MEDIA AND SOLUTIONS

Casein Agar
Skim milk 500ml
Nutrient agar (double strength) 500ml

Frazier’s solution
Mercuric chloride 12g
Distilled water 80ml
Conc. HCl 16ml

Gelatin Agar
Gelatin 30g
Caesin enzyme hydrolysate 10g
NaCl 10g
Agar 15g
Water 1 litre

Gram Staining reagents
Crystal Violet
Solution A
Crystal violet 10g
Ethanol 100ml

Solution B
Ammonium oxalate 1% aq. Solution
For use mix 20ml of solution A with 80ml of solution B

Gram’s iodine
Iodine 5g
KI 10g
Water 100ml

**Safranin solution**

Safranin 0.5g
Water 100ml

**Kovac's reagent**

p-dimethylaminobenzaldehyde 5g
Amyl alcohol 75ml
Conc. HCl 25ml

**Luria Bertani Medium (LB) (pH 7.2)**

Tryptone 10g
Yeast extract 5g
Sodium chloride 10g
Water 1L

**Media for sugar utilization (pH 7.2)**

K$_2$HPO$_4$ 0.06g
(NH$_4$)$_2$ SO$_4$ 0.5 g
MgSO$_4$ 0.06 g
KCl 0.025g
Yeast extract 0.025g
Bromothiophenol 0.01g
Water 250ml

**Methyl Red solution**

Methyl red 0.04g
Ethanol 40ml
Distilled water 100ml
Minimal Media (pH 7.2)

Na₂HPO₄ 4 g
KH₂PO₄ 2 g
(NH₄)₂ SO₄ 0.8 g
MgSO₄ 0.8 g
TES 1 ml
Water 1 litre

Trace Element Solution (TES)

Molish reagent

15% a- naphthol (in ethanol) 10.5
Sulfuric acid 6.5
Ethanol 40.5
Water 4.0

MR-VP test medium

Peptone 5g
K₂HPO₄ 5g
Water 1 litre

Nitrate Broth

Beef extract 3g
Peptone 5g
KNO₃ 1g
Water 1 litre

Nitrate reduction test solutions

Solution 1
0.8% Sulphanilic acid in 5N Acetic acid

Solution 2
0.6% dimethyl-α-naphthylamine in 5N Acetic acid
Nutrient Agar (pH 7.4)
Peptone 5.0 g
Sodium chloride 5g
Beef extract 1.5 g
Yeast extract 1.5g
Water 1 litre

Nutrient solution for field application
NaNO₃ 2g
K₂HPO₄ 2g
(NH₄)₂SO₄ 0.8g
MgSO₄ 0.8 g
Yeast Extract 0.1g
TES 1ml
Water 1 litre
TES: Trace Element Solution (same as used for minimal media)

O/F Medium (Hugh Leifson medium) (Ph 6.8)
Peptic digest of animal tissue 2g
NaCl 5g
K₂PO₄ 0.3g
Glucose 10g
Bromothymol Blue 0.05g
Agar 2g
Water 1 liter

Peptone Water (pH 7.2-7.4)
Peptone 10g
NaCl 5g
Water 1 litre
PUM Buffer (pH 7.1)
K$_2$HPO$_4$ 16.87g (22.2gm K$_2$HPO$_4$.3H$_2$O)
KH$_2$PO$_4$  7.26g
MgSO$_4$.7H$_2$O  0.2g
Urea  1.8g
Water  1 litre

PPGAS Media (pH 7.2)
NH$_4$Cl  0.02M
KCl  0.02M
Tris-HCl  0.12M
Glucose  0.5%
Proteose peptone  1%
MgSO$_4$  0.0016M

Phosphate Buffer Saline (pH 7.2)
NaCl  8g
KCl  0.2g
Na$_2$HPO$_4$  1.44g
KH$_2$PO$_4$  0.24g
Water  1Litre

Sodium phosphate buffer: 10mM (pH 7.0/7.2)
Prepare 0.2M solutions of monobasic sodium phosphate (solution A) and 0.2M solution of dibasic sodium phosphate (solution B).
39.0ml of solution A and 61.0ml of solution B diluted to a total of 200ml gives 10mM sodium phosphate buffer (pH 7.0).
28ml of solution A and 72ml of solution B diluted to a total of 200ml gives 10mM sodium phosphate buffer (pH 7.2)
**Starch Agar**

Peptone 5g  
NaCl 5g  
Yeast extract 1.5g  
Beef extract 1.5g  
Starch soluble 2g  
Agar 15g  
Water 1 litre

**STE buffer**

NaCl 0.1M  
Tris.Cl 10mM (pH 8.0)  
EDTA 1mM (pH 8.0)

**TAE (50X)**

Tris Base 242g  
Glacial acetic acid 57.1ml  
0.5M EDTA (pH 8.0) 100ml  
Water 1 litre

**TE (pH 8.0)**

Tris.Cl 10mM  
EDTA 1mM

**Thornley’s Medium**

Peptone 1g  
NaCl 5g  
K₂HPO₄ 0.3g  
Phenol red 0.01g  
l-arginine-HCl 10.0g  
Agar 3g
Water 1 litre

**Trace Element Solution**

<table>
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<tr>
<th>Chemical</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Al(OH)₃</td>
<td>0.1g</td>
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<tr>
<td>SnCl₂.2H₂O</td>
<td>0.05 g</td>
</tr>
<tr>
<td>KI</td>
<td>0.05g</td>
</tr>
<tr>
<td>LiCl</td>
<td>0.05 g</td>
</tr>
<tr>
<td>MgSO₄.4H₂O</td>
<td>0.08 g</td>
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<tr>
<td>H₃BO₃</td>
<td>0.5 g</td>
</tr>
<tr>
<td>ZnSO₄.7H₂O</td>
<td>0.1 g</td>
</tr>
<tr>
<td>CoCl₂.6H₂O</td>
<td>0.1 g</td>
</tr>
<tr>
<td>NiSO₄.6H₂O</td>
<td>0.1 g</td>
</tr>
<tr>
<td>BaCl₂</td>
<td>0.05 g</td>
</tr>
<tr>
<td>(NH₄)I</td>
<td>0.05 g</td>
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</table>

Water 1 Litre

**Urease medium**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Yeast extract</td>
<td>0.1g</td>
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<tr>
<td>K₂HPO₄</td>
<td>9.1g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>9.5g</td>
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<tr>
<td>Urea</td>
<td>20g</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.01g</td>
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Water 1 litre