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APPENDIX

APPENDIX

MANIPULATION OF THE TESTING STRAINS

1. Preparation of Stock Solution and Media

1.1 Vogel-Bonner medium E stock solution (VB salt)

Ingredient	1 Litre
Warm distilled H ₂ O (45°C)	670 ml
Magnesium sulfate (MgSO ₄ ·7 H ₂ O)	10 g
Citric acid. H ₂ O	100 g
Potassium phosphate, dibasic (anhydrous) (K ₂ HPO ₄)	500 g
Sodium ammonium phosphate (NaNH ₄ HPO ₄ ·4 H ₂ O)	175 g

Add salts in the order indicated to warm water in a 2-liter beaker or flask placed on a magnetic stirring hot plate. Allow each salt to dissolve completely before adding the next. Adjust the volume to 1 liter. Filter the solution and autoclave, loosely capped, for 20 min at 121 °C. When the solution is cool, tighten the cap.

1.2 40% Glucose

Dissolve 40 g of glucose in 100 ml distilled water. Autoclave at 121°C for 20 min.

1.3 Minimal glucose agar plate

Ingredient	300 ml	350 ml
Bacto agar	4.5 g	5.25 g
Distilled H ₂ O	280 ml	330 ml
VB salts	6 ml	7 ml
40% glucose	15 ml	17.5 ml

Add agar to distilled water in an Erlen-Mayer flask. Autoclave at 121 °C for 20 min. When the solution is cool slightly, add sterile VB salts and sterile 40% glucose. Mix and pour about 30 ml into each sterile petri plate. Minimal glucose agar plates are kept in incubator at 37°C before using.

1.4 Oxoid nutrient broth No.2

Ingredient	100 ml
Nutrient broth No.2	2.5 g
Distilled water	100 ml

Dissolve 2.5 g of nutrient broth No.2 in 100 ml distilled water.

Transfer 12 ml of nutrient broth for each flask (covered with sterile gauze).

Autoclave at 121 °C for 20 min.

1.5 Top agar

Ingredient	200 ml
Bacto agar	1.2 g
Sodium chloride (NaCl)	1.0 g
Distilled H ₂ O	200 ml
0.5 mM Histidine-HCl plus 0.5 mM biotin	20 ml

Dissolve ingredients in distilled water. Store in a glass bottle.

Autoclave at 121 °C for 20 min and then add 20 ml 0.5 mM histidine HCl-0.5 mM biotin and 30 ml for 200 ml and 300 ml of top agar respectively.

1.6 0.1M L-histidine HCl stock

Ingredient	100 ml
L-histidine HCl	2.096 g
Distilled H ₂ O	100 ml

Dissolve 2.096 g of L-histidine HCl (MW 209.63) in 100 ml distilled water. Autoclave at 121 °C for 20 min.

1.7 1 mM L-histidine HCl stock

Ingredient	100 ml
0.1 ML-histidine HCl	1 ml
Distilled H ₂ O	99 ml

Dilute 1 ml of 0.1 ML-histidine HCl in 99 ml of distilled water.

Autoclave at 121 °C for 20 min.

1.8 1mM Biotin stock

Ingredient	100 ml
Biotin	24.43 mg (0.02443 g)
Distilled H ₂ O	100 ml

Dissolve biotin (MW 244.3) in distilled water. Warm it until dissolve completely. Autoclave at 121 °C for 20 min.

1.9 0.5 mM L-histidine HCl-0.5 mM biotin

Ingredient	200 ml
1 mM L-histidine HCl	100 ml
1 mM biotin	100 ml

Mix and autoclave at 121 °C for 20 min.

1.10 1 M potassium chloride (KCl)

Ingredient	1000 ml
Potassium chloride (KCl)	74.56 g
Distilled H ₂ O	1000 ml

Mix and autoclave at 121 °C for 20 min.

1.11 0.5 M sodium dihydrogen phosphate (NaH₂PO₄)

Ingredient	500 ml
NaH ₂ PO ₄ (MW 120)	30 g
Distilled H ₂ O to	500 ml

Dissolve NaH₂PO₄ in distilled water. Stir it until dissolve completely.

Adjust the final volume to be 500 ml.

1.12 0.5 M sodium phosphate (Na₃PO₄) pH 7.4

Ingredient	500 ml
Disodium hydrogen phosphate dehydrate (Na ₂ HPO ₄ ·2 H ₂ O)	44.5 g
Distilled water to	500 ml

Dissolve $\text{Na}_3\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in 300 ml of distilled water. Add 0.5 M Na_3PO_4 until to pH 7.4, and then adjust the final volume to be 500 ml.

Autoclave at 121 °C for 20 min.

1.13 152 mM Na_3PO_4 -KCl buffer

Ingredient	330 ml
0.5 M Na_3PO_4 pH7.4	100 ml
1 M KCl	16.5 ml
Distilled H_2O	213.5 ml

Mix and autoclave at 121 °C for 15 min.

1.14 8 mg/ml Ampicillin solution

Ingredient	4 ml
Ampicillin (sodium)	32 mg (0.032 g)
Distilled H_2O	4 ml

It is necessary to sterilize ampicillin solution but it can be filtered through a 0.22 μl membrane filter. Store it in a glass bottle with a screw cap at 0°C

1.15 0.1% Crystal violet

Ingredient	10 ml
Crystal violet	10 g
Distilled H_2O	10 ml

Store at 0°C in glass bottle with screw cap. Wrap the bottle with metal foil to protect against light.

2. Recipes for Some Reagents

2.1 2M sodium nitrite

Ingredient	10 ml
Sodium nitrite	1.38 g
Distilled water to	10 ml

Store in a glass bottle with a screw cap (wrap the bottle with metal foil to protect against light). Autoclave at 121 °C for 20 min.

2.2 2M ammonium sulfamate

Ingredient	10 ml
Ammonium sulfamate	2.28 g
Distilled water to	10 ml

Dissolve ammonium sulfamate in distilled water and adjust the final volume to be 10 ml. Autoclave at 121 °C for 20 min.

2.3 0.2N hydrochloric acid

Ingredient	10 ml
Conc. Hydrochloric acid	1.66 ml
Sterile distilled water	10 ml

Dissolve conc. hydrochloric acid in sterile distilled water. Store with sterile technique because hydrochloric acid can not be autoclaved.

2.4 0.0375 mg/ml 1-aminopyrine

Ingredient	4 ml
0.3 mg/ml 1-aminopyrine	250 µl
Acetonitrile	1750 µl

Dissolve 3 mg of 1-aminopyrine in 300 µl of acetonitrile and mix; and subsequently dilute 300 µl of this solution (3 mg/ml 1-aminopyrine) in 2,700 µl of acetonitrile, the solution obtained will be 0.3 mg/ml 1-aminopyrine. Then, dilute 250 µl of 0.3 mg/ml 1-aminopyrine in 1,750 µl of acetonitrile and mix. Store all solutions in sterile glass vials with screw caps in a freezer. The preparation must be used sterile technique.



BIOGRAPHY

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