Kirkhouse Trust

PROTOCOLS FOR MEDIA PREPARATION

January 2020

1. Yeast Dextrose Chalk (YDC) agar medium:

WHAT YOU NEED FOR 1 LITRE	
Agar	15 g
Yeast Extract	10 g
CaCO ₃ (light powder)	20 g
D-Glucose (Dextrose)	20 g

- 1. Weigh out all ingredients into a suitable container.
- 2. Add 750 mL distilled water. Bring to boil to dissolve; (note: CaCO₃ will not completely dissolve).
- 3. Make up to 1 litre volume with water.
- Dispense into bottles (300 mL or 500 mL medical flats supplied), with constant swirling to ensure even distribution of CaCO₃. Fill bottle half to two thirds full.
- 5. Autoclave at 121°C, 115 psi for 15 minutes.
- 6. Allow medium to cool to approximately 50°C.
- Swirl to ensure even distribution of CaCO₃ and avoid air bubbles. Pour into sterile petri plates (22 mL per 90 mm plate).
- 8. Leave plates to set in a dust hood, or similar, before use.
- 9. Store prepared plates inverted in polythene bags at room temperature. Prepared plates can be stored for several months provided they do not dry out.

2. MXP medium:

To prepare medium for *Xanthomonas Campestris pv phaseoli* (MXP) see the instructions in the article provided in the Lab Manager's Manual:

Claflin, L.E., Vidavar, A.K. and Sasser, M. (1987). MXP, a semi-selective medium for *Xanthomonas campestris* pv. Phaseoli. Phytopathology 77:730-734.

3. Directions for corn meal agar & other media supplied:

Product information from manufacturers can be found online, for example Oxoid brand product information can be found at www.oxoid.com or ask the P&D team for a Technical Data Sheet if one is not provided.

Corn Meal Agar:

Suspend 17g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes.

Potato Dextrose Agar:

Suspend 39 g of powder in 1 litre of purified water and bring to the boil. Distribute into suitable containers and sterilise in the autoclave at 121°C for 15 minutes. Do not overheat.

Malt Extract Agar:

Suspend 50 g in 1 litre of distilled water and bring to the boil to dissolve. Sterilize by autoclaving at 115°C for 10 minutes. in order to suppress the bacterial growth. For fungi a pH value of 3.5 is recommended, but this depends on the microorganism.