.C 9001
Growth Index : Growth
INCUBATION CONDITION: 30 ± 1°C / 48 hours

6.0 DEFINITIONS / ACRONYMS

N/A

7.0 REFERENCES

- 7.1 Sampling Plan and Acceptance Criteria for Microbiological Culture Media.
- 7.2 Media Quality Control Report.
- 7.3 Determination of Expiry Date for Microbiology Media.