APPENDIXES

Appendix A: Equipment, Materials, Media and Reagents

1. Lab equipment and materials

Usual laboratory equipment and in addition : Routine servicing of the cabinet should be carried out to ensure sterile conditions inside the cabinet. Thoroughly clean the room or cabinet with disinfectant. Irradiation with UV light prior to harvesting vaccine will help kill contaminating organisms. Technicians should wear clean laboratory coats, clean hair covers, facemasks and scrub their hands with an antimicrobial soap. sufficient work area, level table with ample surface in room that is clean, well light and well ventilated, and reasonably free of dust and drafts.

- Glassware; Pipettes; 1 ml, 5 ml 10 ml Glass Pasteur pipettes.
- Conical flasks; 50 ml 100 ml 500 ml 1 L, 2 L, Beakers; a range of sizes.
- Tubes for serial dilutions; to 10 ml. sterile 250, 500, 1000 ml. Duran bottle.
- Measuring cylinders; a range of sizes.
- Glass bottles with screw caps 20–30 ml 5–10 ml
- Assorted sterile pipettes and pipetting device
- Autoclavable containers for discarding cultures
- Class II biological safety cabinet
- Water baths, 37ºC and 56°C
- Incubator, 35-37ºC
- pH meter
- Vortex mixer
- Incubator, 37ºC, 5% CO2
- Inverted microscope or standard microscope for the observation of cells

- Freezer, - 70ºC (for long term virus storage) or - 20ºC 4ºC refrigerator
- Low speed, bench top centrifuge preferably with refrigeration
- Liquid nitrogen for cell storage
- T-75 and T-25 tissue culture flasks, canted neck corning
- DNA Engine, PTC-220 DNA Engine Tetrad Cycler
- UV Transilluminature (Alpha innotech)
- Consumables; Syringes ;1 ml, 2.5 ml, 5 ml, 10 ml, Needles; 23 G" 23 mm, 25 G" 16 mm. Tips for micropipettor. Eppendorf tubes. Cryotubes. 96-well V-bottomed microtitre plates. Plastic centrifuge tubes; 10 ml 50 ml
- Single channel and Multichannel pipettors, pipett boys, pipettes and pipettor
- Egg candling lamp. Egg incubator. Shell punch
- Bunsen burner.
- Electronic balance
- Magnetic stirrer
- Centrifuge
- Test tube racks

2. Equipment and Material for sample collection

- Scissors, forceps, plastic bottles, conical tubes, Sterile cotton wool swabs
- Maker pens, Alcohol, Cotton, lighter, Gloves
- Mask, Cap. lab coat. Ice box with ice

3. Media, reagents and chemicals

- Dulbecco's Modified Eagle Medium (D-MEM)* GIBCO BRL, Hyclone
- Penicillin-Streptomycin, stock solution (10,000 U/ml penicillin G; 10,000 µg/ml streptomycin sulfate). Gentamicin reagent solution (50mg gentamicin sulfate/ml). Fetal bovine serum, 40 nm filtered GIBCO BRL, Hyclone. Serum Free Media, GIBCO BRL.

APPENDIX B: Stock solution

1. Buffer solution

Phosphate buffered saline (PBS)

Recipe to prepare five litres of PBS

Reagents

- Sodium chloride NaCl 40.0g
- Potassium chloride KCl 1.0g
- Potassium dihydrogen phosphate anhydrous KH2PO4 1.0g
- Disodium hydrogen phosphate anhydrous Na2HPO4 4.6g
- Distilled water to make up to 5L

Method

1. Weigh out the reagents and place in a 5 L conical flask.
2. Add distilled water to make 5 L. Mix well.
3. Check pH. Adjust to pH 7.2 to 7.4.
4. Pour into storage bottles.
5. Autoclave at 121°C for 15 minutes. Use a slow exhaust.
6. Allow to cool, then tighten the lids and label the bottles.
7. Store opened PBS, pH 7.2 at 4°C for no longer than 3 weeks.

2. Bacterial culture media

Tryptic Soy Broth (TSB)

This is a general purpose broth medium prepared for the cultivation of fastidious and non fastidious organisms.

Materials for preparing 1L of TSB

- 30 g dehydrated TSB medium
- 1 L distilled or deionized water
- Sterile 20 mL glass bottles with lids.

Method

1. Dissolve media in 1 L of water.
2. Warm slightly to dissolve completely.
3. Dispense 9 ml aliquots into the glass bottles.
4. Sterilize in the autoclave at 121°C for 15 minutes.

Sabouraud dextrose agar

Sabouraud dextrose agar is recommended for cultivating fungi. It is readily available and is supplied by Oxoid and Difco with instructions for its preparation. Choramphenicol is a bacterial inhibitor that can be added.

Materials

- Sabouraud dextrose agar 65 g
- Oxoid agar No. 1 or Difco Bacto agar 5 g
- Chloramphenicol (optional) 0.05 g dissolved in 10 ml of 95 percent ethanol.
- 1 L distilled water
- Sterile Petri dishes

Method

1. Suspend the agar in the distilled water. Heat to boiling point.
2. Add the chloramphenicol mixture to the agar. Stir thoroughly.
3. Dispense into 100 ml bottles.
4. Sterilize by autoclaving at 115C for 10 minutes. Allow to cool to 50°C.
5. Pour into sterile Petri dishes. Allow approximately 25 ml for a 90 mm Petri dish.
6. Incubate plates at 37°C overnight and check for growth of bacterial contaminants.
7. Store at 4°C.

3. Antibiotic Solution

The recipe given is for a solution of Penicillin, Streptomycin and Gentamycin dissolved in PBS. In this manual, this solution is given the abbreviation PSG. Benzyl penicillin (Penicillin G) is a broad range antibiotic active against Gram positive and Gram negative aerobic cocci and most spirochaetes. It comes in various forms, which vary in solubility. Streptomycin is an antibiotic affective against Gram negative bacteria. Gentamycin is a broad spectrum antibiotic mainly affecting Gram negative aerobes.

Reagents

- Benzyl penicillin 6 g

- Streptomycin 500 mg
- Gentamycin 250 mg
- Sterile PBS 1 L

Method

1. Dissolve reagents in approximately 800 ml of PBS.
2. Make up to 1 L with PBS.
3. Cold sterilize by passing solution through a 0.2 micron filter.

Dispense into 100 ml sterile glass bottles, lid and label.

4. Anticoagulants

Acid Citrate Dextrose (ACD)

Reagents

- Citric Acid $C(OH)(COOH)(CH_2.COOH)_2.H_2O$ 4.0g
- Sodium Citrate $Na_3C_6H_5O_7.2H_2O$ 11.3g
- D-Glucose $C_6H_{12}O_6$ 11.0g

Method

1. Weigh out reagents into a conical flask.
2. Dissolve in 300 mL of distilled water.
3. Make up to 500 mL with distilled water.
4. Dispense into 100 mL bottles and put on lids. Do not tighten.
5. Sterilize by autoclaving at 116ºC for 10 minutes. Use a slow exhaust
6. Allow to cool, then tighten the lids and label the bottles.
7. Store in the refrigerator.

Alsever's Solution

Reagents

- Citric acid C(OH)(COOH)(CH2.COOH)2.H2O 0.055g
- Sodium Citrate Na3C6H5O7.2H2O 0.8g
- D-Glucose C6H12O6 2.05g
- Sodium chloride NaCl 0.42g
- Distilled water to make up to 100 mL

Method

1. Weigh out reagents into a conical flask.

2. Dissolve of distilled water and make up to100 mL.
3. Dispense into sterile 10 mL bottles.
4. Sterilize by autoclaving at 116ºC for 10 minutes. Use slow exhaust.
5. Allow to cool, then tighten the lids and label the bottles.
6. Store in the refrigerator.

5. Preparation of TPCK-trypsin stock solution

a. Dissolve 20 mg TPCK-trypsin* in 10 ml of dH_2O.

b. Filter through 0.2 µm membrane.

c. Store in aliquots at -20°C

6. 10 X TBE Buffer:

Tris 107.8 g

Boric Acid 55.0 g

EDTA(Na2) 8.2 g

7. Gel loading buffer

20% (w/v) glycerol

1mg/ml (w/v) bromphenol blue 1Mm EDTA

DECLARATION

I, the under signed, declare that the thesis is my original work and has not been presented for a degree in any University.

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