

## **MEDIA RECIPES**

### **Maximum Recovery Diluent (MRD)**

#### **Generic diluent**

Formulation:

Bacteriological Peptone (L37)	1.0g
Sodium Chloride	8.5g
Deionised water	to 1000ml

Stir to dissolve ingredients; Autoclave at 121°C for 15 min.  
pH should be 7.0 after sterilisation.

Aseptically dispense into appropriate volumes.

## **Glucose Agar**

### ***Enterobacteriaceae* confirmation medium**

Formulation:

Tryptone	10.0g
Yeast extract	1.5g
Glucose	10.0g
Sodium chloride	5.0g
Bromocresol purple	0.015g
Agar No 3	12.0g
Deionised water	to 1000ml

Heat to dissolve; dispense in 15ml quantities in test tubes and autoclave at 121°C for 15 min.

pH 7.0 after sterilisation.

Shelf life 1 week at 0 - 5°C.

## Brilliant green agar (modified) (BGA)

### Selective *Salmonella* agar

#### Formulation:

Beef extract	5.0g
Balanced Peptone No 1	10.0g
Yeast extract	3.0g
Disodium hydrogen phosphate	1.0g
Sodium dihydrogen phosphate	0.6g
Lactose	10.0g
Sucrose	10.0g
Phenol red	0.09g
Brilliant green	0.0047g
Agar No 2	12.0g

Add 52.0g of the dehydrated medium to one litre of deionised water. Allow to soak for 10 minutes, and then bring to the boil using a magnetic stirrer to dissolve the solids. Whilst the media is warming make the pH  $6.9 \pm 0.2$  at 35°C. Dispense in sterile 500ml Durans and cool to 47°C in a waterbath. Pour plates and store in the refrigerator. Plates should be surface-dried before use.

Sterility check: 30°C, 48 hr.

Positive control = *Salmonella* spp.

Negative control = *E. coli*.

## Violet red bile glucose agar (VGA)

### *Enterobacteriaceae* enumeration

Formulation:

Yeast extract	3.0g
Peptone	7.0g
Sodium chloride	5.0g
Bile salts No 3	1.5g
Glucose	10.0g
Neutral red	0.03g
Crystal violet	0.002g
Agar	12.0g

Add the 38.5g of dehydrated medium to 1 litre of deionised water and bring to the boil using a hotplate stirrer to dissolve the agar completely.

No further sterilisation is necessary or desirable.

Dispense in sterile Duran bottles and place in the agar cooling bath at 47°C and use within 3 hours.

Performance check  $44 \pm 1^\circ\text{C}$  24 – 48 hr

Positive control = *E. coli*.

Negative control = *Staphylococcus aureus*.

## **Buffered Peptone Water (BPW)**

### ***Salmonella* pre-enrichment**

#### **Buffered Peptone Water**

Formulation:

Peptone	10.0g
Sodium Chloride	5.0g
Disodium hydrogen orthophosphate.12H <sub>2</sub> O	9.0g
Potassium dihydrogen orthophosphate	1.5g
Deionised water	to 1000ml

Stir to dissolve; adjust pH if necessary. Dispense and autoclave at 121°C for 15 min.

pH after sterilisation should be pH 7.0 ± 0.2.

## **Semi-solid nutrient agar (SSN)**

### ***Salmonella* confirmation: examination for H antigens**

Formulation:

Meat extract	3.0
Peptone	5.0
Agar	6.5
Deionised water	to 1000ml

Add dehydrated ingredients to 1 litre of deionised water. Boil to dissolve completely. Dispense in 15 ml quantities in glass universals and sterilise by autoclaving at 121°C for 15 min. Final pH should be pH 7.0 ± 0.2 at 25°C

Slope the universals while still molten. Do not allow to dry out.

## Plate Count Agar (PCA)

### Total Aerobic Counts on food products

#### Formulation:

Tryptone	5.0g	20.0g
Yeast extract	2.5g	10.0g
Glucose	1.0g	4.0g
Agar No. 1	9.0g	36.0g
Deionised water	to 1000ml	to 4000ml

Dissolve the dry ingredients in the water.

Boil to dissolve using a magnetic stirrer.

Dispense in approximate 100ml quantities. Autoclave at 121°C for 15 min.

Final pH should be pH = 7.00 ± 0.2 at 25°C

Sterility check: 37°C, 24 hr.

Positive control = *E. coli*.

Negative control = uninoculated.

## Nutrient Agar (NA)

Formulation:

Lab Lemco	1.0g
Yeast extract	2.0g
Peptone	5.0g
Sodium chloride	5.0g
Agar No 3	15.0g
Deionised water	to 1000ml

Add dried media to water, heat and stir to dissolve; dispense in approx 500ml amounts and autoclave at 121°C for 15 min.

pH should be pH 7.4 +/- 0.2 at 25°C after sterilisation.

## **Tryptone Bile Glucuronic agar (TBX)**

Formulation:

Tryptone	20g
Bile salts No. 3	1.5g
5-Bromo-4-chloro-3-indolyl b-D-glucuronic acid (BCIG)	0.075g (if cyclohexylammonium salt is used, otherwise use 144 $\mu$ moles)
Dimethyl sulfoxide (DMSO)	3ml
Agar No 3	15g
Deionised water	to 1000ml

Dissolve the BCIG in the DMSO. Add all the components to the water and bring to the boil.

Dispense in approx 500ml amounts and autoclave at 121°C for 15 min.

pH should be pH 7.2 +/- 0.2 at 25°C after sterilisation.