

This section lists the culture medium referred to by number in individual strain entries in the main body of the Catalogue. Detailed formulae are given for all media except those readily available commercially in dehydrated form, in which case only the name of the medium is given. It can be assumed that dehydrated media from any reputable manufacturer are suitable, if made up according to the manufacturer's instructions, unless a specific brand is recommended in particular cases.

1 NUTRIENT AGAR (OXOID CM3)

Lab-Lemco beef extract.....	1.0 g
Yeast extract.....	2.0 g
Peptone.....	5.0 g
NaCl.....	5.0 g
Agar.....	15.0 g
Distilled water.....	1.0 L

Autoclave..... 121°C/15min

2 GLUCOSE NUTRIENT AGAR

Medium 1 plus 1% glucose

Autoclave..... 115C/20min

3 GLUCOSE BROTH

Oxoid nutrient broth No. 2 (CM67).....	2.5 g
Glucose.....	1.0 g
Distilled water.....	100.0 ml

Dissolve and mix thoroughly. Distribute approx 7ml into 5"x 5/8" tubes

Autoclave..... 121C/10min

4 PANTOTHENATE AGAR

KH ₂ PO ₄	0.27 g
MgSO ₄ .7H ₂ O.....	0.26 g
FeSO ₄ .7H ₂ O.....	2.80 mg
MnSO ₄	1.50 mg
Na ₂ MoO ₄	2.10 mg
Agar.....	25.00 g
Distilled water.....	1.00 L

Adjust to pH 6.8-7.0 with KOH and autoclave at 121C/10 min. Cool to 50°C and add concentrated, filter-sterilized solution of potassium pantothenate to give a final concentration of 2.57 g/l.

5 YEAST GLUCOSE AGAR

Glucose.....	20.0 g
Yeast extract.....	10.0 g
Agar.....	15.0 g
Distilled water.....	1.0 L

Autoclave..... 121°C/15min

6 SALT NUTRIENT AGAR

Medium 1 plus 2% NaCl.

Autoclave..... 121°C/15min

7 NITROGEN FREE MEDIUM

K ₂ HPO ₄	1.0 g
MgSO ₄ .7H ₂ O.....	0.2 g
CaCO ₃	1.0 g
NaCl.....	0.2 g
FeSO ₄ .7H ₂ O.....	0.1 g
Na ₂ MoO ₄ .2H ₂ O.....	5.0 mg
Agar.....	15.0 g
Distilled water.....	1.0 L
Glucose.....	10.0 g

Dissolve the salts in water and adjust the pH to approximately 7.0. Add the agar, dissolve by steaming, and autoclave the medium at 121°C/15 min. Add the glucose aseptically as 50 ml of a filter-sterilized 20% (w/v) solution to 1.0 L of medium.

8 UREA NUTRIENT AGAR

Medium 1 plus 2% urea.

ml filter-sterilized 20% urea solution is added aseptically post autoclaving to 100 ml cooled, molten, agar. The medium is then immediately dispensed aseptically.

9 DILUTE PEPTONE WATER

Peptone.....	1.0 g
NaCl.....	1.0 g

Adjust pH to 7.0. Autoclave at a 121C/15 min.

10 CHROMATIUM/THIOCAPSA MEDIUM

(a) Trace element solution:

FeCl ₃ .6H ₂ O.....	2.7 g
H ₃ BO ₃	0.1 g
ZnSO ₄ .7H ₂ O.....	0.1 g
Co(NO ₃) ₂ .6H ₂ O.....	50.0 mg
CuSO ₄ .5H ₂ O.....	5.0 mg
MnCl ₂ .6H ₂ O.....	5.0 mg
Distilled water.....	1.0 L

(b) Basal medium:

KH ₂ PO ₄	1.0 g
NH ₄ Cl.....	1.0 g
MgCl ₂ .6H ₂ O.....	0.5 g
Trace elements solution.....	1.0 ml
Distilled water.....	999.0 ml

(c) NaHCO₃..... 10%

(d) Na₂S.9H₂O..... 10%

- (e) $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ 10%
- (f) Sodium malate..... 10%

All solutions are autoclaved separately at 121C/15 min.

For every 10 ml of freshly boiled medium (b), are added, aseptically, 0.2 ml solution (c), 0.02 ml solution (d), 0.1 ml solution (e) and 0.1 ml solution (f).

The complete medium should be used immediately.

11 CHLOROBIUM THIOSULFATOPHILUM MEDIUM

(a) Trace element solution:

- $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 2.7 g
 H_3BO_3 0.1 g
 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1 g
 $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ 50.0 mg
 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 5.0 mg
 $\text{MnCl}_2 \cdot 6\text{H}_2\text{O}$ 5.0 mg
 Distilled water 1.0 L

(b) Basal medium:

- KH_2PO_4 1.0 g
 NH_4Cl 1.0 g
 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 0.5 g
 NaCl 10.0g
 Trace elements solution..... 1.0 ml
 Distilled water 999.0 ml

- (c) NaHCO_3 10%
- (d) $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ 10%
- (e) $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ 10%

All solutions are autoclaved separately at 121C/15 min.

For every 10 ml of freshly boiled medium (b), are added, aseptically, 0.2 ml solution (c), 0.02 ml solution (d) and 0.1 ml solution (e)

Adjust pH to 7.0-7.2 with sterile phosphoric acid.

The complete medium should be used immediately.

12 COOKED MEAT GLUCOSE MEDIUM

Cooked meat medium (Southern Group Laboratories:0503) with the supernatant removed and replaced with an equal volume of medium 3. To prepare this medium from basic ingredients, heat 500 g fat-free minced beef in 1 L distilled water containing 25 ml 1M NaOH, stirring until the mixture boils. Cool to room temperature, skim fat from the surface and filter. Dispense 1 volume meat particles to 4 volumes medium 3 in screw-capped bottles and sterilize at 121C/15 min.

13 CLOSTRIDIUM STICKLANDII MEDIUM

- K_2HPO_4 1.75 g
 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.00 mg

- $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.20 g
 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 10.00 mg
 L-arginine HCl 2.00 g
 L-lysine HCl 2.00 g
 Yeast extract 5.00 g
 Sodium formate 2.00 g
 NH_4Cl 2.00 g
 Distilled water 1.00 L

Adjust pH to 7.0. Autoclave at 121C/15 min. Add 3 ml/100 ml sterile $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ solution.

14 TPYG MEDIUM

- Trypticase 10.0 g
 Peptone 5.0 g
 Yeast extract 5.0 g
 Distilled water 1.0 L

Bottle in 19 ml amounts. Autoclave at 121C/15 min.

Add 1 ml of filter sterilized 20% glucose solution aseptically to each 19 ml of base.

15 CLOSTRIDIUM OROTICUM MEDIUM

- Tryptone 5.00 g
 Yeast extract 0.50 g
 KH_2PO_4 1.36 g
 K_2HPO_4 6.95 g
 Sodium orotate 2.50 g
 Riboflavin 15.00 mg
 Sodium thioglycollate 0.50 g
 Distilled water 1.00 L

Adjust pH to 7.5. Autoclave at 121C/15 min. Add aseptically 100 ml, filter-sterilized, 5% L-arabinose solution. Dispense the complete medium separately.

16 DUBOS SALTS MEDIUM

- NaNO_3 0.5 g
 K_2HPO_4 1.0 g
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g
 KCl 0.5 g
 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 10.0 mg
 Agar 15.0 g
 Distilled water 1.0 L

Adjust pH to 7.2. Autoclave at 121C/15 min. Dispense into slopes. When the agar has solidified, place a strip of sterile filter paper on to the surface of each slope. Inoculate on to the filter paper.

17 POSTGATE'S MEDIUM

- Basal medium
 K_2HPO_4 0.5 g
 NH_4Cl 1.0 g
 CaSO_4 1.0 g
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 2.0 g
 Sodium lactate(70%) 3.5 ml

Yeast extract..... 1.0 g
Distilled water 1.0 L

Dissolve above and gas with oxygen free nitrogen for 10 - 15 minutes, then add:-

Thioglycolic acid..... 0.1 g
Ascorbic acid 0.1 g
FeSO₄.7H₂O 0.5 g

Still gassing pH to 7.4, dispense and autoclave at 115C/10 min.

18 THIOBACILLUS FERROOXIDANS MEDIUM

Solution I

(NH₄)₂SO₄ 0.5 g
K₂HPO₄ 0.5 g
MgSO₄.7H₂O 0.5 g
1N H₂SO₄ 5.0 ml
Distilled water 1.0 L

Solution II

FeSO₄.H₂O 167.0 g
1N H₂SO₄ 50.0 ml
Distilled water 1.0 L

Autoclave solution I at 121°C/15 min and sterilize solution II by filtration. After sterilization 4 parts of solution I are added to 1 part of solution II.

19 SODIUM CASEINATE AGAR

Sodium caseinate 2.0 g
Yeast extract..... 0.5 g
Peptone..... 0.5 g
K₂HPO₄ 0.5 g
Agar 15.0 g
Distilled water 1.0 L

Dissolve all except the agar and adjust pH to 7.4-7.6. Add the agar, steam to dissolve, dispense in bottles or tubes and autoclave at 121°C/15 min.

20 MRS BROTH (DE MAN, ROGOSA, SHARPE (OXOID CM359)

Peptone..... 10.0 g
'Lab-Lemco' powder 8.0 g
Yeast extract..... 4.0 g
Glucose 20.0 g
Sorbitan mono-oleate 1.0 ml
K₂HPO₄ 2.0 g
Sodium acetate.3H₂O 5.0 g
Triammonium citrate..... 2.0 g
MgSO₄.7H₂O 0.2 g
MnSO₄.4H₂O 0.05 g
Distilled water 1.0 L

Adjust pH to 6.2 ±0.2. Dispense in bottles and autoclave at 121C/15 min.

21 MRS SALT

Medium 20 plus 10% salt.

22 MRS CHALK

Medium 20 plus 3% CaCO₃.

23 PEPTONE YEAST GLUTAMATE MEDIUM

Peptone 20.0 g
Yeast extract 10.0 g
Monosodium glutamate 4.0 g
Sodium thioglycollate..... 1.0 g
Distilled water 1.0 L

Adjust pH to 7.0-7.2. Autoclave at 121C/15 min.

24 KREBS' YEAST LACTATE MEDIUM

Yeast extract 10.0 g
KH₂PO₄ 1.0 g
Na₂HPO₄.2H₂O 3.0 g
Sodium lactate(70%) 40.0 ml
Distilled water 960.0 ml

Adjust pH to 7.0. Autoclave at 121C/15 min.

25 SALT NUTRIENT AGAR No 2

Nutrient agar (Medium 1) plus 10% NaCl.

26 PSEUDOMONAS SACCHAROPHILA MEDIUM

Solution (a)

KH₂PO₄ 0.44 g
Na₂HPO₄ 0.48 g
NH₄Cl 0.10 g
MgSO₄.7H₂O 50.00 mg
Distilled water 100.00 ml

Solution (b)

Ferric ammonium citrate 0.1 g
CaCl₂ 10.0 mg
Distilled water 10.0 ml

Solution (c)

20% sucrose in distilled water.

Sterilize the three solutions separately. Aseptically add 0.5 ml (b) and 1 ml (c) to 100 ml (a). If a solid medium is required, prepare (a) with 2% agar. For a completely inorganic medium, substitute ferric chloride for ferric ammonium citrate.

27 YEAST PEPTONE MEDIUM

Yeast extract 2.5 g
Peptone 2.5 g
Agar 15.0 g
Distilled water 1.0 L

Adjust pH to 7.0-7.4. Autoclave at 121C/15 min.

28 SPIRILLUM MEDIUM

Peptone.....	5.0 g
Beef extract	3.0 g
Yeast extract.....	3.0 g
Calcium lactate.....	10.0 g
Agar (if required)	15.0 g
Distilled water	1.0 L

Adjust pH to 7.0. Autoclave at 115C/20 min. There is no need to filter off the precipitate formed during preparation and sterilization.

29 YEAST MALT AGAR (ISP2)

Yeast extract.....	4.0 g
Malt extract	10.0 g
Glucose	4.0 g
Agar	20.0 g
Distilled water	1.0 L

Adjust pH to 7.2. Autoclave at 121C/15 min.

30 STARCH SALTS AGAR

Solution (a)

K ₂ HPO ₄	1.0 g
MgSO ₄ .7H ₂ O	1.0 g
NaCl.....	1.0 g
(NH ₄) ₂ SO ₄	2.0 g
CaCO ₃	2.0 g
Trace salts	1.0 ml
Distilled water	500.0 ml

Solution (b)

Soluble starch.....	10.0 g
Distilled water	500.0 ml

Trace salts

FeSO ₄ .7H ₂ O	0.1 g
MnCl ₂ .4H ₂ O	0.1 g
ZnSO ₄ .7H ₂ O.....	0.1 g
Distilled water	100.0 ml

To prepare (b) make a paste of the starch with a little of the water and then gradually add the remaining water. Mix (a) and (b), adjust the pH to 7.2, add 20 g agar and steam to dissolve the agar. Autoclave at 121C/15 min. Before pouring slopes or plates, mix the medium thoroughly to ensure reasonable distribution of the chalk.

31 THIOBACILLUS AGAR (NON-ACIDURIC)

(NH ₄) ₂ SO ₄	0.1 g
K ₂ HPO ₄	4.0 g
KH ₂ PO ₄	4.0 g
MgSO ₄ .7H ₂ O	0.1 g
CaCl ₂	0.1 g
FeCl ₃ .6H ₂ O	2.0 mg
MnSO ₄ .4H ₂ O	2.0 mg
Na ₂ S ₂ O ₃ .5H ₂ O.....	10.0 g
Purified agar.....	12.0 g
Distilled water	1.0 L

Dissolve all except the agar in distilled water and adjust the pH to 6.6. Add the agar and autoclave at 115C/20 min.

32 THIOBACILLUS AGAR (ACIDURIC)

NH ₄ Cl.....	0.1 g
KH ₂ PO ₄	3.0 g
MgCl.6H ₂ O	0.1 g
CaCl ₂	0.1 g
Na ₂ S ₂ O ₃ .5H ₂ O.....	5.0 g
Purified agar.....	20.0 g
Distilled water	1.0 L

Dissolve all except the agar in distilled water and adjust the pH to 4.2. Add the agar and steam to dissolve. Autoclave at 121C/15 min.

33 ZYMOMONAS MEDIUM

Yeast extract.....	10.0 g
Glucose.....	10.0 g
Tap water.....	1.0 L

Dissolve the above ingredients in tap water and adjust the pH to 4.8. Autoclave at 115C/20 min. Boil the medium immediately before use.

34 CLOSTRIDIUM ACIDI-URICI MEDIUM

KOH.....	0.67 g
K ₂ HPO ₄	0.91 g
Uric acid.....	2.00 g
MgSO ₄ .7H ₂ O.....	0.25 g
CaCl ₂ .2H ₂ O.....	0.02 g
FeSO ₄ .7H ₂ O	6.00 mg
Yeast extract.....	1.00 g
Sodium thioglycollate.....	0.50 g
NaHCO ₃	5.00 g
Distilled water	1.00 L
Agar.....	15.00 g

Add KOH and K₂HPO₄ to 900 mls H₂O and heat. Add uric acid and boil until acid is dissolved. Add all the other chemicals except the sodium thioglycollate and NaHCO₃ at this stage. Adjust pH to 7.4 - 7.8 with NaOH. Add agar if plates/slopes are required.

Autoclave.....121C/15min

Dissolve sodium thioglycollate in 50 mls H₂O and autoclave separately at 121°C/15min. Dissolve NaHCO₃ in 50 mls H₂O and filter sterilize. Add thioglycollate and NaHCO₃ solutions to uric acid base aseptically when the base has cooled to approximately 50°C. Dispense in 20 ml amounts in McCartney bottles or as plates.

Do not allow the temperature of the medium to fall below 35C as this will cause the precipitation of uric acid.

Store the medium at 35C to 45C, preferably anaerobically and use within 7 to 10 days of preparation.

35 CASITONE PHOSPHATE AGAR

Casitone (Difco).....	20.00 g
MgSO ₄ ·7H ₂ O	2.00 g
K ₂ HPO ₄	1.25 g
KH ₂ PO ₄	0.48 g
Agar	20.00 g
Distilled water	1.00 L

Adjust pH to 7.2. Autoclave at 121C/15min

36 CASITONE AGAR

Casitone	3.0 g
CaCl ₂	1.0 g
Agar	15.0 g

Adjust pH to 7.2, autoclave at 121C/15 min.

37 NEOMYCIN MEDIUM No 1

Medium 1 plus 0.05% filter-sterilised sucrose and 1 mg/ml neomycin sulphate.

38 LACTOBACILLI AOAC MEDIUM (DIFCO 0901)

Peptonized milk.....	15.0 g
Yeast extract.....	5.0 g
Glucose	10.0 g
Tomato juice	5.0 g
K ₂ HPO ₄	2.0 g
Sorbitan monooleate complex	1.0 g
Distilled water	1.0 L

Autoclave 121C/15min

39 LACTOBACILLUS CHLORAMPHENICOL MEDIUM No1

LACTOBACILLUS AOAC Medium (Medium 38) plus 300 mcg/ml chloramphenicol.

Autoclave at 121C/15 min.

When resuscitating NCIMB 10463 allow up to 72h incubation at 37C and when subculturing use at least 1 ml heavy suspension in a Pasteur pipette. Use of loops will result in weak cultures.

40 B12 NUTRIENT AGAR

Medium 1 plus 400 mcg/L vitamin B12.

41 GLYCEROL ASPARAGINE AGAR

L-asparagine.....	1.0 g
Glycerol.....	10.0 g
K ₂ HPO ₄	1.0 g
Agar	20.0 g
Trace salts solution.....	1.0 ml
Distilled water	1.0 L

Dissolve all except the agar in distilled water. Adjust pH to 7.0-7.4, add agar and steam to dissolve. Autoclave at 121C/15 min.

Trace salts solution - see Medium 30.

42 OATMEAL AGAR

Oatmeal	20.0 g
Agar.....	8.0 g
Trace salts solution	1.0 ml
Distilled water	1.0 L

Steam the oatmeal for 20 min in the required volume of distilled water. Filter through cheese-cloth and make up the filtrate to its original volume with distilled water. Add trace salts, adjust the pH to 7.2, add the agar and steam to dissolve. Autoclave at 121C/15 min. Mix well before pouring plates.

Trace salts solution - see Medium 30.

43 BDELLOVIBRIO MEDIUM

Host medium:

Yeast extract.....	3.0 g
Peptone.....	0.6 g
Distilled water	1.0 L

Adjust pH to 7.2

Base layer agar:

As host medium, but with the addition of 1.9% agar.

Semi-solid agar:

As host medium, but with the addition of 0.6% agar.

Distribute in 10 ml amounts and autoclave at 115C/20 min. Grow up the appropriate host (see individual catalogue entries) in the host medium for 24-48 h at 30C. Melt the base layer agar and semi-solid agar. Pour the base layer into a petri dish and allow to set. Cool the semi-solid agar to 40-45C, add 1 ml host culture, mix and pour over the base layer agar. Incubate at 30C for 18-24 h, agar surface uppermost. Spot the BDELLOVIBRIO culture, resuspended in a small quantity of host medium on to the surface of the plate and incubate at 30C until zones of clearing appear in the host organism layer (3-5 days).

44 FASTIDIOUS ANAEROBE AGAR (F.A.A.) (LAB90)

Ppeptone mix	23.00 g
NaCl	5.00 g
Soluble starch	1.00 g
Agar No.2	12.00 g
Sodium bicarbonate.....	0.40 g
Glucose.....	1.00 g
Sodium pyruvate.....	1.00 g
Cysteine HCl.monohydrate.....	0.50 g
Haemin	0.01 g
Vitamin K.....	0.001 g
L-arginine	1.00 g
Soluble pyrophosphate	0.25 g
Sodium succinate	0.50 g
Distilled water	1.00 L

Adjust pH to 7.2 ± 0.2. Autoclave at 121C/15 min.

45 ACETATE AGAR

Yeast extract.....	2.0 g
Tryptone	1.0 g
Sodium acetate	1.0 g

Agar 15.0 g
 Distilled water 1.0 L

Adjust pH to 7.4-7.6. Autoclave at 121C/15 min.

46 SULFOLOBUS MEDIUM

Yeast extract..... 1.00 g
 (NH₄)₂SO₄..... 1.30 g
 KH₂PO₄..... 0.28 g
 MgSO₄.7H₂O 0.25 g
 CaCl₂.2H₂O..... 0.07 g
 FeCl₃.6H₂O 0.02 g
 MnCl₂.4H₂O..... 1.80 mg
 Na₂B₄O₇.10H₂O 4.50 mg
 ZnSO₄.7H₂O..... 0.22 mg
 CuCl₂.2H₂O..... 0.05 mg
 NaMoO₄.2H₂O 0.03 mg
 VOSO₄.2H₂O 0.03 mg
 CoSO₄ 0.01 mg
 Freshly distilled water 1.00 L

Adjust pH to 2-3 with 5M H₂SO₄. Autoclave at 121C/15 min.

Warm medium to 70C before inoculation with SULFOLOBUS.

47 TOMATO JUICE AGAR (OXOID CM113)

Tomato juice (solids from 400 ml) 20.0 g
 Peptone..... 10.0 g
 Peptonized milk..... 10.0 g
 Agar 12.0 g
 Distilled water 1.0 L

Adjust pH to 6.1. Autoclave at 121C/15 min.

48 CZAPEK PEPTONE AGAR

Sucrose..... 30.00 g
 K₂HPO₄..... 1.00 g
 MgSO₄.7H₂O 0.50 g
 KCl..... 0.50 g
 FeSO₄.7H₂O..... 0.01 g
 Peptone..... 5.00 g
 Agar 15.00 g
 Distilled water 1.00 L

Dissolve all except the agar, adjust the pH to 7.0-7.3, add the agar and steam to dissolve. Autoclave at 121C/15 min.

49 CZAPEK PEPTONE YEAST AGAR

Medium 48 plus 0.2% yeast extract.

Autoclave at 121C/15 min.

50 URIC ACID MEDIUM

Medium 1 plus 0.5% uric acid

Make up the nutrient agar in seven eighths of the volume of distilled water normally required. Prepare a 4% suspension of uric acid in the remaining one eighth of the volume of distilled water. Autoclave both solutions at 121C/15 min and mix aseptically immediately before pouring. Keep well mixed during pouring.

51 ALKALOPHILE MEDIUM

Medium 1 adjusted to about pH 9.5 with 9% Na sesquicarbonate. If the agar is sterilised in 12 ml amounts, 0.2 ml sterile sesquicarbonate solution added aseptically should produce a suitable pH.

52 BLOOD AGAR BASE (OXOID CM55)

'Lab-Lemco' beef extract..... 10.0 g
 Peptone 10.0 g
 NaCl 5.0 g
 Agar 15.0 g
 Distilled water 1.0 L

Autoclave 121C/15min

53 CHOCOLATE AGAR

Blood agar base (Medium 52) plus 10% horse blood.

Add 10 ml horse blood to 100 ml sterile molten agar at 55C, mix well and steam for about 10 min. Dispense as slopes or plates and allow to set.

54 FLUID THIOGLYCOLLATE MEDIUM

Trypticase 15.0 g
 L-cystine..... 0.5 g
 Glucose..... 5.0 g
 Yeast extract..... 5.0 g
 NaCl 2.5 g
 Sodium thioglycollate..... 0.5 g
 Resazurin 1.0 mg
 Distilled water 1.0 L

Add the trypticase and after dissolving add all the other ingredients before adjusting pH to 7.1. Finally add the resazurin and bottle in 15 - 20 ml amounts in screw capped bottles and autoclave at 121C/15 min.

55 THIOBACILLUS THERMOPHILUS MEDIUM

Na₂S₂O₃ 5.0 g
 NaHCO₃ 1.0 g
 MgCl₂ 0.1 g
 NH₄Cl..... 0.1 g
 Na₂HPO₄ 0.2 g
 Distilled water 1.0 L

Adjust pH to 7.0-7.2. Autoclave 109C/20 min or filter sterilize.

56 TYG MEDIUM

Tryptone 20.0 g
 Glucose..... 5.0 g
 Yeast extract..... 0.5 g
 Sodium thioglycollate..... 0.5 g
 KH₂PO₄ 4.0 g

MgSO ₄ ·7H ₂ O	0.2 g
FeSO ₄ ·2H ₂ O	5.0 mg
MnSO ₄ ·4H ₂ O	5.0 mg
NH ₄ MoO ₄	5.0 mg
Distilled water	1.0 L

Adjust pH to 7.4 with NaOH. Autoclave 116C/10 min.

57 S-8 MEDIUM

Na ₂ HPO ₄	1.20 g
KH ₂ PO ₄	1.80 g
MgSO ₄ ·7H ₂ O	0.10 g
(NH ₄) ₂ SO ₄	0.10 g
CaCl ₂	0.03 g
FeCl ₃ ·6H ₂ O	0.02 g
MnSO ₄ ·4H ₂ O	0.02 g
Na ₂ S ₂ O ₃ ·5H ₂ O.....	10.00 g
NaHCO ₃	0.50 g
KNO ₃	5.00 g
Agar, if required.....	15.00 g
Distilled water	1.00 L

Adjust pH to 7.0. Autoclave 121C/15 min.

58 CAULOBACTER MEDIUM

Peptone.....	2.0 g
Yeast extract.....	1.0 g
MgSO ₄ ·7H ₂ O	0.2 g
Riboflavin	1.0 mg
Agar powder.....	10.0 g
Distilled water	1.0 L

Dissolve all except the agar in the water and adjust the pH to 7.0. Add the agar and dissolve by steaming. Distribute as required and sterilize by autoclaving at 121C/15 min.

59 SPORULATION MEDIUM FOR STREPTOMYCES

Yeast Extract.....	1.0 g
Beef extract	1.0 g
Tryptose	2.0 g
FeSO ₄	Trace
Glucose	10.0 g
Agar	15.0 g
Distilled water	1.0 L

Adjust pH to 7.2. For broth, eliminate agar and reduce concentration to 1/3 of the given quantities.

60 TRYPTONE/YEAST EXTRACT MEDIUM

Tryptone.....	10.0 g
Yeast extract.....	1.0 g
Distilled water	1.0 L
Autoclave.....	121C/15min

61 SC MEDIUM

Corn meal agar	17.0 g
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Phytone.....	8.0 g
K ₂ HPO ₄	1.0 g
KH ₂ PO ₄	1.0 g
MgSO ₄ ·7H ₂ O.....	0.2 g
Haemin (0.1% in 0.05M NaOH)	15.0 ml
Distilled water	1.0 L

Adjust pH to 6.6 with NaOH. Autoclave at 121C/15 min and cool to 50C. Aseptically add the following filter-sterilized components.

20% bovine serum albumin fraction V (SigmaA-9647).....	10.0 ml
50% glucose	1.0 ml
10% cysteine	10.0 ml

62 MANGANOUS ACETATE AGAR

Manganous acetate	0.1 g
Purified agar	10.0 g
Distilled water	1.0 L

Dissolve the manganous acetate in the water and adjust pH to approximately 7.0. Add agar and steam medium to dissolve it. Dispense the medium into screw-capped bottles and autoclave at 121C/15 min and slope.

63 VAN NIEL'S MEDIUM

K ₂ HPO ₄	1.0 g
MgSO ₄	0.5 g
Yeast extract (Difco)	10.0 g
Agar.....	20.0 g
Tap water.....	1.0 L

Adjust pH to 7.0-7.2. Autoclave at 121C/15 min.

64 YEAST MALATE MEDIUM

Yeast extract.....	1.0 g
KH ₂ PO ₄ ·12H ₂ O	1.0 g
NaHCO ₃	1.0 g
(NH ₄) ₂ SO ₄	0.5 g
Sodium malate.....	1.0 g
Trace elements solution	1.0 ml
Distilled water	1.0 L

Adjust pH to 6.8-7.0. Autoclave 121C/15 min.

Trace elements solution:

ZnSO ₄ ·7H ₂ O.....	0.10 g
MnCl ₂ ·4H ₂ O.....	0.03 g
H ₃ BO ₃	0.30 g
CoCl ₂ ·6H ₂ O.....	0.20 g
CuCl ₂ ·2H ₂ O.....	0.01 g
NiCl ₂ ·6H ₂ O.....	0.02 g
Na ₂ MoO ₄ ·2H ₂ O.....	0.03 g
Distilled water	1.00 L

65 HALF STRENGTH NUTRIENT AGAR

Medium 1 with all ingredients except agar at half concentration.

66 CORN STEEP STARCH NUTRIENT AGAR

Medium 65 plus 0.1% corn steep liquor and 1% soluble starch.

67 STARCH NUTRIENT AGAR

Medium 1 plus 1% starch.

68 YEAST NUTRIENT AGAR

Medium 1 plus 0.2% yeast extract.

69 DAVIS AND MINGIOLI MEDIUM A

K ₂ HPO ₄	7.0 g
KH ₂ PO ₄	3.0 g
(NH ₄) ₂ SO ₄	1.0 g
Trisodium citrate.....	0.5 g
MgSO ₄	48.0 mg
Distilled water.....	1.0 L

Autoclave at 121C/15 min, then add filter-sterilized solutions of the following to give the final concentrations indicated:-

Glucose.....	2.5 g/L
L-histidine.....	20.0 mcg/ml
L-leucine.....	40.0 mcg/ml
L-methionine.....	20.0 mcg/ml

70 CASEIN AGAR

Skim milk powder.....	10.0 g
Agar.....	4.5 g
Distilled water.....	1.0 L

Add agar to 200 ml water and heat to dissolve. Dissolve milk powder in 100 ml distilled water, add to agar and autoclave at 121C/15 min.

71 MODIFIED PALLERONI AND DOUDOROFF MINERAL BASE MEDIUM

Na ₂ HPO ₄ .12H ₂ O.....	6.00 g
KH ₂ PO ₄	2.40 g
NH ₄ Cl.....	1.00 g
MgSO ₄ .7H ₂ O.....	0.50 g
FeCl ₃ .6H ₂ O.....	0.01 g
CaCl ₂ .6H ₂ O.....	0.01 g
Agar.....	15.00 g
Distilled water.....	1.00 L

Adjust pH to 6.8. Autoclave at 121C/15 min.

For autotrophic ALCALIGENES SPP. incubate in an atmosphere of H₂/CO₂/Air (4:1:5).

72 ONE TENTH NUTRIENT AGAR

Nutrient Broth No.2 (Oxoid CM67).....	2.5 g
Agar.....	15.0 g
Distilled water.....	1.0 L

Autoclave at 121C/15 min.

73 SOIL EXTRACT NUTRIENT AGAR

Medium 1 made up with soil extract instead of water. To prepare soil extract, autoclave 1 Kg of soil in 1 L of tap water at 121C/30 min. Add 2 g CaCO₃, filter and make filtrate up to 1 L with tap water.

74 MALT YEAST AGAR

Yeast extract.....	3.0 g
Malt extract.....	3.0 g
Peptone.....	5.0 g
Glucose.....	10.0 g
Agar.....	20.0 g
Distilled water.....	1.0 L

Adjust pH to 7.0. Autoclave at 115C/20 min.

75 SOAP AGAR

Beef extract.....	0.30 g
Yeast extract.....	0.60 g
Peptone.....	1.50 g
NaCl.....	1.50 g
Stearic acid.....	30.00 g
Agar.....	0.33 g
NaOH(7M).....	15.00 ml
Distilled water.....	300.00 ml

Adjust pH to 8.5-9.5. Autoclave at 121C/15 min.

76 GLYCEROL ASPARAGINE MEAT AGAR

Medium 41 plus 1% beef extract.

77 BRAIN HEART INFUSION AGAR (LAB 48)

Brain heart infusion solids.....	17.5 g
Glucose.....	2.0 g
NaCl.....	5.0 g
Na ₂ HPO ₄	2.5 g
Agar No.2.....	12.0 g
Distilled water.....	1.0 L

Adjust pH to 7.4. Autoclave at 121C/15 min.

78 CASAMINO ACIDS AND YEAST EXTRACT AGAR

Casamino acids.....	7.5 g
Yeast extract.....	10.0 g
MgSO ₄ .7H ₂ O.....	20.0 g
Na ³ citrate.....	3.0 g
KCl.....	2.0 g
NaCl.....	200.0 g
FeSO ₄ .7H ₂ O (4.98% in 0.001M HCl).....	1.0 ml
Agar.....	15.0 g
Distilled water.....	1.0 L

Adjust pH to 7.4. Autoclave at 121C/10 min.

79 GLUCOSE BROTH BUFFERED

Oxoid CM1 broth powder.....	13.00 g
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Glucose	1.00 g
K ₂ HPO ₄	3.68 g
KH ₂ PO ₄	1.32 g
Distilled water	1.00 L

Adjust pH to 7.2. Autoclave at 121C/15 min

80 CZAPEK (SUCROSE NITRATE) AGAR

Sucrose.....	30.00 g
NaNO ₃	2.00 g
K ₂ HPO ₄	1.00 g
MgSO ₄ .7H ₂ O	0.50 g
KCl.....	0.50 g
FeSO ₄ .7H ₂ O	0.01 g
Agar	15.00 g
Distilled water	1.00 L

Adjust pH to 7.3. Autoclave at 121C/15 min.

81 MICROCYCLUS-SPIROSOMA AGAR

Glucose	1.0 g
Peptone.....	1.0 g
Yeast extract.....	1.0 g
Agar	15.0 g
Distilled water	1000 ml

Adjust pH to 6.8-7.2. Autoclave at 115C/10 min.

82 BACILLUS RACEMILACTICUS AGAR

Glucose	5.0 g
Peptone.....	5.0 g
Yeast extract.....	5.0 g
CaCO ₃	5.0 g
Agar	15.0 g
Distilled water	1.0 L

Adjust pH to 6.8. Autoclave at 121C/15 min.

83 COLBY AND ZATMAN MEDIUM

K ₂ HPO ₄	1.20 g
KH ₂ PO ₄	0.62 g
CaCl ₂ .6H ₂ O.....	0.05 g
MgSO ₄ .7H ₂ O	0.20 g
NaCl.....	0.10 g
FeCl ₃ .6H ₂ O	0.001 g
(NH ₄) ₂ SO ₄	0.50 g
CuSO ₄ .5H ₂ O.....	5.00 mcg
MnSO ₄ .5H ₂ O	10.00 mcg
Na ₂ MoO ₄ .2H ₂ O.....	10.00 mcg
H ₃ BO ₃	10.00 mcg
ZnSO ₄ .7H ₂ O	70.00 mcg
CoCl ₂ .6H ₂ O	5.00 mcg
Oxoid purified agar	20.00 g
Distilled water	1.00 L

Adjust pH to 7.0. Autoclave at 121°C /15 min. Cool to 50°C. Add a filter-sterilized solution of trimethylamine to give a final concentration of 0.1%.

84 ANTIBIOTIC MEDIUM NO.1 (Difco 0263)

85 MODIFIED THORNE MEDIUM

K ₂ HPO ₄	0.5 g
Ferric ammonium citrate	0.5 g
MgSO ₄ .7H ₂ O	1.0 g
Glycerol	20.0 g
Citric acid.....	2.0 g
L-glutamic acid	4.0 g
Distilled water	1.0 L

Adjust pH to 7.4 with NH₄OH (NOT NaOH). Autoclave at 121°C /15 min.

86 ALICYCLOBACILLUS ACIDOCALDARIUS MEDIUM

Solution (a)	
(NH ₄) ₂ SO ₄	1.30 g
KH ₂ PO ₄	0.37 g
MgSO ₄ .7H ₂ O	0.25 g
CaCl ₂ .6H ₂ O.....	0.10 g
FeCl ₃ .6H ₂ O	30.00 mg
Yeast extract.....	1.00 g
Distilled water	500.00 ml

Adjust pH to 3.5 with 0.5M H₂SO₄.

Solution (b)

Glucose.....	1.0 g
Agar.....	20.0 g
Distilled water	500.0 ml

Autoclave solutions (a) and (b) separately at 121°C /15 min, cool to 50°C, then combine.

89 BACILLUS THERMOGLUCOSIDASIUS MEDIUM

Distilled water	1000ml
Agar (Oxoid Purified).....	20.0g

Adjust pH to 7.0. Autoclave at 121C/15 min. Cool to 50C. Add a filter-sterilized solution of trimethylamine to give a final concentration of 0.1%. Make 10% sol., add 1ml per 100ml base.

Solution A (per litre):

CuSO ₄ .5H ₂ O.....	5mcg
MnSO ₄ .5H ₂ O.....	10mcg
NaMoO ₄ .2H ₂ O	10mcg
H ₃ BO ₃	10mcg
ZnSO ₄ .7H ₂ O.....	70mcg
CoCl ₂ .6H ₂ O.....	5mcg

Distilled water

Adjust pH to 8.0 and autoclave at 121C/15 min.

90 TRYPTONE SOYA BROTH (OXOID CM129; LAB4)

Tryptone	15.0 g
Soya peptone	5.0 g
NaCl	5.0 g
K ₂ HPO ₄	2.5 g
Distilled water	1.0 L

Adjust pH to 7.3 and autoclave at 121C/15 min. For solid medium add 15.0 g agar.

91 ENRICHED BLOOD AGAR

Medium 52	400 mls
Medium 77	400 mls
Horse blood	25 mls

Melt Medium 52 and Medium 77. Pour a thin layer of Medium 52 and leave to set. Then after the Medium 77 has cooled to 50C, the blood is aseptically added to it, both are well mixed and then poured as a layer on top of the Medium 52.

92 POTATO DEXTROSE AGAR

Potato infusion	200.0 g
Glucose	20.0 g
Agar	15.0 g
Distilled water	1.0 L

Adjust pH to 5.6 and autoclave at 121C/15 min.

93 NEOMYCIN AGAR No 2

Medium 52 plus 50mcg/ml neomycin.

94 YEAST MANNITOL MEDIUM

Mannitol	10.00 g
Yeast extract	0.25 g
MgSO ₄ .7H ₂ O	0.25 g
CaSO ₄ .2H ₂ O	30.00 mg
Na ₂ HPO ₄ .12H ₂ O	1.20 g
KH ₂ PO ₄	0.55 g
NaCl	0.25 g
FeSO ₄ .7H ₂ O	3.50 mg
ZnSO ₄ .7H ₂ O	160.00 mcg
CuSO ₄ .5H ₂ O	80.00 mcg
H ₃ BO ₃	500.00 mcg
MnSO ₄ .4H ₂ O	400.00 mcg
Agar	20.00 g
Distilled water	1.00 L

Adjust pH to 7.0 and autoclave at 121C/15 min.

95 CLOSTRIDIUM THERMOCELLUM MEDIUM

Cellobiose	2.00 g
Cellulose	10.00 g
Yeast extract	2.00 g
(NH ₄)SO ₄	1.30 g
KH ₂ PO ₄	1.50 g

K ₂ HPO ₄ .3H ₂ O	2.90 g
MgCl ₂ .6H ₂ O	1.00 g
CaCl ₂	0.15 g
Resazurin(0.2%)	1.00 ml
FeSO ₄ .7H ₂ O(5%)	25.00 ml
Distilled water	1.00 L

Adjust pH to 7.8 with NaOH and autoclave at 121C/15 min. Add 5ml/100 ml of the following reductants solution, freshly prepared and filter-sterilized:-

L-cysteine HCl	0.5 g
NaHCO ₃	5.0 g
Distilled water	50.0 ml

96 CLOSTRIDIUM KLUYVERI MEDIUM

Potassium acetate	1.0 g
K ₂ HPO ₄	31.0 mg
KH ₂ PO ₄	23.0 mg
NH ₄ Cl	25.0 mg
CaCl ₂ .2H ₂ O	1.0 mg
MgSO ₄ .7H ₂ O	20.0 mg
MnSO ₄ .H ₂ O	0.2 mg
FeSO ₄ .7H ₂ O	2.0 mg
Na ₂ MoO ₄ .2H ₂ O	0.2 mg
Biotin	10.0 mcg
p-Aminobenzoic acid	20.0 mcg
Resazurin	50.0 mcg
Agar	50.0 mg
Distilled water	100.0 ml

Adjust pH to 7.0. Boil 5 min, cool quickly to 50C, then add 50 mg sodium thioglycollate and 2 ml ethanol. Dispense into tubes containing little CaCO₃, autoclave at 121C/15 min and store anaerobically.

97 CLOSTRIDIUM THERMOHYDROSULFURICUM MEDIUM

Tryptone	10.0 g
Sucrose	10.0 g
Yeast extract	2.0 g
FeSO ₄ .7H ₂ O	0.2 g
Na ₂ SO ₃	0.2 g
Na ₂ S ₂ O ₃ .5H ₂ O	80.0 mg
Distilled water	1.0 L

Adjust pH to 6.8-7.8 and autoclave at 121C/15 min.

98 ENRICHED CYTOPHAGA AGAR

Tryptone	2.0 g
Beef extract	0.5 g
Yeast extract	0.5 g
Sodium acetate	0.2 g
Agar	15.0 g
Distilled water	1.0 L

Adjust pH to 7.2-7.4 and autoclave at 121C/15 min.

For soft agar for the maintenance of active cultures reduce agar content to 4.0 g/L. Dispense in 3 to 4 ml amounts in 7 ml screw-capped bottles (bijou bottles).

99 COOKED MEAT CARBOHYDRATE MEDIUM

Medium 12 with the supernatant replaced with the following:-

Peptone.....	30.0 g
Yeast extract.....	5.0 g
K ₂ HPO ₄	5.0 g
Glucose	4.0 g
Cellobiose.....	1.0 g
Maltose.....	1.0 g
Starch.....	1.0 g
Resazurin (0.1%).....	1.0 ml
Distilled water	1.0 L

Boil, cool, add 0.5 g cysteine, adjust pH to 7.0 and dispense. Autoclave at 121C/15 min. Use immediately or store anaerobically.

100 ACTINOMYCES BROTH (DIFCO 0840)

Heart infusion broth	25.00 g
Yeast extract.....	5.00 g
Casitone	4.00 g
Cysteine HCl.....	1.00 g
Glucose.....	5.00 g
Starch	1.00 g
KH ₂ PO ₄	15.00 g
(NH ₄) ₂ SO ₄	1.00 g
MgSO ₄ .7H ₂ O	0.20 g
CaCl ₂	0.02 g
Distilled water	1.00 L

Adjust pH to 7.2. Autoclave at 121C/15 min.

101 CASITONE YEAST EXTRACT AGAR

Casitone	5.0 g
Yeast extract.....	1.0 g
Agar	15.0 g
Distilled water	1.0 L

Adjust pH to 7.2. Autoclave at 121C/15 min.

102 CHONDROMYCES VYZ MEDIUM

Fresh baker's yeast cake	5.0 g
CaCl ₂	1.0 g
Agar	15.0 g
Distilled water	1.0 L

Adjust pH to 7.2 with KOH. Autoclave at 121C/15 min.

103 HALOPHILIC CHROMATIUM MEDIUM

Medium 10 plus 6% NaCl.

104 POSTGATE'S SALT MEDIUM

Medium 17 plus 2.5% NaCl.

105 ALKALINE PPYG MEDIUM

Peptone.....	5.00 g
Yeast extract.....	1.50 g
Glucose.....	5.00 g
Na ₂ HPO ₄ .12H ₂ O	1.50 g
NaCl	1.50 g
MgCl ₂ .6H ₂ O	0.10 g
Na ₂ CO ₃	5.03 g
Agar.....	15.00 g
Distilled water	1.00 L

Adjust pH to 10.5-11.0. Sterilize solutions of glucose and Na₂CO₃ separately and add to the rest of the medium after autoclaving at 121C/15 min.

106 ACTINOBOLIN MEDIUM

Medium 38 plus 1mg/ml actinobolin.

107 CYTOSINE NUTRIENT AGAR

Medium 1 plus 20mcg/ml cytosine.

108 AC BROTH (DIFCO 0317)

Difco proteose peptone no.3	20.0 g
Bacto beef extract.....	3.0 g
Bacto yeast extract	3.0 g
Difco malt extract.....	3.0 g
Glucose	5.0 g
Ascorbic acid.....	0.2 g
Distilled water	1.0 L

Adjust pH to 7.2. Autoclave at 121C/15 min

109 STREPTOMYCIN NUTRIENT AGAR

Medium 1 plus 50mcg/ml streptomycin.

110 M56 MEDIUM

Na ₂ HPO ₄	8.70 g
KH ₂ PO ₄	5.30 g
(NH ₄) ₂ SO ₄	2.00 g
MgSO ₄ .7H ₂ O.....	0.10 g
Ca(NO ₃) ₂	5.00 mg
ZnSO ₄ .7H ₂ O.....	5.00 mg
FeSO ₄ .7H ₂ O	5.00 mg
Glucose.....	4.00 g
L-leucine.....	0.05 g
L-histidine	0.05 g
Uracil.....	0.03 g
Agar.....	15.00 g
Distilled water	1.00 L

Adjust pH to 7.0 Autoclave at 121C/15 min.

111 L-BROTH DAP THYMIDINE

Tryptone	10.00 g
Yeast extract.....	5.00 g
NaCl	5.00 g

Diaminopimelic acid.....	0.10 g
Thymidine.....	0.01 g
Distilled water.....	1.00 L

Make up without DAP and thymidine and autoclave at 121C/15 min.
Add filter-sterilized solutions of DAP and thymidine to give the final concentrations shown.

112 L (LURIA) AGAR

Bacto tryptone.....	10.0 g
Yeast extract.....	5.0 g
NaCl.....	0.5 g
Glucose(10%).....	20.0 ml
Agar.....	15.0 g
Distilled water to.....	1.0 L

Autoclave..... 121C/15 min

Sterile glucose solution added aseptically after sterilization.

113 LB (LURIA-BERTANI) MEDIUM

Tryptone.....	10.0 g
Yeast extract.....	5.0 g
NaCl.....	10.0 g
Distilled water.....	1.0 L

Adjust pH to 7.0. Autoclave at 121C/15 min.

For solid medium add 15.0 g agar.

114 LBE MEDIUM

Medium 113 plus 4ml/L 50X medium E and 10ml/L 20% glucose.

Medium E(50X)

MgSO ₄ .7H ₂ O.....	10.0 g
Citric acid.H ₂ O.....	100.0 g
K ₂ HPO ₄	500.0 g
NaNH ₄ HPO ₄ .4H ₂ O.....	175.0 g
Distilled water.....	670.0 ml

Dissolve ingredients in the order shown.

115 YT MEDIUM

Tryptone.....	8.0 g
Yeast extract.....	5.0 g
NaCl.....	5.0 g
Distilled water.....	1.0 L
Autoclave.....	121C/15min

116 TSY MEDIUM

Trypticase Soy Broth (BBL 11768).....	30.0 g
Yeast extract.....	5.0 g
Agar.....	20.0 g
Distilled water.....	1.0 L
Autoclave.....	121C/15min

117 LB STREPTOMYCIN MEDIUM

Medium 113 plus 200 mcg/ml streptomycin.

118 CREATININE MEDIUM

K ₂ HPO ₄	2.0 g
Creatinine.....	5.0 g
Fumaric acid.....	2.0 g
Yeast extract.....	1.0 g
Salt solution (see below).....	10.0 ml

Adjust pH to 6.8 with NaOH. Autoclave at 121C/15 min.

Salt solution

MgSO ₄	12.20 g
CaCl ₂ .2H ₂ O.....	0.76 g
MnSO ₄ .H ₂ O.....	1.70 g
FeSO ₄ .7H ₂ O.....	2.80 g
ZnSO ₄ .7H ₂ O.....	0.06 g
NaCl.....	0.60 g
Na ₂ MoO ₄ .2H ₂ O.....	0.10 g
0.1M HCl.....	1.00 L

119 TRYPTONE SOYA AGAR (OXOID CM131)

Tryptone.....	15.0 g
Soya peptone.....	5.0 g
NaCl.....	5.0 g
Agar.....	15.0 g
Distilled water.....	1.0 L

Adjust pH to 7.3. Autoclave at 121C/15 min.

120 MMA SALTS MEDIUM

Medium 83 containing 0.1% monomethylamine instead of trimethylamine.

121 METHANOL SALTS MEDIUM

Medium 83 containing 0.1% methanol instead of trimethylamine.

122 LACTOBACILLUS 8664 MEDIUM

Yeast extract.....	10.0 g
Peptone.....	10.0 g
Maltose.....	20.0 g
Glucose.....	5.0 g
Distilled water.....	1.0 L
Autoclave.....	121C/15min

123 PANTOTHENATE-FREE MEDIUM

Half strength Difco pantothenate medium AOAC USP(0816) with the following additions per litre:-

Folic acid.....	30.0 mcg
Asparagine.....	0.1 g
D-pantethine.....	250.0 mcg
Casamino acids (vitamin free).....	15.0 g
10% K ₂ HPO ₄	0.2 ml

Adjust pH to 6.0 with NaOH. Autoclave at 121C/15 min.

124 LACTOBACILLUS OROTIC ACID MEDIUM

Medium 38 with the following additions per 100 ml.

Orotic acid..... 2.5 mg
D-pantothine 20.0 mcg

Distribute in screw-capped bottles in 20 ml amounts, heat to boiling, add 0.15 ml of 1.5% cysteine HCl per bottle, autoclave at 121C/15 min. Immediately after autoclaving screw caps down to maintain the reduced form pantotheine.

125 LACTOBACILLUS CHLORAMPHENICOL MEDIUM NO.2

Medium 20 plus 100 mg/L chloramphenicol.

When resuscitating NCIMB 11295 allow up to 4 days incubation at 37C to reach a suitable density.

126 MRS CYSTEINE MEDIUM

Medium 20 plus 0.5g/L cysteine HCl.

127 MRS FRUCTOSE MEDIUM

Peptone..... 10.0 g
Beef extract 10.0 g
Yeast extract..... 5.0 g
Fructose..... 10.0 g
Tween 80..... 1.0 ml
K₂HPO₄..... 2.0 g
Triammonium citrate..... 2.0 g
MgSO₄.7H₂O 0.2 g
MnSO₄.4H₂O 0.2 g
Distilled water 1.0 L

Adjust pH to 6.2-6.6. Autoclave at 121C/15 min.

128 ACIDIC TOMATO JUICE AGAR

Medium 47 at pH 4.8.

129 COOK'S CYTOPHAGA AGAR

Tryptone..... 2.0 g
Agar 10.0 g
Distilled water 1.0 L

Adjust pH to 7.3. Autoclave at 121C/15 min.

130 VCR MEDIUM

NaNO₃..... 2.00 g
NH₄Cl..... 0.50 g
KH₂PO₄..... 1.50 g
K₂HPO₄..... 1.20 g
MgSO₄.7H₂O 0.20 g
CaCl₂.2H₂O..... 15.00 mg
FeCl₃ 0.01 g

CuSO₄.5H₂O..... 1.00 mg
Vitamin B12..... 1.00 mcg
Purified agar 15.00 g
Distilled water 1.00 L
Adjust pH to 7.2. Autoclave all ingredients except vitamin B12 at 121C/15 min, then add vitamin B12 aseptically.

131 NITRATE MINERAL SALTS (NMS) MEDIUM

Solution 1. 10x NMS salts

Dissolve in approximately 700 mls of distilled water (in this order):

KNO₃..... 10.0 g
MgSO₄.6H₂O..... 10.0 g
CaCl₂ (anhydrous)..... 2.0 g

Dilute to 1 litre.

Solution 2. Iron EDTA

FeEDTA 3.8 g

Made up to 100 mls with distilled water.

Solution 3. Sodium molybdate

Na₂MoO₄.2H₂O..... 0.26 g

Made up to 1 litre with distilled water.

Solution 4. Trace elements

CuSO₄.5H₂O..... 1.000 g
FeSO₄.7H₂O 2.500 g
ZnSO₄.7H₂O..... 2.000 g
H₃BO₃..... 0.075 g
CoCl₂.6H₂O..... 0.250 g
EDTA di sodium salt 1.250 g
MnCl₂.4H₂O 0.100 g
NiCl₂.6H₂O..... 0.050 g

Dissolve the above in the specified order in distilled water and dilute to 5 litres. Store in the dark.

Solution 5. Phosphate buffer.

Na₂HPO₄.12H₂O 71.6 g
KH₂PO₄ 26.0 g

Dissolve the above in the specified order in 800 mls of distilled water.

Adjust pH to 6.8 and dilute to 1 litre.

Preparation of NMS Medium

1. Dilute 100 mls of solution 1 (10x salts) to 1 litre.
2. Add 1 ml of solution 3 (Na molybdate) and 1 ml of solution 4 (trace elements).
3. Add 0.1 ml of solution 2 (Fe EDTA).
4. Add 1.5% agar for plates.
5. Autoclave at 15 psi for 15 minutes.
6. Autoclave separately 10 mls of solution 5 (phosphate buffer) for every litre of NMS.
7. When the NMS is cool enough to hold in the hand, aseptically add the phosphate buffer. If this is done too early the phosphate will precipitate out.

132 STREPTOMYCIN NUTRIENT AGAR NO.2

Medium 1 plus 125 mcg/ml streptomycin sulphate.

133 STREPTOMYCIN NUTRIENT AGAR NO.3

Medium 1 plus 500 mcg/ml streptomycin sulphate.

134 ENRICHED NUTRIENT AGAR

Heart infusion (Difco) 12.5 g
 Nutrient broth (Difco) 5.4 g
 Yeast extract (Difco) 2.5 g
 Agar 15.0 g
 Distilled water 1.0 L

Adjust pH to 7.0. Autoclave at 121C/15 min.

135 GLYCEROL NUTRIENT AGAR

Medium 1 plus 1% glycerol.

136 MYCOBACTERIUM MEDIUM

(NH₄)₂SO₄ 1.0 g
 Na₂HPO₄ 0.5 g
 KH₂PO₄ 0.5 g
 MgSO₄ 0.2 g
 FeSO₄.7H₂O 5.0 mg
 MnSO₄ 2.0 mg
 Liquid paraffin 5.0 ml
 Distilled water 1.0 L

Homogenize, add 1.5% agar and autoclave at 121C/15 min.

137 TRYPTONE GLUCOSE EXTRACT AGAR

Beef extract 3.0 g
 Tryptone 5.0 g
 Glucose 1.0 g
 Agar 15.0 g
 Distilled water 1.0 L

Adjust pH to 7.0 and autoclave at 121C/15 min.

138 MYCOBACTERIUM YEAST EXTRACT MEDIUM

Yeast extract 2.5 g
 Tryptone 5.0 g
 Glucose 1.0 g
 Agar 15.0 g
 Distilled water 1.0 L

Adjust pH to 7.0 and autoclave at 121C/15 min.

139 L (LURIA) BROTH

Tryptone 10.0 g
 Yeast extract 5.0 g
 NaCl 5.0 g
 Glucose 1.0 g
 Distilled water 1.0 L

Autoclave at 121C/15min. For solid medium add 15.0 g agar.

140 TETRACYCLINE L BROTH MEDIUM

Medium 139 plus 12.5 mcg/ml tetracycline.

141 AMPICILLIN L BROTH MEDIUM

Medium 139 plus 50 mcg/ml ampicillin.

142 KANAMYCIN L BROTH MEDIUM

Medium 139 plus 50 mcg/ml kanamycin.

143 STREPTOMYCIN L BROTH MEDIUM

Medium 139 plus 25 mcg/ml streptomycin.

144 YGCB SALT MEDIUM

Peptone 10.0 g
 Beef broth 10.0 g
 Yeast extract 5.0 g
 Glucose 10.0 g
 Triammonium citrate 5.0 g
 Sodium acetate 2.0 g
 MgSO₄.7H₂O 0.2 g
 MnSO₄.4H₂O 50.0 mg
 NaCl 50.0 g
 Tween 80 1.0 ml

Adjust pH to 6.7 and autoclave at 121C/15 min.

145 PSEUDOMONAS MEDIUM

Solution (a)
 K₂HPO₄ 2.56 g
 KH₂PO₄ 2.08 g
 NH₄Cl 1.00 g
 MgSO₄.7H₂O 0.50 g
 Agar 20.00 g
 Distilled water 1.00 L

Adjust pH to 6.8 and autoclave at 121C/15 min.

Solution (b)
 Ferric ammonium citrate 1.0 g
 CaCl₂ 0.1 g
 Distilled water 100.0 ml

Sterilize by filtration.

Solution (c)
 1M Succinic acid.

Adjust pH to 6.0 with NaOH and autoclave at 121C/15 min.

To solution (a) add 5ml solution (b) and 15ml solution (c)

146 ACID NUTRIENT AGAR

Medium 1 adjusted to pH 5.0 with HCl.

147 THIOCYANATE AGAR

Solution (a)

KH ₂ PO ₄	1.0 g
K ₂ HPO ₄	1.0 g
MgSO ₄ .7H ₂ O.....	0.2 g
CaCl.....	20.0 mg
FeCl ₃ .6H ₂ O (60%).....	0.1 ml
Purified agar.....	30.0 g
Distilled water.....	800.0 ml

Solution (b)

KCNS.....	3.6 g
Distilled water.....	100.0 ml

Solution (c)

Disodium succinate.....	1.5 g
Distilled water.....	100.0 ml

Autoclave the three solutions separately at 121C/15 min, then mix.

148 SSM MEDIUM

Solution (a)

MgSO ₄ .7H ₂ O.....	0.2 g
Na ₂ HPO ₄	6.0 g
KH ₂ PO ₄	3.0 g
Purified agar.....	15.0 g
Distilled water.....	850.0 ml

Solution (b)

Glucose.....	5.0 g
Distilled water.....	50.0 ml

Solution (c)

Casein hydrolysate.....	20.0 g
Distilled water.....	100.0 ml

Filter solution (c) through Whatman no. 1 paper. Autoclave the three solutions separately at 121C/15 min, then mix.

149 PSEUDOMONAS MEDIUM No 2

Medium 71 plus 0.5 % succinic acid. pH to 6.8, agar 2%.

150 PHENYLOBACTERIUM MEDIUM

Antipyrin.....	1.00 g
KH ₂ PO ₄	0.30 g
Na ₂ HPO ₄ .12H ₂ O.....	0.70 g
NH ₄ H ₂ PO ₄	0.30 g
(NH ₄) ₂ HPO ₄	0.70 g
(NH ₄) ₂ SO ₄	0.10 g
CaCl ₂ .6H ₂ O.....	50.00 mg
MgSO ₄ .7H ₂ O.....	0.25 g
H ₃ BO ₃	0.50 mg
CuSO ₄ .5H ₂ O.....	0.10 mg

KI.....	0.10 mg
FeCl ₃ .6H ₂ O.....	0.20 mg
Mn.4H ₂ O.....	0.40 mg
ZnSO ₄ .7H ₂ O.....	0.40 mg
(NH ₄) ₂ MoO ₄	0.20 mg
Biotin.....	0.10 mg
Vitamin B12.....	30.00 mcg
Agar.....	15.00 g
Distilled water.....	1.00 L

Adjust pH to 6.8-7.0 and autoclave at 121C/15 min.

151 SORANGIUM MEDIUM

KNO ₃	1.0 g
K ₂ HPO ₄	1.0 g
MgSO ₄ .7H ₂ O.....	0.2 g
CaCl ₂ .2H ₂ O.....	0.1 g
FeCl ₃ .6H ₂ O.....	20.0 mg
Agar.....	10.0 g
Tap water.....	1.0 L
Autoclave.....	121C/15min

Add sterile strip of filter paper aseptically to slants or broth. For plates add 4-6 strips of sterile filter paper. Inoculate on to filter paper.

152 YMA MEDIUM

Mannitol.....	10.0 g
KH ₂ PO ₄	0.5 g
MgSO ₄ .7H ₂ O.....	0.2 g
NaCl.....	0.1 g
Yeast extract.....	0.4 g
CaCO ₃	4.0 g
Agar.....	15.0 g
Distilled water.....	1.0 L

Adjust pH to 6.8-7.0 and autoclave at 121C/15 min.

153 ACIDIC RHODOSPIRILLACEAE MEDIUM

Yeast extract.....	0.2 g
Disodium succinate.....	1.0 g
Ferric citrate (0.1%).....	5.0 ml
KH ₂ PO ₄	0.5 g
MgSO ₄ .7H ₂ O.....	0.4 g
NaCl.....	0.4 g
NH ₄ Cl.....	0.4 g
CaCl ₂ H ₂ O.....	50.0 mg
Trace element solution.....	1.0 ml
Distilled water.....	1.0 L

Adjust pH to 5.7 and autoclave at 121C/15 min.

Trace element solution:

See Medium 64.

154 YEAST EXTRACT WATER

Yeast extract.....	10.0 g
Distilled water.....	1.0 L

Autoclave.....121C/15min

155 ACIDIPHILUM MEDIUM

MgSO₄.7H₂O.....0.5 g
(NH₄)₂SO₄.....0.1 g
KH₂PO₄.....50.0 mg
KCl.....50.0 mg
Ca(NO₃)₂.....10.0 mg
Mannitol.....1.0 g
Tryptone soya broth0.1 g
Agar12.0 g
Distilled water1.0 L

Prepare medium at double strength without agar. Adjust pH to 3.5 and autoclave at 121C/15 min. Add to an equal volume of hot sterile, double strength agar solution.

156 RHODOSPIRILLUM MEDIUM

Medium 153 plus 0.8g/L yeast extract and 0.5ml/L ethanol. Adjust pH to 6.8.

157 NOVOBIOCIN AGAR

Medium 1 plus 10 mcg/ml novobiocin.

158 APPLE JUICE MEDIUM

Apple juice500.0 ml
Difco yeast extract.....5.0 g
Agar15.0 g

Adjust pH to 4.8 with acetic acid. Steam at 110C for 10 min to sterilize.

159 HISTIDANS MEDIUM

KH₂PO₄.....0.91 g
Na₂HPO₄.....0.95 g
Yeast extract.....10.00 g
Glucose10.00 g
MgSO₄.7H₂O0.50 g
Agar20.00 g
Distilled water1.00 L

Adjust pH to 7.0 and autoclave at 121C/15 min.

160 CYC MEDIUM

Czapek Dox liquid medium powder.....33.4 g
Yeast extract.....2.0 g
Casamino acids (vitamin free).....6.0 g
Agar16.0 g
Distilled water1.0 L

Adjust pH to 7.2 and autoclave at 121C/15 min.

161 TY SALTS MEDIUM

Tryptone1.0 g
Yeast extract.....1.0 g

Salts solution100.0 ml
Distilled water900.0 ml
Autoclave.....121C/15min

Salts solution:

Nitritotriacetic acid.....1.00 g
CaSO₄.2H₂O.....0.60 g
MgSO₄.7H₂O.....1.00 g
NaCl80.00 mg
KNO₃.....1.03 g
NaNO₃.....6.89 g
Na₂HPO₄1.11 g
FeCl₃ (0.028%).....10.00 ml
Trace elements solution10.00 ml
Distilled water1.00 L

Adjust pH to 8.2 with 1M NaOH.

Trace elements solution:

H₂SO₄.....0.5 ml
MnSO₄.H₂O.....2.2 g
ZnSO₄.7H₂O.....0.5 g
H₃BO₃.....0.5 g
CuSO₄.....16.0 mg
Na₂MoO₄.2H₂O.....25.0 mg
CoCl₂.6H₂O.....46.0 mg
Distilled water1.0 L

162 ACID GLUCOSE SALTS MEDIUM

(NH₄)₂SO₄0.15 g
KCl50.00 mg
MgSO₄.7H₂O.....0.50 g
KH₂PO₄0.10 g
Ca(NO₃)₂10.00 mg
Glucose5.00 g
Distilled water1.00 L

Adjust pH to 3.0 and autoclave at 121C/15 min.

163 THERMOACTINOPOLYSPORA MEDIUM

Phytone.....15.0 g
Maltose20.0 g
Yeast extract2.0 g
Agar.....15.0 g
Tap water.....1.0 L

Adjust pH to 7.2 and autoclave at 121C/15 min.

164 THERMOMONOSPORA MEDIUM

Medium 80 plus 0.2% yeast extract and 0.6% casamino acids.
Adjust pH to 8.0.

165 KYE MEDIUM

NaNO₃.....2.50 g
KH₂PO₄1.00 g
CaCl₂.6H₂O0.15 g
MgSO₄.7H₂O.....0.30 g
NaCl0.10 g

FeCl ₃	10.00 mg
Yeast extract.....	1.00 g
Agar	15.00 g
Distilled water	1.00 L

Adjust pH to 6.5 and autoclave at 121C/15 min.

166 DEEP LIVER BROTH

Liver infusion.....	1.0 g
Yeast extract.....	5.0 g
Tryptone.....	10.0 g
K ₂ HPO ₄	2.0 g
Glucose	5.0 g
Distilled water	1.0 L

Adjust pH to 7.4 and autoclave at 121C/15 min.

167 SUCROSE YEAST EXTRACT MEDIUM

Sucrose.....	20.0 g
Yeast extract.....	4.0 g
K ₂ HPO ₄	2.5 g
MgSO ₄ .7H ₂ O	1.0 g
Trace elements solution.....	4.0 ml

Trace elements solution:

MnSO ₄ .4H ₂ O	3.0 g
FeSO ₄ .7H ₂ O	9.0 g
ZnSO ₄ .7H ₂ O.....	18.0 g
CuSO ₄ .5H ₂ O	0.8 g
CoCl ₂ .6H ₂ O.....	0.9 g
Conc H ₂ SO ₄	5.0 ml
Distilled water	1.0 L

168 MODIFIED DAVIS AND MINGIOLI MEDIUM

KH ₂ PO ₄	3.0 g
K ₂ HPO ₄	7.0 g
(NH ₄) ₂ SO ₄	1.0 g
MgSO ₄ .7H ₂ O	0.1 g
Lactose	2.0 g
Agar	15.0 g

Medium is made up with 990 ml distilled water and 10 ml tap water. Dissolve MgSO₄ before adding to avoid precipitation. Adjust pH 7.0 and autoclave at 115C/20 min.

169 ALKALINE STARCH MEDIUM

Peptone.....	6.0 g
Yeast extract.....	3.0 g
K ₂ HPO ₄	1.0 g
MgSO ₄ .7H ₂ O	0.2 g
MnSO ₄	40.0 mg
Na ₂ CO ₃	10.0 g
Starch	20.0 g
Agar	16.0 g
Distilled water	1.0 L

Adjust pH to 9.7 and autoclave at 121C/15 min.

170 ALKALINE POLYPECTATE MEDIUM

Sodium polypectate	5.0 g
Peptone.....	6.0 g
Yeast extract.....	3.0 g
K ₂ HPO ₄	1.0 g
MgSO ₄ .7H ₂ O.....	0.2 g
MnSO ₄	40.0 mg
Na ₂ CO ₃	10.0 g
Agar.....	16.0 g

Adjust pH to 10.0 and autoclave at 121C/15 min.

171 IE MEDIUM

Phosphate solution:

K ₂ HPO ₄	95.0 g
NaH ₂ PO ₄ .2H ₂ O	78.0 g
Distilled water	1.0 L

Basal salts medium:

Phosphate solution.....	20.0 ml
(NH ₄) ₂ SO ₄ (36%).....	5.0 ml
MgSO ₄	0.5 g
Distilled water up to	1.0 L

Adjust pH to 6.8-7.0.

Medium made up as follows:

Yeast extract.....	1.0 g
Bacto peptone.....	5.0 g
Lactose	10.0 g
Agar.....	15.0 g
Basal salts.....	1.0 L
Autoclave.....	121C/15min

Add, aseptically, lactose and 1 ml trace elements (e.g. as in Medium 131) after sterilization.

172 TETRACYCLINE LURIA AGAR NO. 1

Medium 112 plus 12 mcg/ml tetracycline.

173 NEOMYCIN LURIA AGAR

Medium 112 plus 12 mcg/ml neomycin.

174 TY MEDIUM

Bacto tryptone	5.0 g
Yeast extract.....	3.0 g
CaCl ₂ .6H ₂ O	1.3 g
Agar.....	15.0 g
Distilled water	1.0 L
Autoclave.....	121C/15min

175 MILK AGAR

350 ml Medium 52 plus 150 ml water containing 10 g milk. Each sterilized separately at 121C/15 min before combining, aseptically.

176 SPIROPLASMA MEDIUM

PPLO broth (minus crystal violet) (Difco)	21.0 g
Yeast extract.....	5.0 g
Sorbitol.....	70.0 g
Fructose.....	1.0 g
Glucose	1.0 g
Phenol red	20.0 mg
Distilled water	800.0 ml
Autoclave.....	121C/15min

Supplement, when cooled, with 100 ml horse serum (previously heated at 60C for 30 min to sterilize). Incubate as static broth in conical flask at 32C until medium begins to turn orange.

Add 1% agar for solid medium.

177 N-ACETYL GLUCOSAMINE MEDIUM

Medium 20 with glucose replaced by an equivalent amount of N-acetyl glucosamine.

178 TETRACYCLINE LURIA AGAR NO. 2

Medium 112 plus 10 mcg/ml tetracycline.

179 KANAMYCIN LURIA AGAR

Medium 112 plus 10 mcg/ml kanamycin.

180 STREPTOMYCIN NUTRIENT AGAR NO. 4

Medium 1 plus 80 mcg/ml streptomycin.

181 MEDIUM FOR AMMONIA-OXIDISING BACTERIA

(NH ₄) ₂ SO ₄	235.0 mg
KH ₂ PO ₄	200.0 mg
CaCl ₂ .2H ₂ O	40.0 mg
MgSO ₄ .7H ₂ O	40.0 mg
*FeSO ₄ .7H ₂ O.....	0.5 mg
*NaEDTA	0.5 mg
*Phenol red	0.5 mg
Distilled water	1.0 L

*Prepared as separate stock solution as follows:

FeSO ₄ .7H ₂ O.....	50.0 mg
NaEDTA	50.0 mg
Distilled water	100.0 ml

Phenol Red	50.0mg
Distilled Water	100.0ml

Add 1 ml/1.0 L medium of the two stock solutions and autoclave at 121°C /15 min. After autoclaving add sterile 5% Na₂CO₃ until medium turns pale pink. Add further Na₂CO₃ during incubation to restore pink colouration. When no further colour change occurs growth is complete. Grow in dark.

182 MEDIUM FOR NITRITE-OXIDIZING BACTERIA

Medium 181 except that NaNO₂ (0.247g/l) replaces (NH₄)₂SO₄. Growth monitored by use of Griess-Ilosay's reagent (BDH) to determine removal of nitrite.

183 ASM MEDIUM

NH ₄ Cl.....	535.00 g
KH ₂ PO ₄	531.00 g
Na ₂ HPO ₄	866.00 g
K ₂ SO ₄	174.00 g
MgSO ₄ .7H ₂ O.....	37.00 mg
CaCl ₂ .2H ₂ O	7.35 mg
Trace elements.....	1.00 ml
Distilled water	1.00 L

Trace elements solution:

ZnSO ₄ .7H ₂ O.....	288.0 mg
MnSO ₄ .7H ₂ O.....	224.0 mg
H ₃ BO ₃	61.8 mg
CuSO ₄ .5H ₂ O.....	125.0 mg
Na ₂ MoO ₄ .2H ₂ O.....	48.4 mg
CoCl ₂ .6H ₂ O.....	47.6 mg
KI.....	83.0 mg
1M H ₂ SO ₄	1.0 ml

Autoclave at 121C/15 min and add 0.2 ml filter sterilized 0.1M FeSO₄ to 1.0 L of ASM.

For solid medium add 15 g agar and 15 mg vitamin B₁₂ to 1.0 L ASM before autoclaving and 0.2 ml filter sterilized 0.1M FeSO₄ after.

184 CAFFEINE MEDIUM

Solution (a):

NaCl	0.58 g
KH ₂ PO ₄	3.00 g
Na ₂ HPO ₄	7.80 g
Caffeine.....	1.00 g

Solution (b):

MgSO ₄ .7H ₂ O.....	0.12 g
CaCl ₂ .2H ₂ O	11.00 mg

Solution (c):

FeCl ₃	0.16 mg
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Dissolve each constituent of solution (a) separately in distilled water, adjust pH to 5.0, followed by the addition of solution (b). Volume is made up to almost 1.0 L. Solution (c) is added, pH adjusted to 5.0 and volume made up to 1.0 L. Add 1.5% agar and autoclave at 121C/15 min.

185 RIFAMPICIN LURIA AGAR

Medium 112 plus 30 mcg/ml rifampicin.

186 ERYTHROMYCIN L BROTH MEDIUM

Medium 139 plus 10 mcg/ml erythromycin.

187 CHLORAMPHENICOL L BROTH MEDIUM NO.1

Medium 139 plus 5 mcg/ml chloramphenicol.

188 CHLORAMPHENICOL L BROTH MEDIUM NO.2

Medium 139 plus 12.5 mcg/ml chloramphenicol.

189 TRYPTOSE BLOOD AGAR BASE

Medium 90 plus 7% sterile defibrinated horse blood added to cooled (50C) but not solidified medium.

190 TY SALT MEDIUM

Tryptone 10.0 g
Yeast extract 5.0 g
NaCl 10.0 g
Distilled water 1.0 L

Adjust pH to 7.0. Autoclave at 121C/15 min.

191 AMPICILLIN TY SALT MEDIUM

Medium 190 plus 50 mcg/ml ampicillin.

192 TETRACYCLINE TY SALT MEDIUM

Medium 190 plus 12.5 mcg/ml tetracycline.

193 LOWENSTEIN-JENSEN MEDIUM (OXOID PM1)**194 TETRACYCLINE LURIA AGAR NO. 3**

Medium 112 plus 20 mcg/ml tetracycline.

195 MODIFIED ROGOSA BROTH

Trypticase 10.0 g
Yeast extract 5.0 g
Tryptose 3.0 g
KH₂PO₄ 3.0 g
K₂HPO₄ 3.0 g
Ammonium citrate 2.0 g
Salt solution (see below) 5.0 ml
Tween 80 1.0 g
Sodium acetate 1.0 g
Glucose 20.0 g
Cysteine 0.2 g
Distilled water 1.0 L

Salt solution

MgSO₄.7H₂O 11.50 g
FeSO₄.7H₂O 1.68 g
MnSO₄ 2.40 g
Distilled water 100.00 ml

Adjust pH to 6.8 and autoclave at 121/15 min.

196 KANAMYCIN BRAIN HEART INFUSION AGAR

Medium 77 plus 25 mcg/ml kanamycin.

197 CHLORAMPHENICOL L BROTH MEDIUM NO.3

Medium 139 plus 50 mcg/ml chloramphenicol.

198 CHLORAMPHENICOL BRAIN HEART INFUSION AGAR

Medium 77 plus 50 mcg/ml chloramphenicol.

199 BACILLUS BENZOEVARANS MEDIUM

50:50 mixture of medium 71 and medium 98 with only 0.5 g tryptone/litre. A filter sterilized solution of 0.05% sodium benzoate was added to the medium after sterilization.

200 THYMINE NUTRIENT BROTH

Nutrient broth (Oxoid CM1) plus 40 mcg/ml thymine.

201 LAB 8 AGAR (LAB M)

Lab M Peptone 5.0 g
Lab M Beef extract 3.0 g
NaCl 8.0 g
Lab M agar 15.0 g

Adjust pH to 7.3. Autoclave at 121C/15 min.

202 BACILLUS THERMOLEOVORANS MEDIUM

(NH₄)₂HPO₄ 1.0 g
KCl 0.2 g
MgSO₄.7H₂O 0.2 g
Yeast extract 1.0 g
Distilled water 1.0 L

Add 0.1% (v/v) n-heptadecane. Autoclave at 121C/15 min.

203 BENNETT'S AGAR

Yeast extract 1.0 g
Beef extract 1.0 g
N-Z Amine A (or casitone) 2.0 g
Glucose 10.0 g
Agar 15.0 g
Distilled water 1.0 L

Adjust pH to 7.3 with NaOH and autoclave at 121C/15 min.

204 SPIRILLUM NITROGEN-FIXING MEDIUM

KH₂PO₄ 0.40 g
K₂HPO₄ 0.10 g
MgSO₄.7H₂O 0.20 g
NaCl 0.10 g
CaCl₂ 20.00 mg
FeCl₃ 10.00 mg
NaMoO₄.2H₂O 2.00 mg
Sodium malate 5.00 g
Yeast extract 0.05 g
Distilled water 1.00 L

Adjust pH to 7.2-7.4 and autoclave at 121C/15 min.

205 MAGNETIC SPIRILLUM GROWTH MEDIUM (MSGM)

Double glass distilled water.....	1.00 L
Wolfe's vitamin solution.....	10.00 ml
Wolfe's mineral solution.....	5.00 ml
0.01M Ferric quinate.....	2.00 ml
0.1% Resazurin.....	0.45 ml
KH ₂ PO ₄	0.68 g
NaNO ₃	0.12 g
Ascorbic acid.....	35.00 mg
Tartaric acid.....	0.37 g
Succinic acid.....	0.37 g
Sodium acetate.....	0.05 g
Agar (for semi-solid media).....	1.30 g

Add components in the order given with stirring. Adjust pH to 6.75 with NaOH.

Liquid medium:

Sterilize medium at 121C/15 min. Aseptically fill screw capped containers to full capacity with sterile medium. Inoculate heavily leaving no headspace of air, and screw down closures tightly.

Semi-solid medium:

Dispense into screw capped tubes/bottles and sterilize at 121C/15 min.

Wolfe's Vitamin Solution:

Biotin.....	2.0 mg
Folic acid.....	2.0 mg
Pyridoxine HCl.....	10.0 mg
Thiamine HCl.....	5.0 mg
Riboflavin.....	5.0 mg
Nicotinic acid.....	5.0 mg
Calcium pantothenate.....	5.0 mg
Cyanocobalamine.....	100.0 mcg
p-Aminobenzoic acid.....	5.0 mg
Thioctic acid.....	5.0 mg
Distilled water.....	1.0 L

Wolfe's Mineral Solution:

Nitritotriacetic acid.....	1.50 g
MgSO ₄ .7H ₂ O.....	3.00 g
MnSO ₄ .H ₂ O.....	0.50 g
NaCl.....	1.00 g
FeSO ₄ .7H ₂ O.....	0.10 g
CoCl ₂ .6H ₂ O.....	0.10 g
CaCl ₂	0.10 g
ZnSO ₄ .7H ₂ O.....	0.10 g
CuSO ₄ .5H ₂ O.....	0.01 g
AlK(SO ₄) ₂ .12H ₂ O.....	0.01 g
H ₃ BO ₃	0.01 g
Na ₂ MoO ₄ .2H ₂ O.....	0.01 g
Distilled water.....	1.00 L

Add nitritotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1 L with remaining water and add remaining compounds one at a time.

0.01M Ferric Quinate:

FeCl ₃	0.27 g
Quinic acid (Sigma).....	0.19 g
Distilled water.....	100.00 ml

Dissolve and autoclave at 121C/15 min.

206 THIOBACILLUS ACIDOPHILUS MEDIUM

Glucose.....	10.00 g
(NH ₄) ₂ SO ₄	3.00 g
KH ₂ PO ₄	0.50 g
MgSO ₄ .7H ₂ O.....	1.00 g
KCl.....	0.10 g
Ca(NO ₃) ₂ .4H ₂ O.....	18.00 mg
FeSO ₄ .7H ₂ O.....	0.01 mg
Agar.....	15.00 g
Distilled water.....	1.00 L

In liquid medium, the pH should be adjusted to 3.5 with H₂SO₄. In solid medium, the pH should be adjusted to 4.5 with H₂SO₄ after autoclaving the medium. Sterilize the glucose solution and the basal solution separately. Autoclave at 121C/15 min.

207 THERMUS BROCKII MEDIUM

Medium 161 adjusted to pH 7.6.

208 THERMOLEOPHILUM MEDIUM

NaNO ₂	2.00 g
MgSO ₄ .7H ₂ O.....	0.20 g
FeSO ₄ .7H ₂ O.....	1.00 mg
Na ₂ HPO ₄	0.21 g
NaH ₂ PO ₄	90.00 mg
CuSO ₄ .5H ₂ O.....	5.00 mcg
H ₃ BO ₃	10.00 mcg
MnSO ₄ .5H ₂ O.....	10.00 mcg
ZnSO ₄ .7H ₂ O.....	70.00 mcg
MoO ₃	10.00 mcg
KCl.....	0.04 g
CaCl ₂	15.00 mg
Distilled water.....	1.00 L

Adjust pH to 7.0. Autoclave at 121C/15 min. Add 1.0 ml n-heptadecane to 1.0 L of medium.

209 SEA WATER AGAR

Beef extract (Lab-Lemco).....	10.0 g
Neutralized bacteriological peptone.....	10.0 g
Filtered, aged sea water.....	750.0 ml
Distilled water.....	250.0 ml

Dissolve ingredients, heating if necessary. Adjust pH to 7.8, boil for 3-5 minutes, filter. Readjust pH to 7.3. Autoclave at 121C/15 mins.

For a solid medium add 15g/L agar after readjusting the pH and steam to dissolve the agar prior to autoclaving.

210 SEA WATER YEAST PEPTONE MEDIUM

Yeast extract.....	3.0 g
Peptone.....	5.0 g
Filtered, aged sea water	750.0 ml
Distilled water	250.0 ml

Adjust pH to 7.3. Prepare in similar manner to sea water agar.

211 BACTO MARINE MEDIUM 2216 (DIFCO)

Bacto peptone.....	5.00 g
Bacto yeast extract	1.00 g
Ferric citrate	0.10 g
Sodium chloride	19.45 g
Magnesium chloride dried.....	8.80 g
Sodium sulphate.....	3.24 g
Calcium chloride.....	1.80 g
Potassium chloride.....	0.55 g
Sodium bicarbonate	0.16 g
Potassium bromide.....	0.08 g
Strontium chloride.....	3.40 mg
Boric acid.....	2.20 mg
Sodium silicate.....	0.40 mg
Sodium fluoride.....	2.40 mcg
Ammonium nitrate	1.60 mcg
Disodium phosphate.....	0.80 mg
Distilled water	1.00 L

Adjust pH to 7.6. For solid medium add 15.0 g Bacto agar.

212 ANDERSON'S MARINE MEDIUM

Peptone.....	2.5 g
Yeast extract.....	2.5 g
FePO ₄	0.1 g
Filtered, aged sea water	750.0 ml
Distilled water	250.0 ml

Adjust pH to 7.4-7.6. For solid medium add 15.0 g agar.

213 JOHNSON'S MARINE MEDIUM

Bacto peptone.....	5.0 g
FeSO ₄ .7H ₂ O	0.2 g
Na ₂ S ₂ O ₃	0.3 g
Yeast extract (Difco).....	1.0 g
Filtered, aged sea water	1.0 L

For solid medium add 15.0 g agar.

Sodium thiosulphate (optional)..... 0.3g/L

214 GLYCEROL CaCO₃ AGAR - FOR LUMINOUS BACTERIA

Blood agar base (Medium 52).....	40.0 g
NaCl.....	25.0 g
CaCO ₃	5.0 g
Glycerol.....	10.0 g
Distilled water	1.0 L

Autoclave at 115C/20 min

215 PLAICE MEDIUM - FOR LUMINOUS BACTERIA

Fresh plaice, minced.....	200.0 g
Peptone.....	20.0 g

NaCl.....	120.0 g
Tap water.....	4.0 L

Soak plaice in water and allow to stand for 2 hours. Boil for 1 hour and filter. Add peptone and salt. Adjust pH to 7.3. Boil for a few minutes and filter.

For solid medium add 20.0 g agar per litre. Steam to dissolve and autoclave at 121C/15 min.

216 YEASTREL AGAR (388)

Lab-lemco (Oxoid)	5.0 g
Yeastrel*.....	7.0 g
Peptone (Difco)	9.5 g
NaCl	5.0 g
Agar.....	15.0 g
Distilled water	1.0 L

Adjust pH to 7.0.

*Yeastrel is produced by Mapleton's Foods Ltd, Moss Street, Liverpool and is available from health food shops.

217 MEDIUM FOR MARINE FLEXIBACTERIA

KNO ₃	0.5 g
Sodium glycerophosphate.....	0.1 g
Trace elements solution	1.0 ml
Tris buffer.....	1.0 g
Tryptone	5.0 g
Yeast extract	5.0 g
Filtered, aged sea water	1.0 L

Adjust pH to 7.0. For solid medium add 10.0 g agar.

Trace elements solution:

H ₃ BO ₃	2.85 g
MnCl ₂ .4H ₂ O	1.80 g
FeSO ₄	1.36 g
CuCl ₂ .2H ₂ O.....	26.90 mg
ZnCl ₂	20.80 mg
CoCl ₂ .6H ₂ O.....	40.40 mg
Na ₂ MoO ₄ .2H ₂ O.....	25.20 mg
Sodium tartrate	1.77 g
Distilled water	1.00 L

218 MEDIUM FOR FRESHWATER FLEXIBACTERIA

MgSO ₄ .7H ₂ O.....	0.1 g
KNO ₃	0.1 g
CaCl ₂ .2H ₂ O	0.1 g
Sodium glycerophosphate.....	0.1 g
Trace elements solution (see Medium 217)	1.0 ml
Tris buffer.....	1.0 g
Thiamine	1.0 mg
Cobalamin	1.0 mcg
Casamino acids (Difco).....	1.0 g
Distilled water	1.0 L

Adjust pH to 7.5. Add 1.0 g glucose aseptically after autoclaving.

For solid medium add 10.0 g agar.

219 PAYNE, SEGHAL & GIBBONS MEDIUM

Casamino acids (Difco).....	7.50 g
Yeast extract.....	10.00 g
Trisodium citrate.....	3.00 g
KCl.....	2.00 g
MgSO ₄ .7H ₂ O.....	20.00 g
FeCl ₂ .4H ₂ O.....	36.00 mg
MnCl ₂ .4H ₂ O.....	0.36 mg
NaCl.....	250.00 g
Distilled water.....	1.00 L

Adjust pH to 7.4. For solid medium add 20.0 g agar.

220 TRYPTONE YEAST EXTRACT SALT MEDIUM

Solution 1:

NaCl.....	125.0 g
MgCl ₂ .6H ₂ O.....	50.0 g
K ₂ SO ₄	5.0 g
CaCl ₂ .6H ₂ O.....	0.2 g
Distilled water.....	500.0 ml

Adjust pH to 6.8

Solution 2:

Tryptone (Oxoid).....	5.0 g
Yeast extract (Difco).....	5.0 g
Distilled water.....	500.0 ml

Sterilize the two solutions separately and mix after sterilization. After mixing, measure pH with a glass electrode and adjust pH to 6.8.

221 CHOLINE MEDIUM

K ₂ HPO ₄	1.00 g
MgSO ₄	0.50 g
FeSO ₄	0.01 g
NaCl.....	30.00 g
Choline chloride.....	5.00 g
Distilled water.....	1.00 L

Adjust pH to 7.4. For solid medium add 15.0 g agar.

222 SEA WATER BASAL MEDIUM

Tris-HCl (pH 7.5).....	50.0 mM
NH ₄ Cl.....	10.0 g
K ₂ HPO ₄ .3H ₂ O.....	75.0 mg
FeSO ₄ .7H ₂ O.....	29.0 mg
Lactate.....	2.0 g
Artificial sea water.....	500.0 ml
Distilled water.....	500.0 ml

Artificial sea water:

NaCl.....	23.37 g
MgSO ₄ .7H ₂ O.....	24.65 g
KCl.....	1.49 g

CaCl ₂ .2H ₂ O.....	2.94 g
Distilled water.....	1.00 L

For solid medium add 20.0 g agar or 10.0 g purified agar.

223 MARINE CYTOPHAGA MEDIUM A

Medium 98 prepared with 70% sea water/30% distilled water.

224 MARINE CYTOPHAGA MEDIUM B

Medium 98 prepared with 50% sea water/50% distilled water.

225 THIOSULPHATE SALTS BROTH

Na ₂ S ₂ O ₃ .5H ₂ O.....	24.81 g
NH ₄ Cl.....	2.20 g
KH ₂ PO ₄	2.00 g
Artificial sea water (Lyman and Fleming).....	500.00 ml
Deionized water.....	500.00 ml

Artificial sea water (Lyman and Fleming):

NaCl.....	23.476 g
MgCl ₂	4.981 g
Na ₂ SO ₄	3.917 g
CaCl ₂	1.102 g
KCl.....	0.664 g
NaHCO ₃	0.192 g
KBr.....	0.096 g
H ₃ BO ₃	0.026 g
Cl ₂ Sr.....	0.024 g
NaF.....	0.003 g
Water to.....	1.000 L

Adjust pH to 5.0.

226 RENIBACTERIUM KDM-2 MEDIUM

Peptone.....	10.0 g
Yeast extract.....	0.5 g
Cysteine HCl.....	1.0 g
Agar.....	15.0 g
Foetal calf serum.....	200.0 ml
Distilled water.....	1.0 L

Dissolve peptone, yeast extract and cysteine HCl in 800 ml final volume distilled water. Adjust pH to 6.5 with NaOH. Add agar and dissolve by heating. Autoclave at 121C/15 min. Cool to 45C and add serum.

227 VON HOFSTEN & MALMQVIST MEDIUM B

NaNO ₃	2.00 g
K ₂ HPO ₄	0.50 g
MgSO ₄ .7H ₂ O.....	0.20 g
CaCl ₂ .H ₂ O.....	0.02 g
MnSO ₄ .H ₂ O.....	0.02 g
FeSO ₄ .7H ₂ O.....	0.02 g
Carbon source.....	2.00 g
Distilled water.....	1.00 L

Adjust pH to 7.5. For solid medium substitute 15.0 g agar for the carbon source.

228 MARINE METHYLOTROPH MEDIUM

KH ₂ PO ₄	0.14 g
Bis-Tris	2.00 g
Ferric ammonium citrate.....	0.06 g
Sea water.....	1.00 L

Adjust pH to 7.4 and autoclave at 121C/15 min. For solid medium add 12.0 g agarose. After cooling to 45C, add 2.0 ml sterile methanol and a filter sterilized solution of vitamin B12 to give a final concentration of 1.0 mcg/L.

229 SEAWATER SPIRILLUM MEDIUM

Medium 28 made up with 750 ml aged sea water and 250 ml distilled water. Adjust pH to 7.0 and autoclave at 115C/20 min.

230 PPES-II MEDIUM

Polypeptone.....	2.0 g
Proteose-peptone No.3	1.0 g
Bacto-soytone.....	1.0 g
Bacto yeast extract	1.0 g
Ferric phosphate, soluble.....	0.1 g
Marine mud extract	100.0 ml
Agar	15.0 g
Aged sea water	900.0 ml

Adjust pH to 7.6-7.8.

231 HALOPHILE MEDIUM

NaCl.....	156.0 g
MgCl ₂ .6H ₂ O.....	13.0 g
MgSO ₄ .7H ₂ O	20.0 g
CaCl ₂ .6H ₂ O.....	1.0 g
KCl.....	4.0 g
NaHCO ₃	0.2 g
NaBr.....	0.5 g
Yeast extract.....	10.0 g
Agar (Difco).....	20.0 g

Adjust pH to 7.0 with 1M KOH and sterilize by autoclaving at 121C/15 min.

232 COLWELLIA PSYCHROERYTHRUS MEDIUM

NaCl.....	29.0 g
MgCl ₂ .6H ₂ O.....	8.0 g
KH ₂ PO ₄	5.4 g
FeCl ₂ .4H ₂ O	2.0 mg
CaCl ₂ .6H ₂ O.....	33.0 mg
Tryptone	8.0 g
Distilled water	1.0 L

Adjust pH to 7.0. For solid medium add 15.0 g agar.

233 MEDIUM FOR HAEMOPHILUS PISCIIUM

Medium 119 + 1% NaCl. Add 1 ml of a 2 mg per cent filter sterilized solution of cocarboxylase per 10 ml of medium.

234 MARINE RHODOPSEUDOMONAS MEDIUM

Bacto yeast extract.....	2.5 g
Bacto peptone.....	2.5 g
NaCl	30.0 g
Distilled water	1.0 L

Adjust pH to 7.0-7.4. For solid medium add 15.0 g Bacto agar. Distribute in 15 ml amounts in 1 oz screw-capped bottles and autoclave at 121C/15 min.

Before resuspending freeze-dried culture in the liquid medium re-heat to drive out O₂ and screw down the cap tightly. Incubate at 30C in an internally illuminated incubator for several days.

235 HALOBACTERIUM SODOMENSE MEDIUM

NaCl	125.00 g
MgCl ₂ .6H ₂ O	160.00 g
CaCl ₂ .2H ₂ O.....	0.13 g
K ₂ SO ₄	5.00 g
Peptone (Difco)	1.00 g
Yeast extract (Difco)	1.00 g
Soluble starch (BDH)	2.00 g
Distilled water	1.00 L

Adjust pH to 7.0 with NaOH before autoclaving.

236 ALKALOPHILIC HALOPHILE MEDIUM

Yeast extract.....	10.00 g
Casamino acids.....	7.50 g
Trisodium citrate	3.00 g
KCl.....	2.00 g
MgSO ₄ .7H ₂ O.....	1.00 g
MnCl ₂ .4H ₂ O	0.36 mg
FeSO ₄ .7H ₂ O	50.00 mg
NaCl	200.00 g
Na ₂ CO ₃ .10H ₂ O.....	50.00 g
Agar.....	20.0g

Add all ingredients, except Na₂CO₃.10H₂O, to 750ml of distilled water, adjust pH to 6.5.

Add Na₂CO₃.10H₂O to 250mls and autoclave to two solutions separately. After autoclaving, combine to solutions, check the pH at 9.5-10.5.

237 GLUCOSE SALTS MEDIUM

(NH ₄) ₂ SO ₄	1.0 g
NaCl	0.5 g
MgSO ₄ .7H ₂ O.....	0.5 g
Na ₂ HPO ₄ .12H ₂ O	0.7 g
NaH ₂ PO ₄ .2H ₂ O	0.3 g
Glucose.....	5.0 g
Trace element solution.....	0.5 ml
Glass distilled water.....	1.0 L

Adjust pH to 6.9. For solid medium add 15.0 g agar. For soft agar add 3.0 g agar.

Trace element solution:

H ₃ BO ₃	2.85g
MnCl ₂ ·4H ₂ O.....	1.8g
FeSO ₄	1.36g
CuCl ₂ ·2H ₂ O.....	26.9mg
ZnCl ₂	20.8mg
CoCl ₂ ·6H ₂ O.....	40.4mg
Na ₂ MoO ₄ ·2H ₂ O.....	25.2mg
Sodium tartrate.....	1.77g
Distilled water	1L

238 GLYCEROL AGAR

Peptone.....	5.0 g
Beef extract	3.0 g
Glycerol.....	70.0 ml
Agar	15.0 g
Soil extract	250.0 ml
Tap water	750.0 ml

Adjust pH to 7.0.

Alternatively a nutrient agar (Medium 52) plus 7% (w/v) glycerol may be used.

239 DORSET EGG MEDIUM

Fresh egg mixture (yolks and whites).....	750.00 ml
Lab-Lemco powder	2.50 g
Peptone.....	2.50 g
Sodium chloride	1.25 g
Distilled water to	1.00 L

240 "ALCALIGENES TOLERANS" AGAR

Medium 1 plus 0.3% ammonium lactate (60% syrup). Autoclave at 115C/20 min. Add 0.02% ferric citrate (sterile solution). Final pH 7.0.

241 QUARTER STRENGTH NUTRIENT MEDIUM

Nutrient broth (Oxoid CM1) made to ¼ regular strength. For solid medium add 1.5% agar to ¼ strength nutrient broth.

242 TGYM MEDIUM

Tryptone.....	5.0 g
Glucose	1.0 g
Yeast extract.....	3.0 g
DL-methionine	0.5 g
Tap water	1.0 L

Adjust pH to 7.3-7.5.

243 BLOOD AGAR BASE WITH 2.5% NaCl

Medium 52 plus 2.5% NaCl.

244 BLOOD AGAR BASE WITH 3.5% NaCl

Medium 52 plus 3.5% NaCl.

245 HALOBIUS MEDIUM

Vitamin-free casamino acid.....	7.5 g
Yeast extract.....	10.0 g
Sodium citrate	3.0 g
KCl.....	2.0 g
MgSO ₄ ·7H ₂ O.....	20.0 g
NaCl	116.0 g
FeCl ₂	23.0 mg
Agar.....	20.0 g
Distilled water	1.0 L

Adjust pH to 6.2. Autoclave at 121C/15 min.

246 MARINE CYTOPHAGA MEDIUM C

Medium 98 prepared with 100% sea water.

247 LEUCOTHRIX MUCOR MEDIUM

NaCl	11.75 g
MgCl ₂ ·6H ₂ O	5.35 g
Na ₂ SO ₄	2.00 g
CaCl ₂ ·6H ₂ O	1.12 g
KCl.....	0.35 g
Tris buffer.....	0.50 g
Na ² HPO ₄	0.05 g
Monosodium glutamate	10.00 g
Sodium lactate	2.00 g
Deionized water	1.00 L

Adjust pH to 7.6. Autoclave at 121C/15 min.

248 MICROMONOSPORA HALOPHYTICA MEDIUM

Glucose.....	10.0 g
Soluble starch	20.0 g
Yeast extract.....	5.0 g
N-Z Amine Type A (or casein hydrolysate).....	5.0 g
CaCO ₃ (reagent grade)	1.0 g
Agar.....	15.0 g
Distilled water	1.0 L

249 BRACKISH PROSTHECOMICROBIUM MEDIUM

Peptone.....	0.25 g
Yeast extract.....	0.25 g
Glucose.....	0.25 g
Modified Hutner's basal salts.....	20.00 ml
Vitamin solution.....	10.00 ml
Agar.....	15.00 g
Distilled water	500.00 ml
Sea water	500.00 ml

Vitamin solution:

Biotin.....	2.0 mg
Folic acid	2.0 mg
Thiamine HCl.....	5.0 mg

D Calcium pantothenate	5.0 mg
Vitamin B12.....	0.1 mg
Riboflavin	5.0 mg
Distilled water	1.0 L

Modified Hutner's Basal Salts:

Nitritotriacetic acid	10.00 g
MgSO ₄ .7H ₂ O	29.70 g
CaCl ₂ .2H ₂ O	3.34 g
Ammonium molybdate.....	9.25 mg
FeSO ₄ .7H ₂ O.....	99.00 mg
Metals "44"	50.00 ml
Distilled water to	1.00 L

Dissolve the nitritotriacetic acid in 700 ml of water and neutralize the solution with KOH. Then add the other ingredients and make up to 1L. Finally adjust the pH to 7.2 with KOH and H₂SO₄. There may be a slight precipitate. Store at 5°C.

Metals "44"

EDTA.....	2.5g
ZnSO ₄ .7H ₂ O	10.95g
FeSO ₄ .7H ₂ O.....	5.0g
MnSO ₄ .H ₂ O	1.54g
CuSO ₄ .5H ₂ O.....	0.392g
Co(NO ₃) ₂ .6H ₂ O.....	0.248g
Na ₂ B ₄ O ₇ .10H ₂ O.....	0.177g
Distilled water	1L

Add one ingredient at a time to approximately 800ml of water and allow each to dissolve individually. A few drops of 1M H₂SO₄ can be added to retard precipitation. Then make up to 1 litre in a volumetric flask.

250 RHODOSPIRILLUM SALINARUM MEDIUM

NaCl.....	100 g
KCl.....	5 g
MgCl ₂ .6H ₂ O.....	5 g
NH ₄ Cl.....	5 g
MgSO ₄ .7H ₂ O	5 g
Trace elements solution.....	5 ml
1% Ferric citrate solution	10 ml
Yeast extract solution (150 g/l)	30 ml
Peptone (150 g/l).....	30 ml
Distilled water	925 ml

Trace elements solution:

CuSO ₄ .5H ₂ O	1.0 mg
ZnSO ₄ .7H ₂ O.....	220.0 mg
CoCl ₂ .6H ₂ O.....	10.0 mg
MgCl ₂ .4H ₂ O.....	180.0 mg
Na ₂ MoO ₄ .H ₂ O.....	6.3 mg
Distilled water	1.0 L

251 SEA WATER AGAR WITH HORSE BLOOD

Medium 209 plus 10% horse blood

252 SEA WATER AGAR WITH FOETAL CALF SERUM

Medium 209 plus 10% foetal calf serum

253 MODIFIED CM+YE MEDIUM

Bacto vitamin assay casamino acids (Difco)	7.5 g
Bacto yeast extract (Difco)	10.0 g
MgSO ₄ .7H ₂ O.....	20.0 g
Trisodium citrate.2H ₂ O	3.0 g
KCl.....	2.0 g
NaCl	150.0 g
Fe ²⁺ solution	1.0 ml
Agar.....	15.0 g
Distilled water	1.0 L

Adjust pH to 7.4.

Fe²⁺ solution:

FeSO ₄ .7H ₂ O	4.98 g
Distilled water	100.00 ml

254 DUBOS SALTS MEDIUM PLUS 1% NaCl

Medium 16 plus 1% NaCl.

255 IONIC MEDIUM WITH PIPECOLATE

KH ₂ PO ₄	2.26 g
K ₂ HPO ₄	4.10 g
NaH ₂ PO ₄	2.24 g
Na ₂ HPO ₄	3.34 g
Salt solution.....	10.00 ml
Distilled water	1.00 L

To 200 ml of hot ionic medium add 6.0 g agar. Boil to dissolve and add 10 ml of neutralized 0.25M pipercolic acid HCl (Sigma Chemical Co.). Autoclave at 121C/15 min.

Salt solution:

MgSO ₄ .7H ₂ O.....	14.80 g
FeSO ₄ .7H ₂ O	0.55 g
MnSO ₄	45.00 mg
Distilled water	1.00 L
Concentrated H ₂ SO ₄	2.00 drops

256 DAP NUTRIENT AGAR

Medium 1 plus 100 mg/ml synthetic diaminopimelic acid (a mixture of LL-, DD- and meso isomers).

257 ACTINOMYCES HUMIFERUS MEDIUM

Medium 90 plus 5% horse blood.

258 VANILLATE MEDIUM

KH ₂ PO ₄	0.40 g
(NH ₄) ₂ SO ₄	1.00 g
MgSO ₄ .7H ₂ O.....	0.01 g
Yeast extract.....	0.10 g
Agar.....	20.00 g

Trace element solution 10.00 ml
 Distilled water 1.00 L

Trace elements solution

H₃BO₃ 0.50 mg
 CuSO₄·5H₂O 0.04 mg
 KI 0.10 mg
 FeCl₃ 0.20 mg
 MnSO₄·4H₂O 0.40 mg
 (NH₄)₆Mo₇O₂₄·4H₂O 0.20 mg
 ZnSO₄·7H₂O 0.40 mg
 Distilled water 1.00 L

Autoclave mineral salts-yeast extract medium at 121C/15 min.

A solution of vanillic acid as sodium salt (to give final concentration of 1.5 g/L) is prepared separately and filter sterilized. Add aseptically to autoclaved mineral salts-yeast extract medium.

259 R2A MEDIUM

Yeast extract 0.50 g
 Proteose peptone 0.50 g
 Casamino acids 0.50 g
 Glucose 0.50 g
 Soluble starch 0.50 g
 Sodium pyruvate 0.30 g
 K₂HPO₄ 0.30 g
 MgSO₄·7H₂O 0.05 g
 Noble agar 15.00 g
 Distilled water 1.00 L

Adjust pH to 7.2 with KH₂PO₄ or K₂HPO₄. Autoclave at 121C/15 min.

260 SUCROSE PEPTONE AGAR

Sucrose 20.00 g
 Difco peptone 5.00 g
 MgSO₄·7H₂O 0.25 g
 K₂HPO₄ 0.50 g
 Agar 15.00 g
 Distilled water 1.00 L

Dissolve ingredients and adjust pH to 7.0-7.2. Autoclave at 121C/15 min.

261 p-HYDROXYBENZOATE MEDIUM

NaCl 0.5 g
 p-hydroxybenzoic acid 3.0 g
 (NH₄)₂HPO₄ 3.0 g
 K₂HPO₄ 1.2 g
 FeSO₄·7H₂O 0.1 g
 MgSO₄·7H₂O 0.2 g
 Agar 20.0 g
 Distilled water 1.0 L

Adjust pH to 7.0 and autoclave at 121C/15 min.

262 CLOSTRIDIUM CELLOBIOPARUM MEDIUM

Ground beef(fat free) 500.0 g
 Distilled water 1.0 L
 1N NaOH 25.0 ml

Use lean beef or horse meat. Remove fat and connective tissue before grinding. Mix meat, water and NaOH, then boil for 15 min stirring. Cool to room temperature, skim fat off surface and filter retaining both meat particles and filtrate. To the filtrate add water to a final volume of 1 L and then add:

Casitone 30.0 g
 Yeast extract 5.0 g
 K₂HPO₄ 5.0 g
 Resazurin 1.0 mg
 Glucose 4.0 g
 Cellobiose 1.0 g
 Maltose 1.0 g
 Soluble starch 1.0 g

Boil, cool, add 0.5 g cysteine and adjust pH to 7.0. Dispense 7 ml into tubes containing meat particles (use 1 part meat particles to 4 or 5 parts fluid). Autoclave at 121C/30 min. For agar slants use 15 g agar per 1 L medium.

263 CLOSTRIDIUM LENTOCELLUM MEDIUM

NH₄SO₄ 1.60 g
 Yeast extract 1.00 g
 Agar 30.00 g
 K₂HPO₄ 1.65 g
 Cysteine hydrochloride 0.50 g
 NaCl 0.96 g
 MgSO₄ 96.00 mg
 CaCl₂ 96.00 mg
 Resazurin solution(0.1% wt/vol) 1.00 ml
 Cellulose suspension 200.00 ml
 Distilled water 1.00 L

Adjust pH to 7.2 with 5M NaOH. For broth medium exclude agar.

Cellulose suspension

4% (wt/vol) Whatman CF cellulose powder.

If cellulose suspension is not available cellulose can be provided by a strip (4.5 x 1 cm) of Whatman No. 1 filter paper in each tube.

Autoclave 121C/15min

264 REINFORCED CLOSTRIDIAL MEDIUM (OXOID CM149)

Yeast extract 3.0 g
 'Lab-Lemco' Powder 10.0 g
 Peptone 10.0 g
 Soluble starch 1.0 g
 Dextrose 5.0 g
 Cysteine hydrochloride 0.5 g
 Sodium chloride 5.0 g
 Sodium acetate 3.0 g
 Agar 0.5 g
 Distilled water 1.0 L

Adjust pH to 6.8. Bring to the boil to dissolve completely. Autoclave at 121C/15 min.

265 CASAMINO ACIDS MEDIUM

Casamino acids (Difco 0230).....	1.00 g
Glucose	1.00 g
Modified Hutner's basal salts (See Medium 249).....	20.00 ml
Biotin	0.02.mg
Distilled water	1.00 L

266 RHAMNOSE SALTS MEDIUM

K ₂ HPO ₄	2.9 g
KH ₂ PO ₄	2.1 g
NH ₄ Cl.....	2.0 g
MgSO ₄ .7H ₂ O	0.4 g
NaCl.....	30.0 mg
CaCl ₂	3.0 mg
FeSO ₄ .7H ₂ O.....	1.0 mg
Yeast extract.....	3.0 g
Rhamnose.....	10.0 g
Distilled water	1.0 L

Adjust pH to 7.0. Autoclave at 121C/15 min.

267 TRYPTONE BILE AGAR (OXOID CM595)

Tryptone	20.0 g
Bile salts No.3	1.5 g
Agar No.1	15.0 g
Distilled water	1.0 L

Suspend ingredients in distilled water, adjust pH to 7.2 and bring gently to the boil to dissolve completely. Autoclave at 121C/15 min.

268 THERMODESULFOBACTERIUM MEDIUM

Na ₂ SO ₄	3.0 g
NH ₄ Cl.....	1.0 g
MgCl ₂ .6H ₂ O.....	0.2 g
KH ₂ PO ₄	0.3 g
Na ₂ HPO ₄ .12H ₂ O.....	2.0 g
Trace mineral solution.....	10.0 ml
FeSO ₄ .7H ₂ O.....	1.5 mg
Vitamin solution.....	5.0 ml
Resazurin	1.0 mg
Yeast extract.....	1.0 g
Sodium lactate.....	4.0 g
Na ₂ S.9H ₂ O.....	0.5 g
Distilled water	1.0 L

Trace mineral solution:

Nitritotriacetic acid	12.800 g
FeCl ₃ .4H ₂ O	0.200 g
MnCl ₂ .4H ₂ O.....	0.100 g
CoCl ₂ .6H ₂ O.....	0.170 g
CaCl ₂ .2H ₂ O.....	0.100 g
ZnCl ₂	0.100 g
CuCl ₂	0.020 g
H ₃ BO ₃	0.010 g
Na ₂ MoO ₄ .2H ₂ O.....	0.010 g
NiCl ₂ .6H ₂ O	0.026 g
NaCl.....	1.000 g
Na ₂ SeO ₃	0.020 g
Distilled water	1.000 L

Firstly adjust nitritotriacetic acid to pH 6.5 with KOH.

Vitamin solution:

Biotin.....	2.0 mg
Folic acid.....	2.0 mg
Pyridoxine-HCl.....	10.0 mg
Thiamine-HCl.....	5.0 mg
Riboflavin.....	5.0 mg
Nicotinic acid.....	5.0 mg
DL-Calcium pantothenate	5.0 mg
Vitamin B12.....	0.1 mg
p-Aminobenzoic acid	5.0 mg
Lipoic acid.....	5.0 mg
Distilled water	1.0 L

Adjust pH to 6.8-7.0. Prepare the medium anaerobically under 100% nitrogen. Prepare concentrated solutions each of yeast extract, sodium lactate and sodium sulphide anaerobically under nitrogen and autoclave separately. Before use, neutralize the sodium sulphide solution by dropwise addition of 1N HCl.

269 DOUBLE STRENGTH CRUDE LACTOBACILLUS MEDIUM

Yeast extract (Basamine).....	20.0 g
Sucrose	20.0 g
Casein hydrolysate.....	15.0 g
Histidine HCl.H ₂ O	2.0 g
Potassium acetate	3.0 g
Ascorbic acid.....	1.0 g
Pyridoxamine HCl.....	33.0 mcg
Salts A	20.0 ml
Salts B	5.0 ml
Distilled water	1.0 L

Adjust pH to 5.4 with acetic acid.

This medium is used double strength for cultivation.

Salts A:

KH ₂ PO ₄ .H ₂ O	16.5 g
K ₂ HPO ₄ .3H ₂ O	16.5 g
Distilled water	1.0 L

Salts B:

MgSO ₄ .7H ₂ O.....	8.0 g
NaCl	0.4 g
FeSO ₄ .7H ₂ O	0.4 g
MnSO ₄ .H ₂ O.....	0.4 g
HCl (concentrated solution).....	0.1 ml
Distilled water	1.0 L

270 VITAMIN B12 MEDIUM (DIFCO 0457-15-1) PLUS COLISTIN

Add 500 mg/litre of colistin sulphate (Pharmax) and 250 nanograms/litre cyanocobalamin (Glaxo) to the prepared medium..

271 BRIGG'S LIVER TOMATO BROTH

Tomato juice	400.0 ml
Neopeptone	15.0 g
Yeast extract.....	6.0 g
Liver extract	75.0 ml
Glucose	20.0 g
Soluble starch.....	0.5 g
NaCl.....	5.0 g
Cysteine HCl.....	0.2 g
Tween 80.....	1.0 g
Distilled water to	1.0 L

Adjust pH to 5.0.

3.0 g of proteolysed liver (Oxoid L25) may be used instead of the liver extract.

272 BLASTOBACTER DENITRIFICANS MEDIUM

Medium 98 plus 0.025% separately sterilized glucose.

273 GPY-SALTS MEDIUM

Glucose	1.0 g
Peptone.....	0.5 g
Yeast extract.....	0.1 g
Modified Hutner's basal salts (see Medium 249)	20.0 ml
Distilled water	1.0 L

274 PROSTHECOBACTER MEDIUM

(NH ₄) ₂ SO ₄	0.25 g
Glucose	0.25 g
Modified Hutner's basal salts (see Medium 249)	20.00 ml
Na ₂ HPO ₄	71.00 mg
Distilled water to	1.00 L

275 PROSTHECOMICROBIUM AND ANCALOMICROBIUM MEDIUM

Ammonium sulphate	0.25 g
Glucose	0.25 g
Vitamin solution (see Medium 249).....	10.00 ml
Modified Hutner's basal salts (see Medium 249)	20.00 ml
Na ₂ HPO ₄	71.00 mg
Distilled water to	1.00 L

276 ANCALOMICROBIUM ADETUM MEDIUM

Medium 275 plus 5.0 mg nicotinamide added to the vitamin solution.

277 ENTEROCOCCUS FAECIUM MEDIUM

Brain Heart Infusion.....	37.00 g
Yeast extract.....	5.00 g
NaCl.....	9.30 g
Sucrose.....	97.30 g
MgSO ₄	0.25 g
Agar (if needed)	13.30 g
Deionized water up to	1.00 L

Adjust pH to 7.4. Autoclave at 121C/15 min.

To 900 ml of solution aseptically add 100 ml of horse serum, gamma globulin-free inactivated (30 minutes at 56C) and 500 units/ml of penicillin.

278 PEPTONE SUCCINATE AGAR

(NH ₄) ₂ SO ₄	1.00 g
MgSO ₄ .7H ₂ O.....	1.00 g
MnSO ₄ .H ₂ O.....	2.00 mg
FeCl ₃ .6H ₂ O.....	2.00 mg
Succinic acid	1.68 g
Peptone.....	5.00 g
Agar.....	1.50 g
Distilled water	1.00 L

Adjust pH cautiously to 7.0 with KOH. Dispense in 20 ml amounts into 1 oz screw capped bottles and autoclave at 121C/15 min.

279 XANTHOBACTER AGILIS MEDIUM

Solution I:

Na ₂ HPO ₄ .12H ₂ O	9.0 g
KH ₂ PO ₄	1.5 g
NH ₄ Cl.....	1.0 g
MgSO ₄ .7H ₂ O.....	0.2 g
Trace elements solution	1.0 ml
Sodium propionate or 3-hydrobutyrate.....	1.0 g
Agar.....	15.0 g
Distilled water	1.0 L

Adjust pH to 7.0.

Solution II:

Ferric ammonium citrate	50 mg
CaCl ₂ .2H ₂ O	100 ml
Distilled water	100 ml

Trace elements in 2 ml:

H ₃ BO ₄	560 mcg
NiCl ₂ .H ₂ O.....	160 mcg
CuSO ₄ .5H ₂ O.....	16 mcg
MnCl ₂ .4H ₂ O	16 mcg
ZnSO ₄ .7H ₂ O	350 mcg
Na ₂ MoO ₄ .2H ₂ O.....	100 mcg

Sterilize solutions I and II separately. Mix aseptically after sterilization. This prevents formation of a precipitate.

280 PENTACHLOROPHENOL MEDIUM

K ₂ HPO ₄	0.65 g
KH ₂ PO ₄	0.19 g
MgSO ₄ .7H ₂ O.....	0.10 g
NaNO ₃	0.50 g
Sodium glutamate.....	4.00 g

Adjust pH to 7.3-7.4. Autoclave and add 2 ml/L of filter sterilized 0.01M FeSO₄ stock solution.

To prepare pentachlorophenol stock (10,000 ppm) add 1 g pentachlorophenol to 100 ml 0.5 N NaOH.

To induce for pentachlorophenol degradation inoculate media and place on shaker at 200 rpm at 25-30C. Monitor growth on spectrophotometer at 560 nm. When A560 = 0.5, add 5 ml of pentachlorophenol stock (final concentration of 50 ppm). To monitor pentachlorophenol degradation spin down 1 ml of media and read in a spectrophotometer at 320 nm. Degradation should begin within 1 hour and be complete within 3-4 hours. The health of the culture decreases when A560 increases above 1.2.

281 FLEXIBACTER MEDIUM

Tryptone (Difco 0123) 1.0 g
 Vitamin-free casamino acids..... 1.0 g
 Monosodium glutamate..... 0.1 g
 Sodium glycerophosphate 0.1 g
 Vitamin B12..... 1.0 mcg
 Ho-le trace element solution..... 1.0 ml
 Agar (if needed) 15.0 g
 Filtered sea water to 1.0 L

Ho-le trace element solution:

H₃BO₃ 2.85 g
 MnCl₂.4H₂O..... 1.80 g
 FeSO₄..... 1.36 g
 Sodium tartrate..... 1.77 g
 CuCl₂.2H₂O..... 26.90 mg
 ZnCl₂..... 20.80 mg
 CoCl₂.6H₂O..... 40.40 mg
 Na₂MoO₄.2H₂O..... 25.20 mg
 Distilled water 1.00 L

282 FE(III)-LACTATE-NUTRIENT AGAR

Mix 5 ml of filter sterilized 5% FeCl₃.6H₂O with 2.5 ml of filter sterilized 5% sodium lactate AR. Add this mixture at the rate of 0.4 ml per melted universal (12-14 ml) of medium 1 and mix in.

283 R AGAR

Bactopectone..... 10.0 g
 Yeast extract..... 5.0 g
 Malt extract 5.0 g
 Casamino acids 5.0 g
 Beef extract 2.0 g
 Glycerol..... 2.0 g
 Tween 80..... 50.0 mg
 MgSO₄.7H₂O 1.0 g
 Agar 20.0 g
 Distilled water 1.0 L

Adjust pH to 7.2. Autoclave at 121C/15 min.

284 YGLPB MEDIUM

Peptone (Oxoid) 1.00 g
 Lemco (Oxoid) 0.80 g
 Yeast extract (Oxoid) 0.30 g
 KH₂PO₄ 0.25 g

K₂HPO₄ 0.25 g
 MgSO₄.7H₂O 0.02 g
 MnSO₄.4H₂O 5.00 mg
 Glucose 0.50 g
 Lactose 0.50 g
 Distilled water 100ml

Adjust pH to 6.8.

285 NITROCOCCUS MOBILIS MEDIUM

(a)NaNO₂..... 10.0 %

(b)K₂HPO₄..... 2.5 %

(c)NaHCO₃ 5.0 %

(d) Chelated Metals Solution:

CoCl₂.6H₂O..... 4.00 mg
 CuSO₄.5H₂O..... 4.00 mg
 FeCl₃.6H₂O..... 1.00 g
 ZnSO₄.7H₂O..... 0.30 g
 MnSO₄.H₂O..... 0.60 g
 Na₂MoO₄.2H₂O..... 0.15 g
 EDTA 6.00 g
 Distilled water 1.00 L

Adjust pH to 7.5 with NaOH. Add 1 ml chelated metals solution to 1 L sea water. Autoclave at 121C/15 min. Add 1 ml of solution a, b and c to the cooled sea water chelated metals solution.

286 NITROCOCCUS OCEANUS MEDIUM

NH₄Cl 0.635 g
 CaCl₂.H₂O..... 20.000 mg
 MgSO₄.7H₂O 0.357 g
 K₂HPO₄ 43.000 mg
 *Phenol red..... 0.50 mg
 Chelated metals solution (See Medium 285) 1.000 ml
 Filtered sea water 1.000 L

*Phenol Red 50.0mg
 Distilled Water 100.0ml

Add 1ml of the above stock solution to achieve 0.50mg of phenol red.

Adjust pH to 7.5. Autoclave at 121°C /15 min. Periodically during growth adjust pH of medium to 7.5 with sterile 0.1M K₂CO₃ (reappearance of original pink/red colour to medium).

287 CHLOROFLEXUS MEDIUM

Nitrilotriacetic acid..... 0.100 g
 Micronutrient solution 1.000 ml
 FeCl₃ solution..... 1.000 ml
 CaSO₄.2H₂O..... 0.060 g
 MgSO₄.7H₂O..... 0.100 g
 NaCl 0.008 g
 KNO₃..... 0.103 g
 NaNO₃..... 0.689 g
 Na₂HPO₄ 0.111 g
 NH₄Cl..... 0.200 g

Yeast extract.....	0.500 g
Glycl-glycine.....	0.500 g
Distilled water.....	1.000 L

Prepare above medium and adjust pH to 8.2-8.4. Add 0.5 g Na sulphide. Readjust pH to 8.2-8.4. For broth, filter-sterilize and dispense in tubes. For solid medium add 15 g agar.

FeCl₃ Solution:

FeCl ₃	0.2905 g
Distilled water.....	1.0000 L

Micronutrient Solution:

H ₂ SO ₄ (concentrated).....	0.500 ml
MnSO ₄ .7H ₂ O	2.280 g
ZnSO ₄ .7H ₂ O.....	0.500 g
H ₃ BO ₃	0.500 g
CuSO ₄ .2H ₂ O	0.025 g
Na ₂ MoO ₄ .2H ₂ O.....	0.025 g
CoCl ₂ .6H ₂ O.....	0.045 g
Water.....	1.000 L

288 COLBY AND ZATMAN THIAMINE MEDIUM

Medium 83 plus 0.5 mg of thiamine per litre.

289 LAB 8 STARCH AGAR

Medium 201 plus 1% starch.

290 WOODS AND WELTON MEDIUM

Casein hydrolysate	17.0 g
Glucose	5.0 g
Glycerol.....	10.0 g
NaCl.....	23.4 g
Na ₂ SO ₃	0.1 g
Nutrient broth.....	8.0 g
Soytone (soy digest).....	3.0 g
Tryptone.....	0.5 g
Casamino acids (Vitfree).....	0.5 g
Yeast extract.....	2.0 g
Agar	15.0 g
Distilled water.....	1.0 L

Adjust to pH 7.6.

291 NBY MEDIUM

Nutrient broth.....	0.800 %
Yeast extract.....	0.200 %
K ₂ HPO ₄	0.200 %
KH ₂ PO ₄	0.050 %
Glucose (5 ml of 10% solution per.100 ml)	0.500 %
MgSO ₄ .7H ₂ O (2.5 ml of 1% solution per.100 ml)	0.025 %

Autoclave glucose and MgSO₄ solutions separately and add aseptically.

Add 1.5 % purified agar for solid medium.

292 THIOBACILLUS THIOOXIDANS MEDIUM

K ₂ HPO ₄	3.500 g
(NH ₄) ₂ SO ₄	0.300 g
MgSO ₄ .7H ₂ O.....	0.500 g
FeSO ₄ .7H ₂ O	0.018 g
CaCl ₂	0.250 g
Flowers of sulphur.....	5.000 g
Distilled water.....	1.000 L

Dissolve the salts in distilled water and adjust the pH to 4.5. Add the sulphur aseptically after sterilization.

293 AZOSPIRILLUM AMAZONENSE MEDIUM

Medium 1 adjusted to pH 6.0.

294 ACETOBACTER DIAZOTROPHICUS MEDIUM

Glucose.....	50.0 g
Yeast extract.....	10.0 g
CaCO ₃	30.0 g
Agar.....	25.0 g
Distilled water	1.0 L

Mix CaCO₃ thoroughly and cool rapidly. Adjust pH to 5.5.

295 DESULFOCOCCUS MULTIVORANS MEDIUM

Medium 17 plus 1% NaCl

296 ACETOBACTERIUM MEDIUM

NH ₄ Cl.....	1.00 g
KH ₂ PO ₄	0.33 g
K ₂ HPO ₄	0.45 g
MgSO ₄ .7H ₂ O.....	0.10 g
Trace element solution.....	20.00 ml
Vitamin solution.....	20.00 ml
Yeast extract.....	2.00 g
Fructose.....	1.00 g
Resazurin.....	1.00 mg
NaHCO ₃	10.00 g
Cysteine hydrochloride.....	0.50 g
Na ₂ S.9H ₂ O	0.50 g
Distilled water	1.00 L

Trace element solution:

Nitrilotriacetic acid.....	1.500 g
MgSO ₄ .7H ₂ O.....	3.000 g
MnSO ₄ .2H ₂ O.....	0.500 g
NaCl	1.000 g
FeSO ₄ .7H ₂ O	0.100 g
CoSO ₄ .7H ₂ O.....	0.180 g
CaCl ₂ .2H ₂ O	0.100 g
ZnSO ₄ .7H ₂ O.....	0.180 g
CuSO ₄ .5H ₂ O.....	0.010 g
KAl(SO ₄) ₂ .12H ₂ O.....	0.020 g
H ₃ BO ₃	0.010 g
Na ₂ MoO ₄ .2H ₂ O.....	0.010 g
NiCl ₂ .6H ₂ O.....	0.025 g
Na ₂ SeO ₃ .5H ₂ O.....	0.300 mg

Distilled water 1.000 L

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

Vitamin solution:

Biotin 2.0 mg
Folic acid..... 2.0 mg
Pyridoxine-HCl 10.0 mg
Thiamine-HCl 5.0 mg
Riboflavin 5.0 mg
Nicotinic acid..... 5.0 mg
DL-Calcium pantothenate 5.0 mg
Vitamin B12..... 0.1 mg
p-Aminobenzoic acid 5.0 mg
Lipoic acid 5.0 mg
Distilled water 1.0 L

Dissolve ingredients except NaHCO_3 , fructose, cysteine and sodium sulphide, bring to the boil for a few minutes and cool to room temperature under $\text{N}_2 + \text{CO}_2$ (80 + 20) gas mixture. Add NaHCO_3 (solid) and equilibrate the medium with the gas until a pH of approximately 7.4 is reached. Then distribute and autoclave under the same gas. Before use adjust the pH to 8.2 by adding sterile anaerobic Na_2CO_3 solution (approximately 0.25 ml of 5% Na_2CO_3 per 10 ml medium) and add fructose, cysteine and sodium sulphide from anaerobic sterile stock solutions.

297 ACIDOBACTERIUM MEDIUM

$(\text{NH}_4)_2\text{SO}_4$ 2.0 g
KCl..... 0.1 g
 K_2HPO_4 0.5 g
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5 g
Glucose 1.0 g
Yeast extract (Difco)..... 0.1 g
Distilled water 1.0 L

Adjust pH to 3.5 with H_2SO_4 before sterilization.

298 ALTEROMONAS DENITRIFICANS MEDIUM

Peptone (Difco)..... 0.5 g
Tryptone (Difco) 0.5 g
Yeast extract (Difco)..... 0.5 g
Aged sea water 800.0 ml
Tap water 200.0 ml

To avoid precipitation, the nutrients should be autoclaved separately in 100 ml of the tap water and added to the medium after autoclaving. Prior to revival of ampoule and subsequent subculturing chill medium. Use broth to rehydrate culture and only subculture onto solid medium when good growth is apparent. For solid medium add 15.0 g Bacto agar (Difco).

299 TRYPTICASE SOY MEDIUM

Trypticase soy broth (BBL 11768)..... 30.0 g
Distilled water 1.0 L

If solid medium is required add 15.0 g/L of BBL agar

300 ALICYCLOBACILLUS ACIDOTERRESTRIS MEDIUM

Solution A:

$\text{CaCl}_2 \cdot 7\text{H}_2\text{O}$ 0.25 g
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.50 g
 $(\text{NH}_4)_2\text{SO}_4$ 0.20 g
Yeast extract 2.00 g
Glucose 5.00 g
 KH_2PO_4 3.00 g
Distilled water (for liquid medium) 1.00 L
Distilled water (for solid medium) 500.00 ml

Adjust to pH 4.0.

Solution B:

Trace element solution SL-6 1.00 ml

Solution C:

Agar 15.00 g
Distilled water 500.00 ml

Trace element solution SL-6:

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.10 g
 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 0.03 g
 H_3BO_3 0.30 g
 $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 0.20 g
 $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ 0.01 g
 $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ 0.02 g
 $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 0.03 g
Distilled water 1.00 L

Sterilize solutions separately. For liquid medium combine solutions A and B. For solid medium combine solutions A, B and C.

301 BACILLUS SCHLEGELII MEDIUM

$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ 4.50 g
 KH_2PO_4 1.50 g
 NH_4Cl 1.00 g
 $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$ 0.01 g
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.20 g
 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.01 g
Ferric ammonium citrate 5.00 mg
Trace element solution SL-6 3.00 ml
Pyruvate (Na salt) 1.50 g
Distilled water 1.00 L

See Medium 300 for trace element solution SL-6.

Adjust to pH 7.1. Autoclave at 121C/15 min. For solid medium add 15 g agar.

302 YEAST PEPTONE SALT MEDIUM

Medium 27 plus 0.125% NaCl.

303 GLUCOSE YEAST PEPTONE MEDIUM

Glucose 10.0 g
Peptone 5.0 g

Yeast extract..... 5.0 g
Distilled water 1.0 L

Adjust pH to 5.0 - 6.0.

304 TRYPTONE SOYA YEAST EXTRACT BROTH

Medium 90 plus 0.3% yeast extract.

305 CARNOBACTERIUM MEDIUM

Oxoid nutrient broth No.2 25.0 g
Yeast extract..... 3.0 g
Glucose 5.0 g
Oxoid agar No.3 15.0 g
Distilled water 1.0 L

Adjust pH to 6.8.

306 YEAST GLUCOSE BROTH

Medium 5 without agar.

307 DELEYA HALOPHILA MEDIUM

NaCl..... 81.000 g
MgCl₂..... 14.000 g
MgSO₄ 19.600 g
CaCl₂..... 0.470 g
KCl..... 2.000 g
NaHCO₃..... 0.060 g
NaBr..... 0.026 g
Proteose peptone No.3 (Difco)..... 5.000 g
Yeast extract (Difco)..... 10.000 g
Glucose 1.000 g
Distilled water 1.000 L

NaHCO₃ is added from a filter sterilized stock solution when the medium has cooled. Adjust pH to 7.5.

Agar is added at a concentration of 15 g/L for solid medium.

308 ARTIFICIAL ORGANIC LAKE PEPTONE MEDIUM

NaCl..... 30.0 g
MgSO₄.7H₂O 9.5 g
KCl..... 5.0 g
CaCl₂.2H₂O..... 0.2 g
(NH₄)₂SO₄..... 0.1 g
KNO₃ 0.1 g
Peptone..... 5.0 g
Yeast extract..... 1.0 g
Distilled water 960.0 ml

Adjust pH to 7.3 with 0.1M KOH and autoclave at 121C/15 min. Cool medium to 60C and add aseptically 20 ml of sterile HMSS, 20 ml of sterile PS and 1 ml of AOLV.

For solid medium add 15 g of agar prior to sterilization.

Hutner's modified salts solution (HMSS):

See Medium 249.

Phosphate supplement (PS):

K₂HPO₄ 2.5 g
KH₂PO₄ 2.5 g
Distilled water 1.0 L

Artificial Organic Lake vitamin solution (AOLV):

Cyanocobalamine 0.1 mg
Biotin..... 2.0 mg
Calcium pantothenate..... 5.0 mg
Folic acid 2.0 mg
Nicotinamide 5.0 mg
Pyridoxine HCl..... 10.0 mg
Riboflavin..... 5.0 mg
Thiamine HCl..... 5.0 mg
Distilled water to 1.0 L

Sterilize by filtration (0.2 mm). Store at 4C.

309 ARTIFICIAL ORGANIC LAKE MEDIUM

NaCl 80.0 g
MgSO₄.7H₂O 9.5 g
KCl..... 0.5 g
CaCl₂.2H₂O..... 0.2 g
(NH₄)₂SO₄..... 0.1 g
KNO₃..... 0.1 g
Yeast extract 1.0 g
Distilled water..... 60.0 ml

Adjust pH to 8.0 and autoclave at 121C/15 min. On cooling add aseptically 20 ml of sterile HMSS, 20 ml of sterile phosphate supplement and 1 ml of vitamin solution.

Hutner's modified salts solution (HMSS):

See Medium 249.

Phosphate supplement:

K₂HPO₄ 50.0 mg
KH₂PO₄ 50.0 mg
Distilled water 20.0 ml

Vitamin solution:

Cyanocobalamine 10.0 mg
Biotin..... 2.0 mg
Calcium pantothenate..... 5.0 mg
Folic acid 2.0 mg
Nicotinamide 5.0 mg
Pyridoxine HCl..... 10.0 mg
Thiamine HCl..... 10.0 mg
Distilled water to 1.0 L

Sterilize by filtration (0.2 mm). Store at 4C.

310 HALOVIBRIO VARIABILIS MEDIUM

NaCl 95.0 g
MgSO₄.7H₂O 81.0 g
KCl..... 1.0 g
Proteose peptone..... 2.5 g
Trace mineral solution SL-4 10.0 ml

Vitamin solution.....	10.0 ml
Yeast extract.....	7.5 g
Distilled water.....	980.0 ml

Adjust pH to 7.5.

Vitamin solution:

Cyanocobalamine.....	0.01 mg
Biotin.....	0.20 mg
Folic acid.....	0.20 mg
Nicotinic acid.....	0.50 mg
Pyridoxine HCl.....	1.00 mg
Riboflavin.....	0.50 mg
Thiamine HCl.....	0.50 mg
Pantothenic acid.....	0.50 mg
p-Aminobenzoic acid.....	0.50 mg
Lipoic acid.....	0.50 mg
Distilled water.....	1.00 L

Sterilize by filtration (0.2 mm). Store at 4C.

Trace element solution SL-4:

EDTA.....	0.5 g
FeSO ₄ .7H ₂ O.....	0.2 g
Trace element solution SL-6.....	100.0 ml
Distilled water.....	900.0 ml

Trace element solution SL-6:

See Medium 300.

311 10% MARINE SALTS MEDIUM

NaCl.....	81.000 g
MgCl ₂	7.000 g
MgSO ₄	9.600 g
CaCl ₂	0.360 g
KCl.....	2.000 g
NaHCO ₃	0.060 g
NaBr.....	0.026 g
Proteose peptone No.3 (Difco).....	5.000 g
Yeast extract (Difco).....	10.000 g
Glucose.....	1.000 g
Distilled water.....	1.000 L

Adjust pH to 7.0 with KOH.

312 UNIVERSAL BEER AGAR (OXOID CM651)

Peptonised milk.....	15.000 g
Yeast extract.....	6.100 g
Dextrose.....	16.100 g
Tomato supplement.....	12.200 g
K ₂ HPO ₄	0.310 g
KH ₂ PO ₄	0.310 g
NaCl.....	0.006 g
FeSO ₄	0.006 g
MnSO ₄	0.006 g
MgSO ₄	0.120 g
Agar.....	12.000 g

Suspend 62 g in 750 ml of distilled water and bring to the boil to dissolve completely. Add 250 ml commercial beer, without degassing,

to the hot medium and mix gently. Adjust to pH 6.1. Distribute into final containers and autoclave at 121C/10 min.

313 THIOSPHAERA MEDIUM

Na ₂ HPO ₄	4.2 g
KH ₂ PO ₄	1.5 g
NH ₄ Cl.....	0.3 g
MgSO ₄ .7H ₂ O.....	0.1 g
Trace element solution.....	2.0 ml
KNO ₃	0.1 g
Distilled water.....	1.0 L

Adjust pH to 8.0 - 8.2. To avoid precipitation, filter sterilize broth.

For agar prepare broth at double strength, filter sterilize and add aseptically to sterile 3% agar.

Vishniac and Santer trace element solution:

Ethylenediamine tetraacetic acid.....	50.00 g
ZnSO ₄ .7H ₂ O.....	22.00 g
CaCl ₂	5.54 g
MnCl ₂ .4H ₂ O.....	5.06 g
FeSO ₄ .7H ₂ O.....	4.99 g
(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O.....	1.10 g
CuSO ₄ .5H ₂ O.....	1.57 g
CoCl ₂ .6H ₂ O.....	1.61 g
Distilled water.....	1.00 L

Adjust pH to 6.0 with KOH.

314 M13 MEDIUM

Glucose.....	0.25 g
Peptone.....	0.25 g
Yeast extract.....	0.25 g

Tris/HCl (0.1 M, pH 7.5).....
 50.00 ml |

Vitamin solution.....
 10.00 ml |

Hutner's mineral salts (see Medium 249).....
 20.00 ml |

Seawater or artificial seawater (optional).....
 250.00 ml |

Distilled water to.....
 1.00 L |

Adjust pH to 7.2 before autoclaving.

Vitamin solution:

p-Aminobenzoic acid.....	5.0 mg
Biotin.....	2.0 mg
Calcium pantothenate.....	5.0 mg
Folic acid.....	2.0 mg
Nicotinamide.....	5.0 mg
Pyridoxine HCl.....	10.0 mg
Thiamine HCl.....	5.0 mg
Riboflavine.....	5.0 mg
Vitamin B12.....	0.1 mg
Distilled water.....	1.0 L

Filter sterilize and store in refrigerator.

315 PSEUDOAMYCOLATA HALOPHOBICA MEDIUM

Peptone.....	5.0 g
Glucose	5.0 g
Yeast extract.....	3.0 g
K ₂ HPO ₄	0.2 g
Distilled water	1.0 L

316 PSEUDOMONAS PICKETTII MEDIUM

1.0 L of Medium 1 plus

K ₂ HPO ₄	0.45 g
Na ₂ HPO ₄ .12H ₂ O	2.39 g

Adjust pH to 6.8.

317 OTTOW MEDIUM

Glucose	1.0 g
Peptone.....	7.5 g
Meat extract	5.0 g
Yeast extract.....	2.5 g
Casamino acids	2.5 g
NaCl.....	5.0 g
Tap water	1.0 L

Adjust pH to 8.5.

318 SPOROMUSA MEDIUM

K ₂ HPO ₄	0.348 g
KH ₂ PO ₄	0.227 g
NH ₄ Cl.....	0.500 g
MgSO ₄ .7H ₂ O	0.500 g
CaCl ₂ .2H ₂ O.....	0.250 g
NaCl.....	2.250 g
FeSO ₄ .7H ₂ O	0.002 g
Vitamin solution (See Medium 296).....	10.000 ml
Trace element solution SL-10.....	1.000 ml
NaHSeO ₃	15.100 mcg
Yeast extract.....	2.000 g
Casitone	2.000 g
NaHCO ₃	4.000 g
Resazurin	1.000 mg
Cysteine hydrochloride.....	0.300 g
Na ₂ S.9H ₂ O	0.300 g
Distilled water	1.000 L

Adjust pH to 7.0. Prepare the medium anaerobically under 80% N₂ + 20% CO₂ gas mixture. Add fructose at 0.5% final concentration from a filter sterilized anaerobic stock solution.

Trace element solution SL-10:

HCl (25%; 7.7 M).....	10.0 ml
FeCl ₂ .4H ₂ O	1.5 g
ZnCl ₂	70.0 mg
MnCl ₂ .4H ₂ O.....	100.0 mg
H ₃ BO ₃	6.0 mg
CoCl ₂ .6H ₂ O.....	190.0 mg
CuCl ₂ .2H ₂ O.....	2.0 mg
NiCl ₂ .6H ₂ O	24.0 mg
Na ₂ MoO ₄ .2H ₂ O.....	36.0 mg
Distilled water	990.0 ml

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1.0 L.

319 AATCC BACTERIOSTASIS MEDIUM

Bacto peptone.....	10.0 g
Bacto beef extract.....	5.0 g
NaCl	5.0 g
Agar.....	15.0 g

Suspend 35 g in 1.0 L distilled water and boil to dissolve. Autoclave at 121C/15 min. Final pH 7.2 at 25C.

320 CHLORAMPHENICOL LB MEDIUM NO.1

Medium 113 plus 50 mcg/ml chloramphenicol.

321 ERYTHROMYCIN LB MEDIUM

Medium 113 plus 300 mcg/ml erythromycin.

322 CHLORAMPHENICOL LB MEDIUM NO.2

Medium 113 plus 5 mcg/ml chloramphenicol.

323 CHLORAMPHENICOL ERYTHROMYCIN LB MEDIUM

Medium 322 plus 10 mcg/ml erythromycin.

324 CHLORAMPHENICOL AMPICILLIN LB MEDIUM

Medium 322 plus 40 mcg/ml ampicillin.

325 AMPICILLIN KANAMYCIN NUTRIENT AGAR

Medium 1 plus 50 mcg/ml ampicillin and 25 mcg/ml kanamycin.

326 MINERAL MEDIUM WITH CRUDE OIL

K ₂ HPO ₄	0.100 g
MgSO ₄ .7H ₂ O.....	0.020 g
NaCl	0.010 g
CaCl ₂	0.010 g
FeCl ₃	0.002 g
(NH ₄) ₂ SO ₄	0.100 g
Crude oil.(v/v)	0.5%
Water.....	100.000 ml

Adjust pH to 7.2 - 7.5.

327 BASIC CULTIVATION MEDIUM

K ₂ HPO ₄	1.000 g
(NH ₄) ₂ PO ₄	1.500 g
MgSO ₄ .7H ₂ O.....	0.200 g
Fe ₂ (SO ₄) ₃ .5H ₂ O.....	0.010 g
ZnSO ₄ .7H ₂ O.....	0.002 g
Yeast extract.....	10.000 g
Glucose.....	5.000 g
Distilled water	1.000 L

Adjust pH to 7.0.

328 DICHLOROACETIC ACID MEDIUM NO 1

Medium 327 plus less than 1 g/L 2,4 dichloroacetic acid.

329 DICHLOROACETIC ACID MEDIUM NO 2

Medium 327 plus 10 mg/L 2,4 dichloroacetic acid.

330 BASIC MINERAL MEDIUM

MgSO ₄ ·7H ₂ O	0.500 g
Na ₂ HPO ₄ ·2H ₂ O	1.000 g
KH ₂ PO ₄	0.500 g
NH ₄ NO ₃	2.500 g
CaCl ₂ ·2H ₂ O	1.000 mg
Fe(SO ₄) ₃ ·5H ₂ O	0.010 g
MnSO ₄ ·2H ₂ O	0.100 mg
Co(NO ₃) ₂ ·6H ₂ O	0.005 mg
(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	0.100 mg
Distilled water	1.000 L.

331 NAPHTHALENE MEDIUM

Medium 330 plus 5 mM naphthalene.

332 PENTACHLOROPHENOL MEDIUM (MODIFIED)

Medium 330 plus 1 mg/L pentachlorophenol.

333 ENRICHED CYTOPHAGA MEDIUM

Medium 98 without agar. Incubate broths at angle of 45 degrees.

334 PYGV MEDIUM

Hutner's mineral salt solution (see medium 249)	20.00 ml
Bacto peptone	0.25 g
Bacto yeast extract	0.25 g
Bacto agar	15.00 g
Distilled water	965.00 ml

Autoclave at 121C/20 min. After cooling down to 60C add:

2.5% Glucose solution (filter sterilized)	10.0 ml
Vitamin solution (See medium 249)	10.0 ml

Adjust pH to 7.5 (carefully, only weakly buffered - approximately 10 drops of 6N KOH per litre of medium required).

335 GLUCOSE YEAST CHALK MEDIUM

Yeast extract	10.0 g
Glucose	50.0 g
CaCO ₃	30.0 g
Agar	25.0 g
Distilled water	1.0 L

Dissolve the CaCO₃ separately in a portion of the total volume of distilled water and autoclave both solutions at 121C/15 min. After sterilization aseptically mix the solutions and dispense.

336 MEIOTHERMUS RUBER MEDIUM

Universal peptone (Merck)	5.0g
Yeast extract	1.0g
Starch, soluble	1.0g
Agar	12.0g
Distilled water	1000.0ml

Adjust pH to 8.0.

337 THERMUS ENHANCED MEDIUM

Yeast extract	2.5g
Tryptone	2.5g
Agar	28.0g
Nitrilotriacetic acid	100.0mg
CaSO ₄ ·2H ₂ O	40.0mg
MgCl ₂ ·6H ₂ O	200.0mg
0.01 M Ferric citrate	0.5ml
Trace Element Solution (see below)	0.5ml
Phosphate Buffer (see below)	100.0ml
Distilled water	900.0ml

Adjust pH to 7.2 with NaOH. Autoclave at 121C for 15 minutes. Autoclave the phosphate buffer separately and then add to the medium.

Phosphate buffer:

KH ₂ PO ₄	5.44g
Na ₂ HPO ₄ ·12H ₂ O	43.0g
Distilled water	1.0L

Trace Element Solution:

Nitrilotriacetic acid	12.8g
FeCl ₂ ·4H ₂ O	1.0g
MnCl ₂ ·4H ₂ O	0.5g
CoCl ₂ ·6H ₂ O	0.3g
CuCl ₂ ·2H ₂ O	50.0mg
Na ₂ MoO ₄ ·2H ₂ O	50.0mg
H ₃ BO ₃	20.0mg
NiCl ₂ ·6H ₂ O	20.0mg
Distilled water	1.0L

338 CURTOBACTERIUM AND PSYCHROBACTER MEDIUM

Peptone	1.0 g
Yeast extract	0.5 g
Glucose	0.1 g
Agar	1.5 g
Distilled water	100 ml

Adjust pH to 7.0. Incubation temperature 10C.

339 YEAST EXTRACT AGAR 1

Yeast extract	3g
Agar	15g
Distilled water	1L

340 M9 SALTS MEDIUM

10 x M9 salts (see below).....	100.0ml
1 M MgSO ₄	1.0ml
0.1 M CaCl ₂	1.0ml
1 M Thiamine Hcl (sterilized by filtration).....	1.0ml
Glucose (20%).....	10.0ml
Proline.....	20.0mg
Distilled water.....	900.0ml

The above solutions should be sterilized separately by filtration (thiamine, glucose) or autoclaving

10 x M9 salts (per l):

Na ₂ HPO ₄	60.0g
KH ₂ PO ₄	30.0g
NH ₄ Cl.....	10.0g
NaCl.....	5.0g

Adjust pH to 7.4

341 GRAPE JUICE MEDIUM g l⁻¹ or ml l⁻¹

Yeast extract.....	10.0
Tween 80.....	1.0
Grape juice.....	170.0ml
Distilled water.....	1litre

For solid medium - 20.0 g l⁻¹ agar. pH to 5,5 with 1 M NaOH before sterilizing 20 mins at 121C. Please note that growth is best in broth, but never as dense as for *Leuconostoc* species.

342 PEPTONE YEAST MEDIUM

Peptone.....	1.0 g
Yeast extract.....	0.2 g
NaCl.....	0.2 g
Glucose.....	0.2 g
Distilled water.....	100 ml

Adjust to pH 7.0.

343 MODIFIED ANACTER & ORDAL'S MEDIUM

Agar (Oxoid).....	0.7 g
or (Gibco).....	1.0 g
Tryptone.....	0.5 g
Yeast extract.....	0.05 g
Sodium acetate.....	0.02 g
Beef extract.....	0.02 g
Newborn calf serum.....	5.00 ml
Distilled water to.....	100 ml

Adjust pH to 7.2 - 7.4.

344 NUTRIENT AGAR + 30 µg/ml CHLORAMPHENICOL**345 9K MEDIUM**

Solution (a)

(NH ₄) ₂ SO ₄	3.0g
KCl.....	0.1g
K ₂ HPO ₄	0.5g
MgSO ₄ .7H ₂ O.....	0.5g
Ca(NO ₃) ₂	10.0mg
10N H ₂ SO ₄	1.0ml
Distilled water.....	700.0ml

Solution (b)

FeSO ₄ .H ₂ O.....	44.0g
Distilled water.....	300.0ml

Prepare solutions (a) and (b) separately. Dispense (a) as 70ml in 250ml screw-capped bottles, and (b) as 30ml amounts in 1oz screw-capped bottles. Autoclave (a) and (b) separately at 121C/15min. Immediately before use add (b) aseptically to (a).

346 MEDIUM 119 + 2/ NaCl**347 LACTOBACILLUS PONTIS MEDIUM**.....g/l

Tryptone.....	10.0
Meat extract.....	5.0
Yeast extract.....	5.0
Glucose.....	7.0
Fructose.....	7.0
Maltose.....	7.0
Sodium gluconate.....	2.0
Sodium acetate 3H ₂ O.....	5.0
diammonium citrate.....	2.0
K ₂ HPO ₄ .3H ₂ O.....	2.6
MgSO ₄ .7H ₂ O.....	0.1
MnSO ₄ .4H ₂ O.....	0.05
cysteine HCl.....	0.5
Tween 80.....	1.0

Adjust pH to 6.3. If solid medium required add 15 g/l agar.

348 CLOSTRIDIUM ACETOBUTYLICUM MEDIUM

Fresh milk.....	100ml
Resazurin.....	0.1mg

Adjust pH to 7.1 Tube and autoclave under 100% N₂ for 12 min at 121C.

349 SPECIAL VIBRIO MEDIUM

Medium 113 with agar + 1% NaCl + 2 mg/ml carbenicillin

350 BLOOD AGAR 2

Blood agar base No 2 (Oxoid) plus 7% cow or sheep blood

Proteose peptone.....	15.0g
Liver digest.....	2.5g
Yeast extract.....	5.0g
Sodium chloride.....	5.0g
Agar.....	12.0g

pH 7.4 ± 0.2

Suspend 40g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 45-50°C and add 7% sterile blood.

Mix with gentle rotation and pour into petri dishes or other containers.

351 PPB - Modification of Caldwell & Bryant 1966

Cellobiose.....	0.1%
Maltose.....	0.1%
Glucose	0.1%
Starch	0.1%
Difco Yeast Extract.....	0.2%
BBL trypticase	0.2%
Mineral I.....	7.5%
Mineral II	7.5%
Haemin Solution	1.0%
VFA mix (optional).....	0.31%
Resazurin Solution	0.1%
Clarified Rumen Fluid.....	15.0%
Water	

Adjusted to pH 6.8 using 1N NaOH final volume 93%

Additions 2% of a 2.5% L-cysteine Hcl solution
5% of an 8% sodium carbonate solution

Note: It is important that the carbonate is thoroughly equilibrated with CO₂ and also that the medium is thoroughly deep gassed with CO₂ before filling out otherwise the pH of the broth can be as high as 9.5.

1. Preparation of a sterile medium

- Medium prepared to 92% of final volume excluding cysteine and carbonate in flask plugged with cotton wool.
 - Cysteine Hcl 2.5% solution in screw capped bottles with little head space
 - Sodium carbonate 8.0% solution in small flask, with large head space and plugged with cotton wool.
- a, b, c are autoclaved at 15 psi for 15 min at 121°C

2. When atmospheric pressure has been reached remove flasks and insert short sterile gassing jets and pass CO₂ over the surface of the medium and carbonate solution. Place flasks in ice bath. When temperature has cooled to 50°C or below add cysteine followed by carbonate and insert long gassing jets and deep gas for at least 30 min.

3. Fill out aseptically into sterile stoppered tubes using standard Hungate technique.

4. Incubate at 37°C at least overnight to check for contamination or oxidised tubes.

352 MALT EXTRACT AGAR (OXOID)

Malt extract	17.0g
Mycological peptone	3.0g

pH 5.4 ± 0.2

Add 20g to 1 litre of distilled water. Mix well, distribute into final containers and sterilise by autoclaving at 115°C for 10 minutes. This

liquid medium is recommended for the cultivation of moulds and yeasts, during tests for sterility, etc.

353 LINEOLA MEDIUM

Yeast extract (Difco)	0.5%
Peptone (Difco)	0.5%
Sodium acetate	0.1%
Agar (Difco)	1.5%
pH 7.4	

354 MANNITOL AGAR

Yeast extract (Oxoid)	0.5%
Peptone (Oxoid)	0.3%
Mannitol	2.5%
Agar	1.5%
pH 6.8	

355 M10 - as per PPB but excluding rumen fluid

356 YGLM

Litmus milk (LM) Skimmed, separated milk or reconstituted powdered separated milk. 0.75g/litre of Difco Powdered Litmus (code 0209-13) is added to the milk and thoroughly stirred for 15-20 min. Autoclave at 10psi for 10 min. Incubated for 1 week at 30°C to check for sterility before use.

Yeast Glucose Litmus Milk (YGLM/YDLM) Prepared as for Litmus Milk plus 1.0% Glucose and 0.3% Yeast Extract (Oxoid).

LM + Chalk, UDLM + Chalk Approximately 2.0% CaCO₃ (thick layer in bottom of tube or bottle) is pre-sterilized in situ at 15 psi for 15 min before the milk media is added. This reduces the risk of contamination by spore formers surviving the lower heat treatment for the milk. Sterilized at 10 psi for 10 min then incubated as for LM.

357 ATB ACID TOMATO BROTH

Glucose.....	1.0%
Peptone (Oxoid)	1.0%
Yeast Extract Oxoid	0.5%
MgSO ₄ .7H ₂ O	0.02%
MgSO ₄ .4H ₂ O	0.005%
Tomato juice.....	25.0%

358 PLUTON MEDIUM

Glucose.....	1.0%
Starch	0.2%
Peptone (Oxoid)	0.25%
Yeast Extract Oxoid	0.25%
Malt extract (Oxoid)	0.5%
Neopeptone (Difco).....	0.5%
Trypticase	0.2%
1 M potassium phosphate buffer pH 7.2	5.0%

Sterile cysteine Hcl 2.5% is added 0.1ml per 10ml tube

359 COOKED MEAT MEDIUM (DIFCO)

Beef heart 454 g
 Bacto proteose peptone 20 g
 Bacto dextrose 2 g
 NaCl 5 g
 Distilled water 1 L
 PH 7.2

360 CYTOPHAGA MEDIUM

Casitone 0.3%
 CaCl₂.H₂O 0.136%
 Yeast Extract 0.1%
 Agar 1.5%
 Cellobiose 0.5%
 pH 7.2

361 TODD HEWITT BROTH (OXOID)

Infusion from fat-free minced meat 10.0g
 Tryptone 20.0g
 Dextrose 2.0g
 Sodium bicarbonate 2.0g
 Sodium chloride 2.0g
 Disodium phosphate anhydrous 0.4g

pH 7.8 ± 0.2

Weigh 36.4g of powder and disperse in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix and warm to dissolve. Dispense into 10ml volumes in screw capped containers and sterilise by autoclaving at 121°C for 15 minutes.

362 COLUMBIA BLOOD AGAR + 5% BLOOD

Columbia Peptone Mixture 23.0g
 Corn Starch 1.0g
 Sodium chloride 5.0g
 Agar No 2 12.0g

Cool agar to 50°C and aseptically add 5% blood.

363 BRAIN HEART INFUSION BROTH (OXOID)

Brain-Heart Infusion Solids (porcine) 17.5g
 Tryptose 10.0g
 Glucose 2.0g
 Sodium chloride 5.0g
 Disodium phosphate 2.5g
 Agar No 2 12.0g

pH 7.4 ± 0.2

Weigh 49g of powder, disperse in 1 litre of deionised water. Allow to stand for 10 minutes then swirl to mix. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C then pour into petri dishes.

364 CORYNEFORM BROTH

Tryptone 1.0%
 Yeast Extract 0.5%
 NaCl 0.5%

Glucose 0.5%
 pH 7.2-7.4

365 CORYNEFORM AGAR

Coryneform Broth + 1.5% Agar

366 YEAST DEXTROSE AGAR

Nutrient Broth No 2 2.5%
 Glucose 0.5%
 Agar 1.5%
 Yeast Extract 0.3%
 pH 6.8

367 HARTLEY DIGEST BROTH (OXOID)

368 SPECIAL REINFORCED CLOSTRIDIAL MEDIUM 1

Medium 264 without Agar + 0.05% cysteine

369 SPECIAL REINFORCED CLOSTRIDIAL MEDIUM 2

Medium 264 + 0.3% Panmede (liver extract) + 0.05% Cysteine Hcl

370 SUPPLEMENT YEAST GLUCOSE BROTH

Medium 306 supplemented with 10ug/ml tetracyclin and 50ug/ml erythromycin

371 YEAST GLUCOSE CYSTEINE MEDIUM

Medium 306 + 0.05% cysteine

372 YEAST GLUCOSE UREA MEDIUM

Medium 5 + 2% urea

373

Medium 351 + 1% sodium lactate

374

Medium 1 + 0.1% starch

375

Medium 1 + 5% NaCl

376

Medium 20 + 1% arabinose + 1% maltose

377

Medium 20 + 1% maltose

378

Medium 20 + 1% lactose

379

Medium 20 + 0.5% Panmede

380

Medium 264 + 1% Tween 80

381

Medium 284 + 1% lactose

382

Medium 284 + 5% serum

383

Medium 284 + 5% serum + 0.05% cysteine

384

Medium 284 + 0.1% Tween 80

385

Medium 357 + 0.05% cysteine

386

Medium 361 + 5% serum

387

Medium 361 + 1% Tween 80

388

Medium 361 + 1% NaCl + 5% serum + 0.05% cysteine

389

Medium 361 + 0.05% cysteine

390

Medium 363 + 0.05% cysteine

391

Medium 90 containing 1M/Litre Kcl

392

Medium 366 + 10% serum

393

Medium 367 + 0.05% cysteine

394

Medium 357 + 0.1% Tween 80

395

Medium 357 + 0.1% Tween 80 + 10% ethanol

396 RHODOCYCLUS MEDIUM

Yeast extract0.2 g
Di-sodium succinate..... 1 g
Ferric citrate solution (0.1g per 100ml) 5 ml
KH₂PO₄0.5 g
MgSO₄.7H₂O.....0.4 g
NaCl0.4 g
NH₄Cl.....0.4 g
CaCl₂.2H₂O50 mg
Ethanol0.5 ml
Yeast extract0.8 g
Trace element solution (see below)..... 1 ml
Distilled water up to 1 L
pH.....5.7

Trace element solution:

ZnSO₄.7H₂O.....0.1 g
MnCl₂.4H₂O..... 30 mg
H₃BO₃.....0.3 g
CoCl₂.6H₂O.....0.2 g
CuCl₂.2H₂O..... 10 mg
NiCl₂.6H₂O..... 20 mg
Na₂MoO₄.2H₂O..... 30 mg
Distilled water 1 L

Adjust pH to 6.8. Distribute 40 ml medium into 50 ml screw-capped bottles. Flush each bottle for 1 to 2 minutes with nitrogen gas, then close immediately with rubber septa and screw-caps. Autoclave. Sterile syringes are used to inoculate and remove the samples. Incubate in light using a tungsten lamp. If needed, add 15 g agar per litre medium.

397 AZORHIZOBIUM MEDIUM

Peptone5 g
Yeast extract 1 g
Beef extract5 g
Sucrose5 g
MgSO₄.....0.24 g
Agar..... 15 g
Distilled water 1 L

398

Medium 119 + 2% NaCl

399 DSM TRYPTICASE SOY AGAR

Peptone from casein 15.0g
Peptone from soymeal5.0g
NaCl5.0g

Agar	15.0g
Distilled water	1000.0ml

Adjust pH to 7.3.

400 LISTERIA SELECTIVE AGAR BASE (Oxoid CM856)

Columbia blood agar base	39.0g
Aesculin	1.0g
Ferric ammonium citrate	0.5g
Lithium chloride	15.0g
Distilled water	1000.0ml
pH 7.0 ± 0.2	

401 RHODOSPIRILLACEAE MODIFIED MEDIUM

Yeast extract	0.3g
Ethanol	0.5ml
Di-sodium succinate	1.0g
Ammonium acetate	0.5g
Ferric citrate solution (0.1% in H ₂ O)	5.0ml
KH ₂ PO ₄	0.5g
MgSO ₄ x 7 H ₂ O	0.4g
NaCl	0.4g
NH ₄ Cl	0.4g
CaCl ₂ x 2 H ₂ O	0.05g
Vitamin B ₁₂ solution (10mg in 100ml H ₂ O)	0.4ml
Trace element solution SL-6 (see below)	1.0ml
Distilled water	1050.0ml

Adjust pH to 6.8. Boil the medium under a stream of nitrogen gas for few minutes and distribute 45ml medium into 50ml screw-capped bottles (already flushed with nitrogen gas). Bubble each bottle with nitrogen gas and close immediately with a rubber septum and screw tight. Autoclave at 121C for 15 minutes. Sterile syringes are used to inoculate and remove samples. Incubate in the light using a tungsten lamp. For brown and other oxygen sensitive *Rhodospirillaceae* add 300mg of L-cysteine (0.03% end concentration) to the boiling medium and readjust the pH to 6.8 or to the prepared medium in bottles inject neutralized sulfide solution (0.005 to 0.01% end concentration).

Trace element solution SL-6:

ZnSO ₄ x 7 H ₂ O	0.1g
MnCl ₂ x 4 H ₂ O	0.03g
H ₃ BO ₃	0.3g
CoCl ₂ x 6 H ₂ O	0.2g
CuCl ₂ x 2 H ₂ O	0.01g
NiCl ₂ x 6 H ₂ O	0.02g
Na ₂ MoO ₄ x 2 H ₂ O	0.03g
Distilled water	1000.0ml

Neutralized sulfide solution:

Distilled water	100.0ml
Na ₂ S x 9 H ₂ O	1.5g

The sulfide solution is prepared in a 250ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min at 121C. After cooling to room temperature the pH is adjusted to about 7.3 by adding sterile 2 M H₂SO₄ drop-wise with a syringe without opening the bottle. Appearance of a yellow colour indicates the drop of pH to about 8. The

solution should be stirred continuously to avoid precipitation of elemental sulfur. The final solution should be clear and is yellow in colour.

402 RUMINOCOCCUS PASTEURII MEDIUM

KH ₂ PO ₄	0.2g
NH ₄ Cl	0.25g
NaCl	1.0g
MgCl ₂ ·6H ₂ O	0.4g
KCl	0.5g
CaCl ₂ ·2H ₂ O	0.15g
Trace Elements Solution SL-7 (see below)	1.0ml
Resazurin	1.0mg
NaHCO ₃	2.5g
Sodium tartrate	2.0g
Na ₂ S·9H ₂ O	0.36g
Distilled water	1.0L

Adjust medium for final pH 7.2. Boil medium without sodium bicarbonate, trace elements or sodium sulfide under 80% N₂ and 20% CO₂; dispense and tube with the same gas phase. Cool and aseptically add the filter-sterilized sodium bicarbonate and trace elements. Aseptically add the sodium sulfide which has been separately autoclaved under N₂.

Trace Elements Solution SL-7:

Hydrochloric acid, 25%	10.0ml
FeCl ₂ ·4H ₂ O	1.5g
CoCl ₂ ·6H ₂ O	190.0mg
MnCl ₂ ·4H ₂ O	100.0mg
ZnCl ₂	70.0mg
H ₃ BO ₃	62.0mg
Na ₂ MoO ₄ ·2H ₂ O	36.0mg
NiCl ₂ ·6H ₂ O	24.0mg
CuCl ₂ ·2H ₂ O	17.0mg
Distilled water	1.0L

Dissolve the FeCl₂·4H₂O in the concentrated HCl, then dilute. Use 1.0ml/L of medium.

403 THERMOTOGA ELFII MEDIUM

NH ₄ Cl	1.0g
K ₂ HPO ₄	0.3g
KH ₂ PO ₄	0.3g
MgCl ₂ x 6 H ₂ O	0.2g
CaCl ₂ x 2 H ₂ O	0.1g
KCl	0.1g
NaCl	10.0g
Trace element solution (see below)	10.0ml
Sodium acetate	0.5g
Yeast extract	5.0g
Trypticase	5.0g
Resazurin	0.5mg
L-Cysteine	0.5g
Na ₂ CO ₃	2.0g
Sodium thiosulfate x 5 H ₂ O	5.0g
Glucose	4.0g
Na ₂ S x 9 H ₂ O	0.5g
Distilled water	1000.0ml

Prepare medium anaerobically under 80% N₂ + 20% CO₂ gas atmosphere. Autoclave separately anaerobic (N₂) stock solutions of Na₂CO₃, thiosulfate, glucose and sulfide. The pH of the completed medium is 7.5.

Trace element solution:

Nitrilotriacetic acid	1.5g
MgSO ₄ x 7 H ₂ O	3.0g
MnSO ₄ x 2 H ₂ O	0.5g
NaCl	1.0g
FeSO ₄ x 7 H ₂ O	0.1g
CoSO ₄ x 7 H ₂ O	0.1g
CaCl ₂ x 2 H ₂ O	0.1g
ZnSO ₄ x 7 H ₂ O	0.18g
CuSO ₄ x 5 H ₂ O	0.01g
KAl(SO ₄) ₂ x 12 H ₂ O	0.02g
H ₃ BO ₃	0.01g
Na ₂ MoO ₄ x 2 H ₂ O	0.01g
NiCl ₂ x 6 H ₂ O	0.025g
Na ₂ SeO ₃ x 5 H ₂ O	0.3mg
Distilled water	1000.0ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

404 ACTINOMADURA MADURAE MEDIUM

Bacto yeast extract (Difco).....	1.0g
Bacto beef extract (Difco)	1.0g
N-Z amine, type A (Sheffield Chem. Co.).....	2.0g
Sucrose.....	10.0g
Agar	15.0g
Distilled water	1.0L

Adjust pH to 7.3.

405 LACTOBACILLUS KEFIRGRANUM MEDIUM

Trypticase.....	5g
Tryptone.....	5g
Yeast extract.....	5g
KH ₂ PO ₄	5g
Diammonium hydrogen citrate.....	2g
MgSO ₄ .7H ₂ O	0.5g
MnSO ₄ .xHO.....	0.5g
Tween 80.....	1ml
Cheese whey.....	1L

406 SP MEDIUM

Raffinose	1.0g
Sucrose	1.0g
Galactose	1.0g
Soluble starch.....	5.0g
Casitone (Difco 0259)	2.5g
MgSO ₄ .7H ₂ O	0.5g
K ₂ HPO ₄	0.25g
Agar	15.0g
Distilled water	1.0L

407 ERYTHROMICROBIUM & ROSEOCOCCUS MEDIUM

Yeast extract (Difco)	1.0g
Bacto Peptone (Difco)	1.0g
Sodium acetate	1.0g
KCl.....	0.3g
MgSO ₄ .7H ₂ O.....	0.5g
CaCl ₂ .2H ₂ O	0.05g
NH ₄ Cl.....	0.3g
K ₂ HPO ₄	0.3g
Vitamin B ₁₂	20µg
Trace elements solution (see below)	1ml
Distilled water	1L

pH 7.5-7.8.

Trace elements solution:

Erthyene diamine tetraacetic acid.....	500mg/l
FeSO ₄ .7H ₂ O	300mg/l
MnCl ₂ .4H ₂ O	3mg/l
CoCl ₂ .6H ₂ O.....	5mg/l
CuCl ₂ .2H ₂ O.....	1mg/l
NiCl ₂ .6H ₂ O.....	2mg/l
Na ₂ MoO ₄ .2H ₂ O.....	3mg/l
ZnSO ₄ .7H ₂ O.....	5mg/l
H ₃ BO.....	2mg/l

Dissolve each compound separately in distilled water, add to the solution of EDTA, adjust the pH to approximately 4, and make up to 1 litre.

408 YEAST MANNITOL EXTRACT MEDIUM

Yeast extract.....	1.0g
Soil extract.....	200.0ml
Mannitol	10.0g
Agar.....	20.0g
Tap water.....	1.0L

Adjust pH to 7.0-7.2.

409 CARBOXYMETHYL CELLULOSE MEDIUM

(NH ₄) ₂ SO ₄	0.1%
MgSO ₄ .7H ₂ O.....	0.1%
CaCl ₂ .2H ₂ O	0.1%
FeCl ₃	0.02%
K ₂ HPO ₄ (autoclaved separately).....	0.1%
Casitone (Difco)	0.2%
Carboxymethyl cellulose	1.5%
Agar.....	0.6%

Carboxymethyl cellulose Simga no. C-5013, sodium salt, high viscosity, works well.

410 DESULFOTOMACULUM THERMOSAPOVORANS MEDIUM

Basal medium	
K ₂ HPO ₄	0.5 g
NH ₄ Cl	1.0 g
CaSO ₄	1.0 g

MgSO ₄ ·7H ₂ O	2.0 g
NaCl.....	15.0g
Yeast extract.....	1.0 g
Distilled water	1.0 L

Dissolve above and gas with oxygen free nitrogen for 10 - 15 minutes, then add:-

Thioglycollic acid.....	0.1 g
Ascorbic acid	0.1 g
FeSO ₄ ·7H ₂ O.....	0.5 g
Sodium pyruvate	1.0g

Still gassing pH to 7.4, dispense and autoclave at 115C/10 min.

411 GORDONIA RUBRIPERTINCTA MEDIUM

Peptone.....	10.0g
NaCl.....	5.0g
Agar	20.0g
Beef water	1000.0ml

Adjust pH to 7.2-7.4. Sterilize at 121C for 30 minutes.

412 MICROLUNATUS PHOSPHOVORUS MEDIUM

Glucose	0.5g
Peptone.....	0.5g
Monosodium glutamate.....	0.5g
Yeast extract.....	0.5g
KH ₂ PO ₄	0.44g
(NH ₄) ₂ SO ₄	0.1g
MgSO ₄ ·7H ₂ O	0.1g

Adjust pH to 7.0 with a diluted NaOH solution. Growth is estimated by measuring the optical density at 600nm with a spectrophotometer.

413 POSTGATE'S SEA WATER MEDIUM

Basal medium	
K ₂ HPO ₄	0.5 g
NH ₄ Cl.....	1.0 g
CaSO ₄	1.0 g
MgSO ₄ ·7H ₂ O	2.0 g
Sodium lactate(70%).....	3.5 ml
Yeast extract.....	1.0 g
Filtered aged sea water	1.0 L

Dissolve above and gas with oxygen free nitrogen for 10 - 15 minutes, then add:-

Thioglycollic acid.....	0.1 g
Ascorbic acid	0.1 g
FeSO ₄ ·7H ₂ O.....	0.5 g

Still gassing pH to 7.4, dispense and autoclave at 115C/10 min.

414 CASTENHOLZ TYE MEDIUM

Castenholz Salts, 2X:	
Nitritotriacetic acid	0.2g
Nitsch's Trace elements (see below)	2.0ml
FeCl ₃ solution (0.03%).....	2.0ml

CaSO ₄ ·2H ₂ O.....	0.12g
MgSO ₄ ·7H ₂ O.....	0.2g
NaCl	0.016g
KNO ₃	0.21g
NaNO ₃	1.4g
Na ₂ HPO ₄	0.22g
Agar (if needed).....	30.0g
Distilled water	1.0L

Adjust pH to 8.2.

Nitsch's Trace elements:

H ₂ SO ₄	0.5ml
MnSO ₄	2.2g
ZnSO ₄	0.5g
H ₃ BO ₃	0.5g
CuSO ₄	0.016g
Na ₂ MoO ₄	0.025g
CoCl ₂ ·6H ₂ O.....	0.046g
Distilled water	1.0L

1% TYE:

Truption (Difco 0123).....	10.0g
Yeast extract	10.0g
Distilled water	1.0L

Mix aseptically 5 parts double strength Castenholz Salts with one part 1% TYE and 4 parts distilled water. Final pH of complete medium should be 7.6.

415 BIFIDOBACTERIUM MEDIUM

Casein peptone, typtic digest.....	10.0g
Meat extract.....	5.0g
Yeast extract	5.0g
Glucose.....	10.0g
K ₂ HPO ₄	3.0g
Tween 80	1.0ml
Distilled water	1.0L

Adjust pH to 6.8. After sterilization, aseptically add solutions of sodium ascorbate and L-cysteine HCl to final concentration of 1.0% and 0.05% respectively. Heat medium not freshly prepared in a steamer for 10 min before addition of the reducing substances.

416 RHODOCOCCLUS PERCOLATUS MEDIUM

Glucose.....	4.0g
Yeast extract	4.0g
Malt extract	10.0g
CaCO ₃	2.0g
Agar.....	12.0g
Distilled water	1000.0ml

Adjust pH to 7.2 with KOH before adding agar (use pH-indicator paper).

417 ALKALINE NUTRIENT AGAR 1

Medium 1 + 0.5% NaCl.

After sterilization add sterile 1M Na-sesquicarbonate solution (1ml in 10ml) to achieve a pH of 9.7.

Na-sesquicarbonate solution:

NaHCO₃ 4.2g
Na₂CO₃ anhydrous 5.3g
Distilled water 100.0ml

418 ALKALINE NUTRIENT AGAR 2

Medium 1 + 10% NaCl.

After sterilization add sterile 1M Na-sesquicarbonate solution (1ml in 10ml) to achieve a pH of 9.7.

Na-sesquicarbonate solution:

NaHCO₃ 4.2g
Na₂CO₃ anhydrous 5.3g
Distilled water 100.0ml

419 THERMOCRISPIUM MEDIUM

Standard I Broth (Merck) 25.0g
Malt extract 10.0g
CaCO₃ 2.0g
Agar 12.0g
Distilled water 1000.0ml

Adjust pH to 7.2.

420 BACILLUS LENTIMORBUS

Mueller-Hinton broth 10.0g
Yeast extract 10.0g
K₂HPO₄ 3.0g
Glucose (autoclaved separately) 0.5g
Sodium pyruvate 1.0g
Distilled water to 1.000ml

The pH is adjusted to 7.1. This medium may be solidified by inclusion of 20g of agar.

421 SPECIAL MINERAL SALTS MEDIUM

(NH₄)₂SO₄ 0.3g
NaCl 5.85g
CaCl₂·2H₂O 0.2g
K₂HPO₄ 0.1g
MgSO₄·7H₂O 0.14g
FeSO₄·7H₂O 0.3mg
CoSO₄·7H₂O 0.11mg
H₃BO₄ 0.6mg
ZnCl₂ 0.22mg
CuSO₄·5H₂O 0.08mg
Distilled water 1 L

Nutrient-Enriched Agar

The nutritive agar contains 20g agar per litre of mineral salts solution.

The carbon source is any *one* of the following added per litre of mineral salts.

Starch 2.4g
m-Toluic acid (neutralized) 1.35g
n-butanol 1.5ml
Lactic acid (85%) 1.35g
Ethanol (95%) 1.5ml
Glucose 2.4g

After the addition of the carbon source, the pH is adjusted to 7.0 with 0.5 N NaOH.

422 FRATEURIA AURANTIA MEDIUM

Potato* 200g
Press yeast 30g
Liver, infusion from* 25g
Meat extract 5g
Thioglycolate medium dehydrated** 10g
Glucose 5g
Glycerol 15g
CaCO₃ 15g
Distilled water, make up to 1L
Agar 15g

Adjust pH to 7.0.

*Gently boil sliced potatoes in 500ml of water (or sliced liver in 150ml of water) for 30 mins and remove solids by filtration through cloth.

**Wako Pure Chemicals Ind. Ltd., Osaka, Japan.

423 SPHINGOMONAS MEDIUM

5% PTYG

Peptone 0.25g
Tryptone 0.25g
Yeast extract 0.5g
Glucose 0.5g
MgSO₄·7H₂O 0.6g
CaCl₂·2H₂O 0.07g

Add agar in usual concentration for solid media.

424

Medium 113 with ampicillin at 25-50 µg ml⁻²

425

Medium 29 + 5% NaCl

426 JANIBACTER MEDIUM

Bacto peptone (Difco) 10.0g
Yeast extract 5.0g
Casamino acids (Difco) 5.0g
Meat extract (Difco) 2.0g
Malt extract (Difco) 5.0g
Glycerol 2.0g
MgSO₄ x 7 H₂O 1.0g
Tween 80 0.05g
Agar 20.0g

Distilled water 1000.0ml

Adjust pH to 7.2. Sterilise 20 at 121°C.

427 BOGORIELLA MEDIUM

Glucose 10.0g
Peptone (Difco) 5.0g
Yeast extract (Difco) 5.0g
KH₂PO₄ 1.0g
MgSO₄ 7H₂O 0.2g
NaCl 40.0g
Na₂CO₃ 10.0g
Agar 20.0g
Aqua dest. 1000.0ml

Adjust pH to 9.6. NaCl and Na₂CO₃ were autoclaved separately and added to the organic compounds at 60°C before pouring the agar medium.

428 PYS MEDIUM

KH₂PO₄ 1.0g
(NH₄)₂SO₄ 1.0g
NaCl 0.2g
MgCl₂ 6H₂O 0.2g
CaCl₂ 2H₂O 0.05g
Trace element solution SL8 1.0ml
Sodium pyruvate 2.2g
Yeast extract 1.0g
Distilled water 1.0L

Trace Element Solution SL8

EDTA-disodium salt 5.2g
FeCl₂ 4H₂O 1.5g
ZnCl₂ 70.0mg
MnCl₂ 4H₂O 100.0mg
H₃BO₃ 62.0mg
CoCl₂ 6H₂O 190.0mg
CuCl₂ 2H₂O 17.0mg
NiCl₂ 6H₂O 24.0mg
Na₂MoSO₄ 2H₂O 36.0mg
Distilled water 1.0L

Adjust pH to 6.8. Separately sterilise sodium pyruvate by filtration and aseptically add to the medium.

429 METHYLOMICROBIUM ALCALIPHILUM MEDIUM

Mineral Base

Na₂CO₃ 20g
NaHCO₃ 10g
NaCl 3g
K₂HPO₄ 1g
KNO₃ 0.5g
Distilled water 1L

Agar Base

Agar 35g
Distilled water 1L

Stock Solutions

- a) MgSO₄·7H₂O 2g/10mls
- b) CuSO₄·5H₂O 0.025g/100 ml
- c) * Trace element solution (Pfennig & Lippert)

*(Pfennig, H G & Lippert (1966) Arch. Microbiol. 55, 245 - 256)

Method

pH mineral base to 10.0. Autoclave mineral base and agar base separately at 15 psi/15 min. Cool both solutions to 50°C and aseptically mix 1 part of mineral base with 1 part of agar base. Additionally aseptically add the following amounts of stock solutions to the final volume of mineral/agar base: a) 0.5 ml/Litre; b) 1ml/Litre; c) 2ml/Litre. Dispense aseptically.

For liquid medium, as above but omit the agar base step (resultant increased concentration of solutes is acceptable).

430 IFO MEDIUM 203

Peptone 10g
Yeast extract 5g
Liver, infusion from* 25g
Glucose 3g
Glycerol 15g
Distilled water, make up to 1L
Agar 15g

Adjust pH to 7.0.

*See Medium 422

431 COLUMBIA AGAR

Columbia Peptone Mixture 23.0g
Corn Starch 1.0g
Sodium chloride 5.0g
Agar No 2 12.0g

Adjust pH to 7.0 - 7.2.

Autoclave at 121°C for 15 minutes.

432 SALT MEDIUM

Tryptone 5.0g
Proteose peptone 5.0g
NaCl 58.4g
Distilled water 1000.0ml

Adjust pH to 6.9.

433 XANTHOBACTER TAGETIDIS MEDIUM

NH₄Cl 0.4g
MgSO₄ 7H₂O 0.1g
Trace metal solution 10ml
Na₂HPO₄ 2H₂O 7.9g
KH₂PO₄ 1.5g
Oxoid bacteriological agar 15g
Sodium acetate 0.4g

Adjust pH to 7.3. Sterilise at 115°C for 10 mins. Can add phneol red to observe pH change if desired. Produces shiny yellow colonies.

Trace metal solution

Dissolve 50g EDTA (disodium salt) in about 400ml of water. Dissolve 9g NaOH in the EDTA solution. Best to do this in a 1-2L beaker on a magentic stirrer.

Dissolve the following salts individually in 30-40ml lots water and add to the EDTA - NaOH solution (plus 5-10ml washings).

ZnSO ₄ 7H ₂ O.....	11g
CaCl ₂ or CaCl ₂ 2H ₂ O	5g or 7.34g
McNL ₂ 4H ₂ O	2.5g
CoCl ₂ 6H ₂ O.....	0.5g
Ammonium molybdate.....	0.5g
FeSO ₄ 7H ₂ O	5g
CuSO ₄ 5H ₂ O	0.2g

Adjust pH to pH6 with N NaH (20-40ml approx. Add gradually with mixing).

Make up to 1L with distilled water. Store in a dark bottle. Do NOT autoclave the stock solution.

434 OM-2

(NH ₄) ₂ C ₂ O ₄ (ammonium oxalate)	10-20g
NaHCO ₃	10g
NaH ₂ PO ₄ .2 H ₂ O	10g
NaCl.....	0.7g
KCl.....	0.57g
MgCl ₂ 6 H ₂ O.....	0.1g
CaCl ₂ 2 H ₂ O.....	0.01g
Na ₂ S ₂ O ₃ 5H ₂ O (sodium thiosulfate).....	1g
Deionized water	1000ml

Adjust pH to 6.8-7.0. The first three salts must be dissolved in the indicated sequence. After autoclaving for 15 min at 121°C the pH of the medium shifted to 8.5-9.0. Solid medium contained 1.5 to 2.0% (wt/vol) of Bacto-Agar.

435 MEDIUM L10

CH ₃ COONa.3H ₂ O (Sodium Acetate Trihydrate)	6.0g
Na ₂ SO ₄	7.0g
NH ₄ Cl.....	0.25g
KH ₂ PO ₄	1.0g
NaCl.....	10g
MgCl ₂ .6H ₂ O.....	3.0g
CaCl ₂ .2H ₂ O.....	0.15g
Trace element solution (SL10)	1ml
Distilled water	1L

Glass vials or tubes, fitted with butyl or neoprene rubber septa should be used. Scrum vials with aluminium crimp seals and neoprene rubber septa are advised.

Prepare the above by dissolving in 1 litre of distilled water, then generously sparge with oxygen free nitrogen (OFN) to facilitate removal of dissolved oxygen for approximately 15 minutes. Displace oxygen from appropriately chosen vials (see above) using a stream of OFN delivered via an hypodermic syringe needle and, while continuing to gas the bottles, dispense medium into them. Immediately seal the vials

in such a way as to minimise the possibility of oxygen re-entering them. Sterilise by autoclaving at 121°C.

Reduce the medium in the vials by injecting 0.1ml filter-sterilised 1.25M Na₂S.9H₂O solution per 10ml medium.

A final pH of 6.8 is required. If adjustment is necessary (as the vials are sterile and sealed, and to ensure anoxic conditions are maintained) this should be done by injecting an appropriate volume of sterile 1M Na₂CO₃ into each vial aseptically via an hypodermic syringe and needle. To determine the volume of 1M Na₂CO₃ required, open one vial and whilst monitoring pH measure the amount required to adjust the medium to pH 6.8 for a known volume of medium.

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.0 ml
FeCl ₂ .4H ₂ O	1.5 g
ZnCl ₂	70.0 mg
MnCl ₂ .4H ₂ O	100.0 mg
H ₃ BO ₃	6.0 mg
CoCl ₂ .6H ₂ O.....	190.0 mg
CuCl ₂ .2H ₂ O.....	2.0 mg
NiCl ₂ .6H ₂ O.....	24.0 mg
Na ₂ MoO ₄ .2H ₂ O.....	36.0 mg
Distilled water	990.0 ml

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1.0 L.

436 MEDIUM L20

CH ₃ CH ₂ COONa.....	3.0g
Na ₂ SO ₄	7.0g
NH ₄ Cl.....	0.25g
KH ₂ PO ₄ .2H ₂ O	1.0g
NaCl	20g
MgCl ₂ .6H ₂ O.....	3.0g
CaCl ₂ .2H ₂ O.....	0.15g
Trace element solution (SL10).....	1ml
Distilled water	1L

Glass vials or tubes, fitted with butyl or neoprene rubber septa should be used. Scrum vials with aluminium crimp seals and neoprene rubber septa are advised.

Prepare the above by dissolving in 1 litre of distilled water, then generously sparge with oxygen free nitrogen (OFN) to facilitate removal of dissolved oxygen for approximately 15 minutes. Displace oxygen from appropriately chosen vials (see above) using a stream of OFN delivered via an hypodermic syringe needle and, while continuing to gas the bottles, dispense medium into them. Immediately seal the vials in such a way as to minimise the possibility of oxygen re-entering them. Sterilise by autoclaving at 121°C.

Reduce the medium in the vials by injecting 0.1ml filter-sterilised 1.25M Na₂S.9H₂O solution per 10ml medium.

A final pH of 6.8 is required. If adjustment is necessary (as the vials are sterile and sealed, and to ensure anoxic conditions are maintained) this should be done by injecting an appropriate volume of sterile 1M Na₂CO₃ into each vial aseptically via an hypodermic syringe and

needle. To determine the volume of 1M Na₂CO₃ required, open one vial and whilst monitoring pH measure the amount required to adjust the medium to pH 6.8 for a known volume of medium.

Trace element solution SL-10:

HCl (25%; 7.7 M)	10.0 ml
FeCl ₂ .4H ₂ O	1.5 g
ZnCl ₂	70.0 mg
MnCl ₂ .4H ₂ O.....	100.0 mg
H ₃ BO ₃	6.0 mg
CoCl ₂ .6H ₂ O.....	190.0 mg
CuCl ₂ .2H ₂ O.....	2.0 mg
NiCl ₂ .6H ₂ O	24.0 mg
Na ₂ MoO ₄ .2H ₂ O.....	36.0 mg
Distilled water	990.0 ml

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1.0 L.

437 MANGANESE SULPHATE NUTRIENT AGAR

Medium 1 plus 5mg per litre of MnSO₄

438 NITRATE STUDIES MEDIUM 1

NaCl.....	20g
KCl.....	0.5g
Na ₂ HPO ₄	5.5g
NH ₄ Cl.....	0.0127g
K ₂ SO ₄	1.75g
NaH ₂ PO ₄	0.775g
Na ₂ EDTA.....	0.75g
MgSO ₄ 7H ₂ O	0.1g
Acetate	8.3mM
NaNO ₂ -	0.0759g (1.1mM)
Wolin's Trace Elements	7ml
Distilled water	1L

439 TRYPTONE AGAR

Tryptone (Difco 0123)	8.0g
NaCl.....	8.0g
Agar	15.0g
Distilled water	1.0L

440 NITRATE STUDIES MEDIUM 2

NaCl.....	20g
KCl.....	0.5g
Na ₂ HPO ₄	5.5g
NH ₄ Cl.....	0.0127g
K ₂ SO ₄	1.75g
NaH ₂ PO ₄	0.775g
Na ₂ EDTA.....	0.75g
MgSO ₄ 7H ₂ O	0.1g
Glycerol.....	5.56mM
KNO ₃	0.111g
Wolin's Trace Elements	7ml

441 CORYNEBACTERIUM AGAR

Trypticase peptone.....	10.0g
Yeast extract	5.0g
Glucose.....	5.0g
NaCl	5.0g
Agar.....	15.0g
Distilled water	1000.0ml

Adjust pH to 7.2 - 7.4

442 DEGRYSE MEDIUM 162

Macronutrients solution 10x.....	mg/1
Nitrilotriacetic acid.....	1000
MgCl ₂ .6H ₂ O	2000
CaSO ₄ .2H ₂ O.....	400

Micronutrients solution 100x

MnSO ₄ .4H ₂ O.....	220
ZnSO ₄ .7H ₂ O.....	50
H ₃ BO ₃	50
CoCl ₂ .6H ₂ O.....	4.6
CuSO ₄ .5H ₂ O.....	2.5
Na ₂ MoO ₄ .2H ₂ O.....	2.5

For liquid medium

Macronutrients solution 10x.....	100ml
Micronutrients solution 100x	5ml
Ferric citrate (C ₈ H ₅ O ₇ Fe.5H ₂ O) 0.01M	0.5ml
Na ₂ HPO ₄ .12H ₂ O 0.2M	15ml
KH ₂ PO ₄ 0.2M.....	10ml
Yeast extract	2.5g
Tryptone	2.5g
Distilled water	Until 1000ml

Adjust to pH with NaOH

Autoclave at 121°C for 15 minutes

For solid medium

The same as liquid with 2% agar

443 TRYPTICASE SOY YEAST EXTRACT

Trypticase Soy Broth	30g
Yeast Extract	3g
Agar.....	15g
Distilled Water	1000ml

Adjust pH to 7.0 - 7.2.

444 TPY MEDIUM

Tryptone	10g
Phytone peptone	5.0g
Glucose.....	5.0g
Yeast extract.....	2.5g
Tween 80	1.0 ml
Cysteine Hydrochloride.....	0.5g
K ₂ HPO ₄	2.0g
MgCl ₂ .6H ₂ O.....	0.5g
ZnSO ₄ .7H ₂ O.....	0.25g
CaCl ₂	0.15g
FeCl ₃	Traces
Agar.....	15.0g

Distilled water 1000ml

Make up salts in 100x stock solutions. Add 1ml of each salt to 100ml of media.

Autoclave media at 15 psi for 25 mins.

445

Medium 113 plus 100µg/ml ampicillin

446

Medium 1 plus 2% glucose plus 50 µg/ml ampicillin

447

Medium 1 plus 2% glucose plus 15 µg/ml kanamycin

448 SODALIS GLOSSINIDIUS MEDIUM

Lactalbumin hydrolysate 8.1g
Yeast extract..... 6.2g
CaCl₂ 2H₂O..... 0.25g
Kcl..... 0.25g
MgCl 6 H₂O 0.12g
NaCl..... 8.7g
NaHCO₃..... 0.15g
NaH₂PO₄ 2H₂O 0.28g
D-glucose 5g
Distilled water 1 litre

Adjust pH to 8, then filter sterilise. To 4 parts of the above, add 1 part sterile foetal calf serum.

449 N-Z AMINE WITH SOLUBLE STARCH AND GLUCOSE

Glucose 10.0g
Soluble starch..... 25.0g
Yeast extract..... 5.0g
N-Z Amine Type A (Sigma C0626) 5.0g
Reagent grade CaCO₃ 1.0g
Agar 15.0g
Distilled water 1.0L

450 COLUMBIA BLOOD AGAR + 5% CITRATED SHEEP BLOOD

Columbia Peptone Mixture 23.0g
Corn Starch 1.0g
Sodium chloride 5.0g
Agar No 2..... 12.0g
Distilled water 1.0L

Cool agar to 50°C and aseptically add 5% citrated sheep blood.

451 SEA WATER BLOOD AGAR

Medium 90 plus 1.5% sea salts or NaCl plus 10% horse or sheep blood

452 CSY-3 MEDIUM

Casitone (Difco) 1.0 g
Bacto soytone (Difco)..... 1.0 g
Yeast extract (Difco) 1.0 g
Ferric ammonium citrate 0.4 g
Agar..... 15.0 g
Seawater 750 ml
Distilled water 250 ml

453 HALOMONAS MAGADIENSIS MEDIUM

Glucose..... 10.0g
Peptone (Difco) 5.0g
Yeast extract (Difco) 5.0g
KH₂PO₄ 1.0g
MgSO₄ 7H₂O..... 0.2g
NaCl 40.0g
Na₂CO₃..... 10.0g
Agar..... 20.0g
Distilled water 1 litre

Adjust pH to 10.0. NaCl and Na₂CO₃ were autoclaved separately and added to the organic components at 60°C before pouring the agar media.

454 HALOBACULUM GOMORRENSE MEDIUM

NaCl 125g
MgCl₂ 160g
K₂SO₄ 5.0g
CaCl₂ 0.1g
Yeast extract (Difco) 1.0g
Casamino acids (Difco)..... 1.0g
Starch 2.0g
Distilled water 1.0 L
Agar (if required) 20.0 g

Adjust pH to 7.0. Leave to dissolve before autoclaving or adding agar. Autoclave at 15 psi for 15 mins.

455 YEAST STARCH AGAR

Yeast extract 2 g
Soluble starch 10 g
Agar 15 g
Distilled water 1 L

Adjust pH to 7.3.

456 POLYPEPTONE YEAST EXTRACT MEDIUM

Polypeptone 10 g
Yeast extract 2 g
MgSO₄.7H₂O 1 g
Agar 15 g
Distilled water 1 L

Adjust pH to 7.0.

457 PYGS MEDIUM (MODIFIED)

Proteose peptone	5.0 g
Tryptone	5.0 g
Yeast extract.....	10.0 g
Lab Lemco powder (Oxoid L29)	10.0 g
Glucose	2.0 g
Soluble starch.....	1.0 g
Salts solution A	40.0 ml
Salts solution B	40.0 ml
Distilled water	to 1 L

Adjust pH to 6.8 with 1 Molar HCl. Autoclave at 121°C for 15 mins.

Salts solution A

	g/L of distilled water
CaCl ₂ ·2H ₂ O.....	0.26 g
MgSO ₄	0.48 g
NaCl.....	2.00 g

Salts solution B

	g/L of distilled water
KH ₂ PO ₄	1.0 g
K ₂ HPO ₄	1.3 g
NaHCO ₃	10.0 g

For liquid medium

1. Steam for 10 minutes with bottle caps loose.
2. Immediately seal caps and allow to cool prior to use

For solid medium:- Add agar at 15g/L.

458 BY AGAR

Peptone.....	5.0 g
Meat extract	5.0 g
Yeast extract.....	5.0 g
NaCl.....	2.5 g
K ₂ HPO ₄	0.1 g
MgSO ₄ 7H ₂ O	0.05 g
Agar	15.0 g
Tap water	1.0 L

Adjust pH to 7.2.

459 NUTRIENT AGAR + 0.5% YEAST EXTRACT

Add 0.5% yeast extract to Nutrient agar

460 PYG MEDIUM

Peptone.....	5.0 g
Yeast extract.....	2.5 g
Glucose	5.0 g
Distilled water	1L

PH 6.5

461 ISP 3*

Oatmeal	20 g
Trace salts solution**	1 ml
Distilled water	1 L
Agar.....	18 g

Adjust pH to 7.2.

*Manufactured by Wako Pure Chemicals Ind Ltd, Osaka, Japan.

** Trace salts solution

FeSO ₄ 7H ₂ O	0.1 g
MnCl ₂ 4H ₂ O.....	0.1 g
ZnSO ₄ 7H ₂ O	0.1 g
Distilled water	100 ml

462 POLYPEPTONE PLUS GLUCOSE

Polypeptone.....	10g
Yeast extract.....	5g
NaCl	5g
Glucose.....	1g
Distilled water	1L

pH 7.2

463 BUSHENLL-HAAS (KEROSENE-MINERAL SALTS MEDIUM

MgSO ₄ 7H ₂ O.....	0.2g
CaCl ₂	0.02g
KH ₂ PO ₄	1.0g
NH ₄ NO ₃	1.0g
K ₂ HPO ₄	1.0g
FeCl ₃ concentrated solution (15g/25 ml distilled water)	2 drops
Distilled water to	1L
Adjust pH to 6.9 – 7.0 with dilute NaOH before sterilisation. Overlay sterile kerosene on medium at time of use (2%)	

464 1/3 STRENGTH TRYPTONE SOYA AGAR

1/3 strength Medium 299

465 ACIDOCELLA MEDIUM

10 x heterotrophic basal salts solution

(g litre⁻¹)

(NH ₄) ₂ SO ₄	4.5g
KCl.....	0.5
MgSO ₄ 7H ₂ O.....	5g
KH ₂ PO ₄	0.5g
Ca(NO ₃) ₂ 4H ₂ O	0.14g

Liquid media for the growth consists of 1 x basal salts plus 1 ml trace elements solution litre⁻¹ of medium. The medium is adjusted to pH 3.0 with H₂SO₄ before autoclaving at 120°C for 20 minutes. After autoclaving, FeSO₄ 7H₂O (100 µM final concentration) and benzoate (1 mM) or fructose (5 mM) are added aseptically.

Trace Elements Solution

(g litre⁻¹ in 0.01M H₂SO₄)

ZnSO ₄ 7H ₂ O.....	10g
CuSO ₄ 5H ₂ O	1g
MnSO ₄ 4H ₂ O	1g
CoSO ₄ 7H ₂ O	1g
Cr ₂ (SO ₄) ₃ 15H ₂ O.....	0.5g
H ₃ BO ₃	0.6g
NaMoO ₄ 2H ₂ O	0.5
NaVO ₃	0.1g
NiSO ₄ 6H ₂ O.....	1g
Na ₂ SeO ₄ 10H ₂ O	1g
Na ₂ WO ₄ 2H ₂ O	0.1g

Add each of the above salts individually until they are dissolved. The vanadium may take up to 3-4 days to dissolve. This solution is autoclaved before use; autoclaving may help dissolve the vanadium more quickly. Do not wait until the vanadium has dissolved before autoclaving.

466 BLOOD AGAR 1

Medium 52 plus 7% debrinated horse blood

467 DESULFOSPOROSINUS MERIDIEI MEDIUM

Medium 17 + 2g per litre pyruvate

468 PEDOBACTER MEDIUM

Peptone.....	10.0 g
Meat extract	10.0 g
NaCl.....	5.0 g
Agar	20.0 g
Distilled water	1.0 L

Adjust pH to 7.0 – 7.2

469 ALKALIBACTERIUM MEDIUM

Solution 1

Take a flask of 1 litre and add:

(NH ₄) ₂ SO ₄	0.5g
MgSO ₄ .7H ₂ O	0.0123g
K ₂ HPO ₄	0.0874g
Na ₂ CO ₃	5.299g
Distilled Water	250ml

Solution 2

Make up 25ml 2% L-glutamic acid monosodium salt monohydrate (MW 187.13) stock solution (Sigma) Caution the compound is the salt and not the acid.

Solution 3

Make up 25ml 10% (w/v) yeast extract (Oxoid) stock solution.

Solution 4

Add 10.0g Bacteriological agar to 200ml H₂O.

Solutions 1, 2, 3 and 4 are autoclaved separately.

Combine the above solutions to give a total volume of 500ml and pour into petri dishes.

470 LUEDEMANN MEDIUM

Yeast Extract	0.5%
Malt Extract Broth.....	1.5%
Soluble Starch	1%
Glucose.....	1%
CaCO ₃	0.2%
NaCl	0.5%
Agar.....	1.5%
H ₂ O distilled.....	100ml
pH.....	8.6

Sterilise at 121°C for 15 mins.

471 TRYPTICASE SOY AGAR + 5% DEFIBRINATED SHEEP BLOOD

Medium 299 + 5% defibrinated sheep blood

472 METHYLOCAPSA ACIDOPHILA MEDIUM

KH ₂ PO ₄	0.10 g
MgSO ₄	0.05g
CaCl ₂ 2H ₂ O	0.01g
KNO ₃	0.10g
Trace element solution.....	1.00 ml
Distilled H ₂ O	1 00 L

pH 5.0-5.8

Trace element solution

EDTA	5.00 g
FeSO ₄ .7H ₂ O	2.00 g
ZnSO ₄ .7H ₂ O.....	0.10 g
MnCl ₂ .4H ₂ O.....	0.03 g
CoCl ₂ .6H ₂ O.....	0.20 g
CuCl ₂ . 5H ₂ O.....	0.10 g
NiCl ₂ . 6H ₂ O.....	0.02 g
NaMoO ₄	0.03 g
Distilled Water	1000ml

473 MARINOCOCCUS ALBUS MEDIUM

NaCl	81.0g
MgCl ₂ .6H ₂ O.....	7.0g
MgSO ₄ .7H ₂ O.....	9.6g
CaCl ₂	0.36g
KCl.....	2.0g
NaHCO ₃	0.06g
NaBr	0.026g
Protease Peptone No 3.....	5.0g
Yeast extract.....	10.0g
Glucose.....	1.0g
Distilled H ₂ O.....	1 Litre

pH 7.2 (Agar 1.5%).

474 VNSS MEDIUM

NSS

.....	Per Litre
NaCl.....	17.6g
Na ₂ SO ₄	1.47g
NaHCO ₃	0.08g
KCl.....	0.25g
Kbr.....	0.04g
MgCl ₂ .6 H ₂ O.....	1.87g
CaCl ₂ . 2H ₂ O.....	0.41g
SrCl ₂ .6 H ₂ O.....	0.008g
H ₃ BO ₃	0.008g

Adjust to pH 7.

Add the following per litre NSS to make VNSS medium

Peptone.....	1.0g
Yeast extract.....	0.5g
Glucose.....	0.5g
Fe SO ₄ .7H ₂ O.....	0.01g
Na ₂ HPO ₄	0.01g

475 XMM MEDIUM

FeSO ₄	0.002%
MgSO ₄	0.02%
KNO ₃	0.075%
K ₂ HPO ₄	0.05%
CaCl ₂	0.004%
Soluble xylan.....	0.5%

(If mostly insoluble xylan is available then 1% would be necessary)

Adjust pH to 7.2 with 1M NaOH or 1M HCl. The agar is prepared by the addition of 1.5% (w/v) Oxoid Bacteriological agar. The medium should be sterilised by autoclaving at 121°C for 15 minutes.

476 BRACKIELLA OEDIPODIS MEDIUM

Medium 119 + 5% sheeps blood

477 MH MEDIUM

Yeast extract (Difco).....	10.0g
Protease peptone no 3 (Difco).....	5.0g
Glucose.....	1.0g
NaCl.....	60.7g
MgCL ₂ 6H ₂ O.....	15.0g
MgSO ₄ 7H ₂ O.....	7.4g
CaCl ₂	0.27g
KCl.....	1.5g
NaHCO ₃	45mg
NaBr.....	19mg
Agar.....	20.0g
Distilled water.....	1 Litre

478 BACTO LACTOBACILLI MRS BROTH

Ingredients per litre

Bacto Proteose Peptone No 3.....	10g
Bacto Beef Extract.....	10g
Bact Yeast Extract.....	5g

Dextrose.....	20g
Sorbitan Monooleate Complex.....	1g
Ammonium Citrate.....	2g
Sodium Acetate.....	5g
Magnesium Acetate.....	0.1g
Manganese Sulfate.....	0.05g
Disodium Phosphate.....	2g

Final pH 6.5 ± 0.2 at 25°C

One pound will make 8.25 litres of medium.

1. Suspend 55g in 1 litre of distilled or deionized water and heat to boiling to dissolve completely.
2. Distribute into tubes, bottles, or flasks as desired.
3. Sterilize in the autoclave for 15 minutes at 15lbs pressure (121°C)

479 GLUCOSE BROTH BUFFERED + YEAS

Medium 79 +10g/L yeast extract

480 RAE MEDIUM

Glucose.....	40.00 g
Peptone.....	10.00 g
Yeast extract.....	10.00 g
Citric acid x H ₂ O.....	1.50 g
Na ₂ HPO ₄ x 2H ₂ O.....	3.38 g
Glacial acetic acid.....	10.00 ml
Absolute ethanol.....	10.00 ml
Distilled water.....	980.00 ml

Final pH ca. 3.8 (pH is not adjusted).

Both liquid and solid media are prepared by autoclaving the medium without the addition of glacial acetic acid and absolute ethanol. Glacial acetic acid and absolute ethanol are sterilized either by filtration (use Teflon filters) or they may be autoclaved in completely closed screw cap glass bottles sealed with a Teflon coated septum. The preparation of solid media employs a double layer system. A layer of growth medium containing 0.5% agar (about 50 ml) is poured into a plate and allowed to solidify. A thin layer (5-10 ml) of the growth medium containing 1% agar is then poured on top. Carefully streak the plates and incubate in closed containers to keep the humidity high (>90%).

481 GLUCOSE SULPHITE MEDIUM

Glucose.....	0.15g
(NH ₄) ₂ SO ₄	0.5g
CaCO ₃	0.1g
Ca(NO ₃) ₂	0.1g
KCl.....	0.05g
K ₂ HPO ₄	0.05g
MgSO ₄ 7H ₂ O.....	0.05g
Na ₂ S 9H ₂ O.....	0.187g
Vitamin Solution.....	1.00ml
Agar (if required).....	15g
Distilled water.....	1L

pH 7.3 (±0.2)

Vitamin solution (Eikelboom 1975)

Biotin	5.0µg
Calcium pantothenate.....	0.1mg
Coccarboxylase.....	0.1mg
Cyanocobalamin (Vitamin B12)	5.0 µg
Folic acid.....	5.0 µg
Inositol	0.1mg
Niacin.....	0.1mg
p-aminobenzoic acid	0.1mg
Pyridoxine	0.1mg
Riboflavin	0.1mg
Thiamine	0.1mg
Distilled water	1L

Filter, sterilise and add aseptically after autoclaving base medium at 121°C for 15 mins. Add the vitamin solution to sterilised solid media before pouring and to liquid media after cooling.

482 CAV MEDIUM

Casamino acids (Difco).....	2.0g
(NH ₄) ₂ SO ₄	0.5g
Basal salt solution	10mls
200mM Potassium phosphate	25mls
buffer (pH 7.0)	
Agar	15.0g
Distilled water	1L

Basal Salt solution

Fe SO ₄ 7H ₂ O.....	1.11g
Mg SO ₄ 7H ₂ O	24.65g
CaCl ₂ 2H ₂ O.....	2.94g
NaCl.....	23.4g
MnSO ₄ 4H ₂ O	111mg
ZnSO ₄ 7H ₂ O.....	28.8mg
Co(NO ₃) ₂ 6H ₂ O.....	29.2mg
CuSO ₄ 5H ₂ O	25.2mg
Na ₂ MoO ₄ 2H ₂ O.....	24.2mg
H ₃ BO ₃	31.0mg
Trisodium EDTA	4.53mg
Distilled water	1L

483 NPG AGAR

Difco Potato dextrose agar	19.5g
Difco Nutrient agar	15.5g
Distilled water	1L

484 TRYPTONE SOY MEDIUM + MNSO4

Tryptone Soy Agar/Broth + 5 mg/l of MnSO₄

485 BFA MEDIUM

Yeast extract.....	4.0g
(NH ₄) ₂ SO ₄	2.0g
KH ₂ PO ₄	3.0g
Solution A	4ml
Solution B	4ml

pH 5.5

For solid medium add 5 mg MnSO₄ 4H₂O and 18g of Agar

Solution A

(NH ₄) ₂ SO ₄	125.0g
MgSO ₄ 7H ₂ O.....	50.0g

Solution B

CaCl ₂	62.5g per litre
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486 GLUCOSE YEAST EXTRACT MEDIUM

Glucose.....	10.0 g
Yeast extract	10.0 g
Distilled water	1.0 L

pH 6.8 ± 0.2

Agar if required

15.0 g

487 MINIMAL MEDIUM + CHLOROMETHANE FOR STRAIN CC495

KH ₂ PO ₄	4.5g
K ₂ H PO ₄	10.5g
MgSO ₄ 7H ₂ O.....	0.15g
NH ₄ NO ₃	1.5g
Trace element solution.....	10ml
Vitamin solution.....	5ml
Distilled water	1L

Adjust pH to 7.2 with 6M NaOH

Incubate in an atmosphere of 2% (vol/vol) chloromethane (CH₃ Cl)

Trace element solution

H ₃ BO ₃	500mg
Cn SO ₄ 5H ₂ O.....	40mg
KI.....	100mg
FeSO ₄ 7H ₂ O	200mg
MnSO ₄ 7H ₂ O.....	400mg
(NH ₄) ₆ Mo ₇ O ₂₄ 4H ₂ O	200mg
Zn SO ₄	400mg
Distilled water	1L

Vitamin solution

Folic acid	4mg
p-aminobenzoic acid	200mg
Cyanocobalamin.....	200mg
Distilled water	1L

488 HCMM2 MEDIUM

Buffer solution.....	10ml
Nutrient solution.....	10ml
Iron-EDTA solution.....	1.0ml
Micronutrient solution	1.0ml
Casamino acid solution	2.0ml
OR Casamino acid	0.30g
Milli-Q water	up to 1L
Autoclave	

Buffer solution

KH₂PO₄ 34.0g
Na₂HPO₄ 39.5g
Milli-Q water..... up to 250ml
Autoclave

Naerings solution

KNO₃ 12.5g
(NH₄)₂ SO₄..... 59.5g
MgSO₄ 7H₂O 1.25g
CaCl₂·2H₂O 0.33g
Milli-Q water..... up to 250ml
Autoclave

Iron-EDTA solution

Fe (III) – EDTA..... 0.643g
Milli-Q water..... up to 250ml
Autoclave

Micronutrient solution

H₃BO₃ 0.725g
MnSO₄ H₂O 0.385g
CuSO₄ 5H₂O 0.00975g
ZnCl 0.00501g
OR ZnSO₄ 7H₂O 0.0149g
CoCl₂ 6H₂O..... 0.0103g
Na₂MoO₄ 2H₂O..... 0.00613g
Milli-Q water..... up to 250ml
Sterile filtrate

Casamino acid solution (150g litre⁻¹)

Casamino acid..... 7.5g
Milli-Q water..... up to 50ml
Sterile filtrate

Phenanthrene is added from an acetone solution (eg 5mg phenanthrene ml⁻¹ (0.5% w/v)). Phenanthrene is soluble at approximately 1 mg/l in water, so if degradation is to be followed on GC, only this amount should be added. If just a growing culture is to be obtained, five times the amount could be added. The solution is added to each culture flask and allowed to evaporate under sterile conditions.

Solid medium is made by addition of 2.0% Noble Agar (Difco). Let plates dry (30-45 min) and apply on top of each plate 750-1000 µl of a phenanthrene solution in acetone (5mg phenanthrene ml⁻¹ (0.5%)), allow acetone to evaporate. An opaque layer of phenanthrene will remain and clearing zones will appear around colonies degrading the phenanthrene.

pH: 7

489 SOIL EXTRACT AGAR 2

Soil Extract - garden soil (1 kg) + 1 litre of water + autoclave 15 psi/ 30 mins.

Add 2 g CaCO₃, filter and make up to 1 litre.

Use 250 mls of soil extract + 750 mls H₂O + add 28 gms CM₃.

Autoclave 121°C /15 mins

490 CIP MEDIUM

Columbia Agar Base.....39.0g
Horse blood 100ml
Distilled water to 1.0L

pH 7.3 ± 0.2. Autoclave 121°C for 15 minutes.

491 MODIFIED BENNETTS AGAR

Yeast Extract 1.0g
Lab Leinco.....0.8g
Casitone.....2.0g
Glucose.....10.0g
Agar.....18.0g
Distilled water 1L

Adjust pH to 4.5 to 5.5 with HCl.

492 MSP MEDIUM

Mannitol 10g
KH₂PO₄ 0.5g
Sodium Glutamate.....0.5g
NaCl 50mg
Solution A 10ml
Solution B..... 1ml
Solution C..... 1ml
Yeast Extract 1g
Agar (if required)..... 20g
Distilled water to 1L

pH 6.8.

Solution A

MgSO₄ 7H₂O..... 1g
Distilled water 100ml

Solution B

CaCl₂ 2H₂O 5.28g
Distilled water 100ml

Solution C

FeCl₃ 6H₂O..... 666mg
Distilled water 100ml

493 YMSA MEDIUM

Yeast Extract 1.0g
Mannitol 10.0g
Agar (if required)..... 15.0g
Soil Extract..... 200ml
Distilled water 800ml

pH 7.0.

Soil Extract

Air-dried garden soil..... 80.0g
Na₂CO₃..... 0.2g
Distilled water 200ml

Autoclave soil suspension for one hour at 121°C. To obtain a clear supernatant allow to settle and centrifuge. Adjust to 7.2 (pH).

494 PYF MEDIUM

Peptone.....	5.0g
Tryptone.....	5.0g
Yeast Extract.....	10.0g
Fructose.....	5.0g
Na ₂ HPO ₄	2.0g
Tween 80.....	1.0g
Cysteine HCl.....	0.5g
Distilled water to.....	1L

pH 7.0.

Add all components except fructose. Stir until dissolved. Steam for 15 minutes then add fructose, pH to 7.0. Add agar (1.5%) if required then autoclave for 15 minutes at 121°C.

495 HALOCOCCUS DOMBROWSKII MEDIUM

Casein hydrolysate.....	5.0g
Yeast extract.....	5.0g
NaCl.....	200.0g
MgCl ₂ .6H ₂ O.....	20.0g
KCl.....	2.0g
CaCl ₂ .2H ₂ O.....	0.2g
Tris.....	12.1g

pH 7.4

Autoclave at 121 °C for 15 minutes.

496 YCFA GSC

Bacto casitone.....	1.00g
Yeast extract.....	0.25g
NaHCO ₃	0.40g
Glucose.....	0.20g
Maltose.....	0.20g
Cellobiose.....	0.20g
Min I.....	15.00ml
Min II.....	15.00ml
VFA mix.....	0.31ml
Haemin solution.....	1.00ml
Vitamin solution 1.....	100.00 mcl
	before autoclaving
Vitamin solution 2.....	100.00mcl
	before autoclaving
Resazurine.....	0.10ml
L-cysteine.....	0.10g
Distilled water.....	up to 100ml

VFA mix

Acetic acid.....	17.00ml
Propionic acid.....	6.00ml
n-Valeric acid.....	1.00ml
Isovaleric acid.....	1.00ml
Isobutyric acid.....	1.00ml

The VFA mix does not contain butyric acid for the purposes of the VFA analysis. The formula of the haemin and vitamin solutions are explained below.

Haemin solution

KOH.....	0.28g
Ethanol 95%.....	25.00ml
Haemin.....	100.00mg
Distilled water.....	up to 100.00ml

Vitamin solution 1

Biotin.....	1.00mg
Cobalamin.....	1.00mg
PABA.....	3.00mg
Folic acid.....	5.00mg
Pyridoxamine.....	15.00mg
Distilled water.....	up to 100.00ml

Vitamin solution 2

Thiamin.....	5.00mg
Riboflavin.....	5.00mg
Distilled water.....	up to 100.00ml

Vitamin solution 2 is added to the medium after autoclaving as a filtered-sterilised solution.

The pH of the medium is adjusted to 7.3 with NaOH before autoclaving and it will drop to about 6.8 after autoclaving.

Mineral I

K ₂ HPO ₄	3.00g
Distilled water.....	1.00L

Mineral II

KH ₂ PO ₄	3.00g
(NH ₄) ₂ SO ₄	6.00g
NaCl.....	6.00g
MgSO ₄	0.60g
CaCl ₂ (dry).....	0.60g
Distilled water.....	1.00L

Boil medium gently while adding ingredients except Cysteine HCl. Add the cysteine last after the medium has been cooled by bubbling oxygen free CO₂ through it. When the medium has cooled to below 60°C, the pH is checked and adjusted if required. The complete medium is dispensed into appropriate vessels under a CO₂ blanket, immediately sealed and autoclaved.

497 ALCANIVORAX BORKUMENSIS MEDIUM

NaCl.....	23.00 g
MgSO ₄ .7H ₂ O.....	5.80 g
MgCl ₂ .2H ₂ O.....	6.16 g
CaCl ₂ .2H ₂ O.....	1.47 g
Na ₂ HPO ₄ .7H ₂ O.....	0.89 g
NaNO ₃	5.00 g

FeSO ₄ ·7H ₂ O	0.03 g
Sodium pyruvate	10.00 g
Distilled water	1.00 L

pH 7.0 – 7.5

498 HORIKOSHI-I MEDIUM

Glucose	10.0g
Polypepton (Nihon Seiyaku)	5.0g
Yeast extract.....	5.0g
K ₂ HPO ₄	1.0g
MgSO ₄ 7H ₂ O	0.2g
Agar	15.0g
Distilled water	900ml

After autoclaving for 15 minutes at 121°C, aseptically add 100ml of a 10% Na₂CO₃ solution to the medium. Check final pH to be about 10.0.

499 CBM MEDIUM

Glucose	10g
MgSO ₄ 7H ₂ O	0.2g
MnSO ₄ 4H ₂ O	0.01g
FeSO ₄ 7H ₂ O	0.01g
<i>p</i> -aminobenzoic acid	1mg
biotin	2ug
thiamine. HCl.....	1mg
casein hydrolysate (Oxoid).....	4g

Sterilise by autoclaving at 15lb/in² for 15 min and K₂HPO₄ plus KH₂PO₄ were then added aseptically from sterile solutions each to final concentrations of 0.5g/l which brought the final pH value to 6.9.

1500 MODIFIED HS-1 MEDIUM

For aerobic growth:

Solution 1

NaCl.....	25.0g
KCl.....	3.8g
MgSO ₄ 7H ₂ O	9.6g
CaCl ₂ 2H ₂ O.....	0.5g
MgCl ₂ 6H ₂ O.....	7.0g
Na ₂ CO ₃	3.0g
K ₂ HPO ₄	0.4g
Glucose	4.0g
NH ₄ Cl.....	1.0g
Solution S1	10ml

pH (adjust with solution KOH 10M) to 7.3

Autoclave @ 121°C for 15 mins.

Add separately sterile stock solutions to give final concentrations of:

Solution 2 - 0.5% glucose

Soluton 3 - 0.1% yeast extract

For anaerobic growth:

Prepare all of the above, but using Hungate procedures. (N₂/CO₂; 70:30 v/v). Also add sterile stock solutions (prepared again using Hungate techniques) to give final concentrations of:

Solution 4 - 0.05% L-cysteine

Solution 5 - 0.3% NaHCO₃

Solution 6 - 0.05% Na₂S 9H₂O

Resazun may be used as a redox indicator if deisred (add 0.001g per litre of solution 1).

1501 DIFCO M17

Dehydrated Difco M17

1502 PEPTONE-SUCCINATE-SALTS MEDIUM (PSS)

Peptone (Difco 0118)	10.0g
Succinic acid	1.0g
(NH ₄) ₂ SO ₄	1.0g
MgSO ₄ 7H ₂ O.....	1.0g
FeCl ₃ 6H ₂ O.....	2.0mg
MnSO ₄ H ₂ O.....	2.0mg
Distilled water	1L

Adjust pH to 6.8 with KOH. Autoclave at 121°C for 15 minutes.

1503 CAMPYLOBACTER MEDIUM

NaCl	150.0g
KN03	2.0g
M Plate Count Broth (Difco 0751)*	17.0g
Agar (for solid medium)	20.0g
Distilled water	1L

* OR

Yeast extract	5.0g
Tryptone	10.0g
Dextrose	2.0g

Autoclave to 121°C for 15 minutes

1504 HALOBACTERIUM MEDIUM

Casamino acids (Difco)	5.0g
Yeast extract (Difco)	5.0g
Sodium glutamate.....	1.0g
Trisodium citrate	3.0g
MgSO ₄ 7H ₂ O.....	20.0g
KCl.....	2.0g
NaCl	200.0g
FeCl ₂ 4H ₂ O.....	36.0mg
MnCl ₂ 4H ₂ O.....	0.36mg
Agar.....	20.0g

Add components to distilled water and bring volume to 1.0L Adjust pH to 7.0 – 7.2. Autoclave to 15 min for 121°C.

1505 MRS + 2.5% NaCl pH 8.5

Medium 20 + 2.5% NaCl.

Adjust pH to 8.5.

1506 ROLLED OATS MEDIUM

Rolled Oats.....	20.0g
Agar	12.0g
Trace Elements Solution	1.0ml
Distilled Water	1000.0ml

Rolled Oats are boiled for 20 min and filtered, then add agar. Make up to 1000ml. Then add trace elements:

Trace Element Solution

FeSO ₄ 7H ₂ O	0.1g
MnCl ₂ 4H ₂ O	0.1g
ZnSO ₄ 7H ₂ O	0.1g
Distilled Water	100ml

Autoclave 121°C for 15 mins

1507 GYM STREPTOMYCES MEDIUM

Glucose	4g
Yeast Extract	4g
Malt Extract	10g
CaCO ₃	2g
Agar	12g
Distilled Water	1000ml

Adjust pH to 7.2 with KOH before adding agar (use pH-indicator paper)

Autoclave 121°C for 15mins.

1508 ALKALINE NUTRIENT AGAR 3

Medium 1 + 2.5% NaCl

For Bacillus strains the addition of 10mg per litre MnSO₄H₂O is recommended

Autoclave 15/15.

After sterilisation add sterile 1M Na-sesquicarbonate solution (1ml in 10ml) to achieve a pH of 9.7.

Na-sesquicarbonate solution

NaHCO ₃	4.2g
NaCO ₃ anhydrous	5.3g
Distilled water	100ml

1509 FLAVOBACTERIUM MEDIUM

Lactose	5g
Yeast Extract	1g
NH ₄ Cl	0.5g
K ₂ HPO ₄	0.5g
NaCl	0.5g
Distilled water	1000ml

Adjust pH to 7.2 and autoclave 15/15.

1510 M-R2A MEDIUM

NH ₄ Cl	0.8g
KH ₂ PO ₄	0.25g
K ₂ HPO ₄	0.4g
KNO ₃	0.505g
CaCl ₂ 2H ₂ O	15.0mg

MgCl ₂ 6H ₂ O	20.0mg
FeSO ₄ 7H ₂ O	7.0mg
Na ₂ SO ₄	5.0mg
MnCl ₂ 4H ₂ O	5.0mg
H ₃ BO ₃	0.5mg
ZnCl ₂	0.5mg
CoCl ₂ 6H ₂ O	0.5mg
NiSO ₄ 6H ₂ O	0.5mg
CuCl ₂ 2H ₂ O	0.3mg
Na ₂ MoO ₄ 2H ₂ O	10.0mcg
Yeast Extract	0.5g
Peptone	0.5g
Casamino acids	0.5g
Dextrose	0.5g
Soluble Strach	0.5g
Sodium Pyruvate	0.5g
Agar	15.0g
Distilled Water	1L

Adjust medium for final pH 7.0.

Autoclave 121°C for 15 minutes.

1511 DESULFITOBACTERIUM HAFNIENSE MEDIUM

NH ₄ Cl	1g
NaCl	0.1g
MgCl ₂ 6H ₂ O	1g
CaCl ₂ 2H ₂ O	0.05g
K ₂ HPO ₄	0.4g
Resazurin	0.5mg
Trace Element Solution SL-10 (see medium 435) with 0.5g/1 Na ₂ EDTA	1ml
Selenite/Tungstate Solution	1ml
Yeast Extract	1g
NaHCO ₃	2.6g
Distilled water	1000ml

pH to 7.0

Prepare medium anaerobically under OFN.

Add bicarbonate after the heating of medium.

Autoclave at 15/15.

Also prepare 10ml stock solutions of

Vitamin solution (see medium 296) to be added at	0.1ml per 10ml
Na-pyruvate (25%, w/v)	0.1ml per 10ml
Na-thiosulfate x 5H ₂ O (25%, w/v)	0.05ml per 10ml
Na ₂ S x 9H ₂ O (3%, w/v)	0.1ml per 10ml

Selenite/Tungstate Solution

NaOH	0.5g
Na ₂ SeO ₃ x 5H ₂ O	3mg
Na ₂ WO ₄ x 2H ₂ O	4mg
Distilled Water	1000ml

1512 ANCYLOBACTER NATRONUM MEDIUM

KH ₂ PO ₄	1.4g
(NH ₄) ₂ SO ₄	3.0g
MgSO ₄ .7H ₂ O	0.2g
Na ₂ HPO ₄	3.0g

Ferric citrate	30mg
CaCl ₂ .2H ₂ O.....	30mg
MnCl ₂ .4H ₂ O.....	5mg
ZnSO ₄ .7H ₂ O.....	5mg
CuSO ₄ .5H ₂ O.....	0.5mg
Yeast Extract.....	0.2g
Methanol.....	10ml
Distilled Water	1000ml

pH to 8.0

1513 FILOBACILLUS MEDIUM

Peptone.....	0.75g
Yeast Extract.....	0.75g
Glucose	0.75g
NaCl.....	100.0g
Modified Hutner's Basal Salts (see medium 249).....	20.0ml
Wolfe's Vitamin Solution (see medium 205).....	20.0ml
Artificial Seawater (see below).....	250.0ml
0.1M Tris/HCl Buffer (pH 7.5).....	50.0ml
Distilled Water	660.0ml

For a solid medium, use 18 grams of agar and adjust the pH of the Tris/HCl buffer to 8.5.

Autoclave at 121 °C for 15 mins.

Artificial Seawater

NaCl.....	23.47g
MgCl ₂	4.98g
Na ₂ SO ₄	3.92g
CaCl ₂	1.10g
KCl.....	0.66g
NaHCO ₃	0.19g
KBr.....	0.10g
H ₃ BO ₄	0.026g
SrCl ₂	0.024g
NaF.....	0.003g
Distilled Water	1000ml

1514 PYA MEDIUM

Peptone.....	8g
Yeast Extract.....	3g
Agar	15g
K ₂ HPO ₄	1g
EDTA.....	3.5mg
ZnSO ₄ .7H ₂ O.....	3mg
FeSO ₄ .7H ₂ O.....	10mg
MnSO ₄ .H ₂ O.....	2mg
CuSO ₄ .5H ₂ O.....	1mg
Co(NO ₃) ₂ .6H ₂ O.....	2mg
H ₃ BO ₃	1mg

Add ingredients to 1 litre NaHCO₃/Na₂CO₃ buffer (100mM in deionized H₂O; pH 10).

Autoclave 121 °C for 15 minutes

1515 CHLOROBIVM LIMICOLA MEDIUM

K ₂ HPO ₄	0.5g
MgSO ₄	0.5g

NH ₄ Cl.....	1g
NaHCO ₃	1g
Distilled Water	1000ml

Dissolve in water and gas with OFN for 15 mins , then add Na₂S.9H₂O at 0.1%.

Adjust pH to 6.0.

Dispense into 'SRB' vials under OFN and autoclave at 121 °C for 15 minutes.

1516 METHYLOCELLA MEDIUM

NaNO ₃	0.2g
MgSO ₄ .7H ₂ O.....	0.2g
CaCl ₂ .2H ₂ O.....	0.06g
Fe EDTA	0.004g
Trace Element Solution (see medium 131 sol. 4).....	0.5ml
Agar.....	15g
Distilled Water	1000ml

Autoclave at 121 °C for 15 minutes.

After autoclaving, cool to 50 °C and aseptically add 1mM of pH5.8 Citric acid/Na₂HPO₄ buffer (see below) before dispensing.

Make up 1.92g citric acid in 100mls water.

Make up 2.84g Na₂HPO₄ in 100ml water.

Add 7.91ml of citric acid to 12.09ml of Na₂HPO₄ check pH 5.8.

Add 10ml of above to 1000ml of medium.

1517 YEAST MOULD MEDIUM (DIFCO)

Yeast Extract	3.0g
Malt Extract.....	3.0g
Peptone.....	5.0g
Dextrose	10.0g
Distilled Water	1000ml

Final pH 6.2

For solid medium add 20grams per litre of Bacto agar.

1518 POLYPEPTONE YEAST GLUCOSE MEDIUM

Polypeptone.....	5g
Yeast Extract	5g
Glucose.....	5g
MgSO ₄ .7H ₂ O.....	1g
Distilled Water	1000ml
Agar.....	15g

pH 6.6 – 7.0

1519 BACILLUS KRULWICHII MEDIUM

Peptone.....	8g
Yeast Extract	3g
K ₂ HPO ₄	1g
Na ₂ CO ₃	10g
Agar.....	15g

Adjust pH to 10.

1520 HALOMONAS MEDIUM

NaCl.....	100g
Bacteriological agar	15g
MgSO ₄ ·7H ₂ O	10g
Casein hydrolysate	5g
KCl.....	5g
Trisodium citrate.....	3g
KNO ₃	1g
Yeast Extract.....	1g
CaCl ₂ · 6H ₂ O.....	0.2g
Distilled Water	1000ml

Adjust pH to 7.4 - 7.6 with 1M NaOH, and sterilise by autoclaving at 121°C for 15 minutes.

1521 TEAG-ASC CORYNEFORM MEDIUM

Peptone.....	1%
Casamino Acids	0.5%
Yeast Extract.....	0.5%
Malt Extract	0.5%
Tween 80.....	1%

1522 TH AGAR

Peptone.....	5g
Yeast Extract.....	3g
NaCl.....	5g
EDTA.....	3.5mg
ZnSO ₄ ·7H ₂ O.....	3.0mg
FeSO ₄ ·7H ₂ O.....	10.0mg
MnSO ₄ ·H ₂ O	2.0mg
CuSO ₄ ·5H ₂ O	1.0mg
Co(NO ₃) ₂ ·6H ₂ O.....	2.0mg
H ₃ BO ₃	1.0mg
Distilled Water	1000ml

pH to 7.5, autoclave 15/15.

1523 NUTRIENT AGAR + 100mg/L AMPICILLIN

Medium 1 + 100mg/L Ampicillin.
Add Ampicillin after nutrient agar has been autoclaved and cooled to 50°C.

1524 PFENNIG'S MEDIUM

Solution A:

CaCl ₂ x 2 H ₂ O	1.25 g
KH ₂ PO ₄	1.70 g
NH ₄ Cl	1.70 g
KCl	1.70 g
MgSO ₄	2.50 g
Distilled water	4000.00 ml

(For marine or estuarine isolates add 100.0 g NaCl to this solution and increase the MgSO₄ x 7 H₂O to 15.0 g).

Solution B:

Distilled water	860.00 ml
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Autoclave in a cotton-stoppered Erlenmeyer flask and cool to room temperature under an atmosphere of N₂ in an anaerobic jar.

Solution C:

Vitamin B ₁₂ solution (0.002% in H ₂ O)	5.00 ml
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Filter sterilize.

Solution D: Trace element solution (SL-12 B) 5.00 ml Autoclave at 121°C for 15 min.

Solution E:

NaHCO ₃	7.50 g
H ₂ O	100.00 ml

Bubble with CO₂ and, after saturation, filter sterilize under CO₂ pressure into sterile, gas-tight, 100 ml screw-cap bottle.

Solution F:

Na₂S x 9 H₂O (10 g in 100 ml) 20.00 ml Prepare in a screw-cap bottle, bubble with N₂ to replace air, close tightly and autoclave.

Trace element solution SL-12 B:

Distilled water	1000.00 ml
Na ₂ -EDTA	3.00 g
FeSO ₄ x 7 H ₂ O	1.10 g
CoCl ₂ x 6 H ₂ O	190.00 mg
MnCl ₂ x 2 H ₂ O	50.00 mg
ZnCl ₂	42.00 mg
NiCl ₂ x 6 H ₂ O	24.00 mg
Na ₂ MoO ₄ x 2 H ₂ O	18.00 mg
H ₃ BO ₃	300.00 mg
CuCl ₂ x 2 H ₂ O	2.00 mg

Adjust pH to 6.0. Autoclave solution A for 45 min. in 5-litre special bottle or flask (with four openings at the top) at 121°C, together with a teflon-coated magnetic bar.

In this 5-litre bottle, two openings for tubes are in the central, silicon rubber stopper; a short, gas-inlet tube with a sterile cotton filter; and an outlet tube for medium, which reaches the bottom of the vessel at one end and has, at the other end, a silicon rubber tube with a pinch cock and a bell for aseptic dispensing of the medium into bottles. The other two openings have gas-tight screw caps; one of these openings is for the addition of sterile solutions and the other serves as a gas outlet. After autoclaving cool solution A to room temperature under a N₂ atmosphere with a positive pressure of 0.05 - 0.1 atm (a manometer for low pressure will be required).

Saturate the cold medium with CO₂ by magnetic stirring for 30 min. under a CO₂ atmosphere of 0.05 - 0.1 atm. Add solution B, C, D, E and F through one of the screw-cap openings against a stream of either N₂ gas or better, a mixture of 95% N₂ and 5% CO₂ while the medium is magnetically stirred.

Adjust the pH of the medium with sterile HCl or Na₂CO₃ solution (2 mol/liter each) to pH 7.3. Distribute the medium aseptically through the medium outlet tube into sterile, 100 ml bottles (with metal caps and autoclavable rubber seals) using the positive gas pressure (0.05 - 0.1 atm) of the N₂ /CO₂ gas mixture: Leave a small air bubble in each bottle to meet possible pressure changes. The tightly sealed, screw-cap bottles can be stored for several weeks or months in the dark. During the first 24 h, the iron of the medium precipitates in the form of black flocks. No other sediment should arise in the otherwise clear medium. Incubate in the light using a tungsten lamp. Feed periodically with neutralized solution of sodium sulfide (see below to replenish sulfide and with other supplement solutions.

Neutralized sulfide solution:

Distilled water	100.00 ml
Na ₂ S x 9 H ₂ O	1.50 g

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.3 by adding of sterile 2 M H₂ SO₄ drop-wise with a syringe without opening the bottle. Appearance of a yellow colour indicates the drop of pH to about 8. The solution should be stirred continuously to avoid precipitation of elemental sulfur. The final solution should be clear and is yellow in colour.

1525 TWEEN 80 AGAR

Solution A

Peptone.....	10g
NaCl.....	5g
CaCl ₂ ·2H ₂ O.....	0.1g
Agar	15g
Distilled Water	900ml

Solution B

Tween 80.....	10g
Distilled Water	100ml

Autoclave solutions A+B separately and combine after cooling to 50°C.

1526 HALOPHILIC METHANOTROPH MEDIUM

MgSO ₄ ·7H ₂ O	0.1g
NH ₄ Cl.....	0.5g
Na ₂ HPO ₄ ·2 H ₂ O	0.7g
KH ₂ PO ₄	0.3g
NaCl.....	50.0g
CaCl ₂ ·6H ₂ O.....	0.01g
FeSO ₄ ·7 H ₂ O	0.005g
Trace Element Solution (Med 131 Sol.4)	1ml
Distilled Water	1000ml
Agar	at 1.5%

Adjust pH to 8.2 with 10% NaOH.

1527 HALOBACTERIUM NORICI MEDIUM

NaCl.....	250.0g
MgSO ₄ ·7H ₂ O	20.0g
Trisodium Citrate 2H ₂ O	3.0g
Tryptone.....	5.0g
Yeast Extract.....	3.0g
Trace Element Solution (Med 131 Sol.4)	1ml
Distilled Water	1000ml
Agar	at 2%

Adjust pH to 7.0.

1528 VAN DROOGENBROOK AND LAUDELOUT'S MEDIUM

NaNO ₂	1.38g
Na ₂ HPO ₄ ·2H ₂ O	6.4g
KH ₂ PO ₄	0.54g
Stock Solution A	1ml
Stock Solution B	1ml
Distilled Water	1000ml

Stock Solution A

FeSO ₄	0.05g
NaEDTA	0.05g
Distilled Water	100ml

Stock Solution B

MgSO ₄ ·7H ₂ O	1g
CuSO ₄ ·5H ₂ O	0.02g
ZnSO ₄ ·7H ₂ O.....	0.02g
NaMoO ₄ ·2H ₂ O.....	0.02g

Distilled Water	100ml
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1529 CYT MEDIUM

Pancreatic Digest of Casein (Casitone)	1.0g
CaCl ₂ ·2H ₂ O.....	0.5g
MgSO ₄ ·7H ₂ O.....	0.5g
Yeast Extract	0.5g
Purified Agar (if required).....	15.0g
Distilled Water	1000ml

Adjust pH to 7.2 and autoclave 15/15.

1530 WILKINS-CHALGREN ANAEROBE MEDIUM

Tryptone	10.0g
Gelatin peptone.....	10.0g
Yeast Extract	5.0g
Glucose.....	1.0g
NaCl	5.0g
L-Arginine	1.0g
Sodium pyruvate.....	1.0g
Menadione.....	0.0005g
Haemin	0.005g
Distilled Water	1000ml

Adjust pH to 7.1±0.2 and autoclave 15/15.

1531 METHYLOCELLA TUNDRAE M2 MEDIUM

KNO ₃	0.25g
KH ₂ PO ₄	0.10g
MgSO ₄ ·7H ₂ O.....	0.05g
CaCl ₂ ·2H ₂ O.....	0.001g
Trace Elements (Med 131 sol.4).....	1ml
Distilled Water	1000ml

1532 ALKALIBACTERIUM PSYCHROKALIPHILUS MEDIUM**Solution 1**

Reinforced Clostridial Agar.....	25.0g
Agar.....	3.0g
Distilled Water	400ml

Solution 2

Na ₂ CO ₃	5.0g
Distilled Water	100ml

Autoclave the above solutions separately and cool to 50-55°C and combine. The pH should be around 10.

1533 PYS-2 MEDIUM

Polypeptone	8.0g
Yeast Extract	3.0g
NaCl	5.0g
Distilled Water	1000ml

Adjust pH to 7.5.

1534 ASN III MODIFIED MEDIUM

NaCl.....	25.0g
MgCl ₂ .6H ₂ O.....	2.0g
KCl.....	0.5g
NaNO ₃	0.75g
MgSO ₄ .7H ₂ O.....	3.5g
CaCl ₂ .2H ₂ O.....	0.5g
Na ₂ CO ₃	0.02g
Yeast Extract.....	1.0g
Trace Elements SL-10 (see recipe 318).....	1.0ml
K ₂ HPO ₃ .3H ₂ O (8g per litre).....	2.5ml
FeNH ₄ -citrate (6g per litre).....	0.5ml
7-Vitamine Solution (see below).....	0.5ml
Distilled Water.....	1000ml

Adjust pH to 8.0±0.2.

7-Vitamine Solution

4-Aminobenzoic Acid.....	80mg
D(+)-Biotin.....	20mg
Nicotinic Acid.....	200mg
Ca-D(+)-Pantothenate.....	100mg
Pyridoxamine hydrochloride.....	300mg
Thiaminedichloride.....	200mg
Cyanocobalamin.....	100mg
Distilled Water.....	1000ml

1535 YEM MEDIUM

Mannitol.....	10g
K ₂ HPO ₄	0.5g
MgSO ₄ .7H ₂ O.....	0.1g
NaCl.....	0.1g
Yeast Extract.....	0.6g
Distilled Water.....	1000ml

Adjust pH to 6.8.

1536 NITRILOTRIACETATE MEDIUM

Na ₂ HPO ₄ .2H ₂ O.....	0.41g
KH ₂ PO ₄	0.26g
MgSO ₄ .7H ₂ O.....	1.00g
CaCl ₂ .2H ₂ O.....	0.20g
Trace Element Solution.....	1.00ml
Vitamin Solution (Filter Sterlized).....	1.00ml
Nitriлотriacetate.....	1.00g
Distilled Water.....	1000.00ml

Adjust pH to 6.5.

Trace Element Solution

FeCl ₂ .4H ₂ O.....	1.50g
ZnCl ₂	68.00mg
MnCl ₂ .4H ₂ O.....	100.00mg
H ₃ BO ₃	62.00mg
CoCl ₂ .6H ₂ O.....	120.00mg
CuCl ₂ .2H ₂ O.....	17.00mg
NiCl ₂ .6H ₂ O.....	24.00mg
Na ₂ MoO ₄ .2H ₂ O.....	24.00mg
HCl 0.05M.....	1000.00ml

Vitamin Solution

Vitamin B ₁₂	50.00mg
Pantothenic Acid.....	50.00mg
Riboflavin.....	50.00mg
Pyridoxine-HCl.....	10.00mg
Biotin.....	20.00mg
Folic Acid.....	20.00mg
Nicotinic Acid.....	25.00mg
Nicotine amide.....	25.00mg
Alpha-Lipoic Acid.....	50.00mg
p-Aminobenzoic Acid.....	50.00mg
Thiamine-HCl.2H ₂ O.....	50.00mg
Distilled Water.....	1000.00ml

1537 OCEANIBULBUS MEDIUM

Tryptone.....	10g
NaCl.....	10g
Yeast Extract.....	5g
Sea Salts (Sigm 59883).....	14g
Distilled Water.....	1000ml

Adjust pH to 7.2 Autoclave 121°C/15 mins.

1538 METHYLOPHAGA MURATA MEDIUM

KNO ₃	1.0g
KH ₂ PO ₄	1.0g
MgSO ₄ .7H ₂ O.....	0.22g
NaCl.....	30.0g
Trace Element Solution (Med 131 Sol4).....	1ml
Methanol.....	10ml
Distilled Water.....	1000ml

Adjust pH to 9.0 by adding 50ml 2M NaHCO₃ and 10ml 1M Na₂CO₃ per litre. Autoclave 15/15.

Cool to 50°C and add 20µg/L vitamin B₁₂.

1539 HETEROTROPHIC NITROBACTER MEDIUM

Yeast Extract.....	1.50g
Peptone.....	1.50g
Sodium pyruvate.....	0.55g
Trace Element.....	1.00ml
Stock Solution.....	100.00ml
Distilled Water.....	899.00ml

Adjust pH to 7.4 with NaOH or KOH.

Trace Element Solution

MnSO ₄ .7H ₂ O.....	33.80mg
H ₃ BO ₃	49.40mg
ZnSO ₄ .7H ₂ O.....	43.10mg
(NH ₄) ₆ Mo ₇ O ₂₄	37.10mg
FeSO ₄ .7H ₂ O.....	97.30mg
CuSO ₄ .5H ₂ O.....	25.00mg
Distilled Water.....	1000ml

Stock Solution

CaCO ₃	0.07g
NaCl.....	5.00g

MgSO ₄ .7H ₂ O	0.50g
KH ₂ PO ₄	1.50g
Distilled Water	1000ml

1539a MIXOTROPHIC NITROBACTER MEDIUM

Medium 1539 with 2g/L of NaNO₂.

1539b 10% MIXOTROPHIC NITROBACTER MEDIUM

Yeast Extract	0.15g
Peptone.....	0.15g
Sodium pyruvate	0.055g
NaNO ₂	2.00g
Trace Element	1.00ml
Stock Solution.....	100.00ml
Distilled Water	899.00ml

Adjust pH to 8.6 with NaOH or KOH.

1539c AUTOTROPHIC NITROBACTER MEDIUM

NaNO ₂	2.00g
Trace Element	1.00ml
Stock Solution.....	100.00ml
Distilled Water	899.00ml

Adjust pH to 8.6 with NaOH or KOH.

Autoclave and leave to stand for 2-3 days so that the pH can adjust itself to pH 7.4-7.6.

1540 RHODOPIRELLULA BALTICA MEDIUM

Peptone.....	0.75g
Yeast Extract	0.75g
Glucose	5.00g
Hutner's Basal Salts (Med 249)	20ml
Vitamin Solution (Filter Sterilise)	10ml
Buffer	50ml
Artificial Sea Water (Lyman and Fleming)	
See Medium 225	250ml
Distilled Water	670ml
Agar	18.0g

Vitamin Solution no 6(Staley,1968)

Biotin	4.0mg
Pyridoxine Hydrochloride	20.0mg
Thiamine Hydrochloride	10.0mg
Ca-pantothenate	10.0mg
P-aminobenzoic Acid.....	10.0mg
Folic Acid.....	4.0mg
Riboflavin	10.0mg
Nicotinamide or Nicotinic Acid	10.0mg
Vitamin B ₁₂	0.2mg
Double Distilled Water.....	1000ml

Stirring of the mixture improves dissolution. Sterilise by filtration only.

1541 MRS + BHI MEDIUM

Medium 20.....	70.0g
Medium 363.....	10.0g
Agar	15.0g
Distilled Water	1000ml

1542 ACIDIFIED OATMEAL AGAR

Medium 42 acidified to pH 5.0.

1543 BACTO MARINE MEDIUM 2216 (Difco) WITH BIPHENYL

Medium 2216 with a few crystals of biphenyl or naphthalene in broth or solid medium.

1544 GLUCONOBACTER OXYDANS MEDIUM

Glucose.....	100.0g
Yeast Extract	10.0g
CaCO ₃	20.0g
Agar.....	15.0g
Distilled Water	1000ml

Adjust pH to 6.8.

1545 LURIA MEDIUM + AMPICILLIN + TETRACYCLINE

Medium 139 (with glucose at 10%). Aseptically add 100µg/ml ampicillin and 12.5µg/ml tetracycline.

1546 M2GSC MEDIUM

The following medium requiring gassing with O₂ free CO₂.

Casitone	10.0g
Yeast Extract	2.5g
NaHCO ₃	4.0g
Glucose.....	2.0g
Soluble starch	2.0g
Cellobiose.....	2.0g
Rumen fluid (clarified)	300ml
Mineral I solution	150ml
Mineral II solution	150ml
Resazurin (0.1% w/w)	1.0ml
Cysteine HCl	1.0g
Distilled Water	to 1000ml

Mineral Solution I

K ₂ HPO ₄	3.0g
Distilled Water	1000ml

Mineral Solution II

KH ₂ PO ₄	3.0g
(NH ₄) ₂ SO ₄	6.0g
NaCl	6.0g
MgSO ₄	0.6g
CaCl ₂	0.6g

Distilled Water

Clarified Rumen Fluid

Collect rumen contents and autoclave 121°C for 15 minutes and filter through muslin gauze to remove the larger particulate material. The liquid fraction is centrifuged at 10000g (Sorvall RC-5B-GSA rotor) for 30 minutes at 4°C. The clarified supernatant fraction is carefully recovered and routinely stored at 4°C prior to use.

1547 EKHO LAKE STRAINS MEDIA

Mineral Salt Solution (Hutner/Cohen-Bazire).....	20ml
Peptone (Bacto).....	0.25g
Yeast Extract (Bacto).....	0.25g
Agar (Bacto).....	15.00g
Distilled Water	965ml

Autoclave 121°C for 20 minutes. After cooling to 60°C add:
 Glucose solution (2.5% filter sterilised) 10ml
 Vitamin solution (double concentrated) 5ml

Adjust pH to 7.5.

Mineral Salt Solution (Hutner/Cohen-Bazire)

Nitroloacetic acid (NTA)	10.00g
MgSO ₄ .7H ₂ O	29.70g
CaCl ₂ .2H ₂ O.....	3.34g
Na ₂ MoO ₄ .2H ₂ O	12.67mg
FeSO ₄ .7H ₂ O	99.00mg
Metal salt solution “44”	50ml
Distilled Water	900ml

Dissolve NTA first by neutralising with KOH then add other salts.
 Adjust pH to 7.2 with KOH or H₂SO₄ then adjust volume to 1000ml
 with distilled water.

Metal Salt Solution “44”

EDTA.....	2.5g
ZnSO ₄ .7H ₂ O.....	10.95g
FeSO ₄ .7H ₂ O.....	5g
MnSO ₄ .H ₂ O or MnSO ₄ .4H ₂ O	1.54g or 2.031g
CuSO ₄ .5H ₂ O	0.392g
Co(NO ₃) ₂ .6H ₂ O.....	0.248g
NaB ₄ O ₇ .10H ₂ O	0.177g

The solution should be made up in approx. 800ml of distilled water
 with one ingredient being added at a time and allowed to dissolve
 completely. Make up to 1000ml stock solution and check pH is 2.6 (do
 NOT adjust). Filter sterilise and store under refrigeration, ideally at
 4°C.

Vitamin Solution (Double Concentrated)

Biotin	4mg
Folic Acid.....	4mg
Pyridoxine-HCl	20mg
Riboflavin	10mg
Thiamine-HCl.2H ₂ O	10mg
Nicotinamide.....	10mg
D-Ca-pantothenate (D-pantothenic acid)	10mg
Vitamin B12 (Cyanocobalamin)	0.20mg
p-Aminobenzoic acid (4-aminobenzoic acid).....	10.00mg
Distilled Water	1000ml

Filter sterilise and store in the dark under refrigeration, preferably 5°C.

1548 ALKALIBACTERIUM IBURENSES MEDIUM

Soln. A.

Peptone	8g
Yeast extract	3g
K ₂ HPO ₄	1g
Agar.....	20g
Distilled water	750ml

Soln. B.

Na ₂ CO ₃	10g
Distilled water	250ml

Autoclave soln. A and B separately at 121°C for 15 minutes. Cool to
 around 50°C; aseptically mix both solutions immediately prior to
 dispensing.

Final pH should be 10 – 10.2.

1549 BRAIN HEART INFUSION WITH THIAMINE HCl

Prepare Brain Heart Infusion as per manufacturer’s instructions.
 Sterilise by autoclaving at 121°C for 15 minutes. Cool to around 50°C.
 Prepare solution of thiamine HCl to give 1 mg per litre when aseptically
 added to the Brain Heart Infusion solution post autoclaving. Filter
 sterilise the Thiamine HCl solution and aseptically mix both solutions
 prior to dispensing.

1550 DEFIBRINATED SHEEP BLOOD AGAR

Nutrient agar	900ml
Defibrinated Sheep Blood	100ml

Prepare Nutrient agar as per manufacturer’s instruction. Cool to 50°C.
 Add blood aseptically. Mix and distribute quickly into sterile tubes or
 plates.

1551 HALOVIBRIO MEDIUM

1 Mineral Base

NaCL.....	120g
K ₂ HPO ₄	3g
NH ₄ Cl.....	0.5g
Trace element solution (Pfenning + Lippert).....	1ml
Distilled Water	1L

2 Buffer

10mM HEPES or TES buffer

Sterilise dispensed quantities of 1+2 at 121°C for 20 minutes.

Aseptically add buffer after autoclaving to give final concentration of 10mM

Adjust pH to 7.5

3 Additives

The following solutions should be prepared and autoclaved at 121°C for 20 minutes then aseptically added to the sterile base and buffer mix in these concentrations:

MgSO ₄ .7 H ₂ O (1M)	2ml/L
Sodium Acetate (2M).....	10ml/L
Yeast Extract (10%)	2ml/L

N.B. It is recommended that the additives and buffer are warmed to 50°C before aseptically adding to the sterile medium.

Pfenning + Lippert Trace Elements:

NaEDTA	500mg/L
FeSO ₄ .7H ₂ O	200mg/L
ZnSO ₄ .7H ₂ O.....	10.0mg/L
MnCl ₂ .4H ₂ O.....	3.0mg/L
H ₃ BO ₃	30mg/L
CoCl ₂ .6H ₂ O.....	20mg/L
CuCl ₂ .2H ₂ O.....	1.0mg/L
NiCl ₂ .6H ₂ O	2.0mg/L
Na ₂ MoO ₄ .2H ₂ O	3.0mg/L

Add to 1 litre distilled water. Dissolve and aliquot to required volumes.

Autoclave at 121°C for 20 minutes.

1552 SPECIAL METHYLMICROBIUM ALCALIPHILUM MEDIUM

NMS Basal medium to which is added 3% NaCl.

Adjust to pH 8-9 (no greater than 9.2) using specified buffers after autoclaving.

Prepare NMS medium Cat. No. 131

Add 3% NaCl.

Prepare two buffer solutions to be used to adjust the pH of the medium after autoclaving:

Solution 1

1M NaHCO₃

Solution 2.

1M Na₂CO₃.

Autoclave all solutions at 121°C for 15 minutes, following recipe instructions for NMS medium.

After autoclaving aseptically add the phosphate buffer (solution 5 in the NMS recipe) to the basal medium as per the instructions for NMS medium.

Aseptically adjust the pH of the medium using solutions 1 and 2.

N.B. It is suggested that 25ml of solution 1 and 2.5ml of solution 2 are added aseptically to begin with and the pH taken. Further adjustment may then be made if necessary until the final pH is between 8 and 9 and no higher than pH 9.2.

1553 MANNITOL YEAST EXTRACT MEDIUM

Yeast Extract	1.0g
Mannitol	10.0g
K ₂ HPO ₄	0.5g
NaCl	0.1g
CaCl ₂ .2H ₂ O solution	1.0ml
FeCl ₃ .7H ₂ O solution	1.0ml
Agar.....	20.0g

CaCl₂.H₂O solution;

Composition per 10.0ml;

CaCl₂.2H₂O.....5.28g

Preparation: Add CaCl₂.2H₂O to distilled water and bring volume to 10.0ml.Mix thoroughly

FeCl₃.6H₂O Solution;

Composition per 10.0ml;

FeCl₃.6H₂O.....0.66mg

Preparation: Add FeCl₃.6H₂O to distilled water and bring volume to 10.0ml.Mix thoroughly

Preparation of Medium;

Add components to distilled water and bring volume to 1.0L.Mix thoroughly. Gently heat and bring to boiling. Distribute into tubes or flasks.

Autoclave at 121°C for 15 minutes

Adjust pH to 7.0

1554 BHI/2 MEDIUM

To 37g per litre B.H.I base (OXOID CM0225) add;

Casein hydrolysate.....	10.0g
Glucose.....	5.0g
Yeast extract.....	5.0g
Distilled water	1.0 litre

Autoclave at 121°C for 15 minutes.

1555 ISP-3 WITH 0.1% YEAST EXTRACT

To medium 461 add 0.1% yeast extract

Autoclave at 121°C for 15 minutes.

1556 MINERAL MEDIUM (BRUNNER)

Na ₂ HPO ₄	2.44g
KH ₂ PO ₄	1.52g
(NH ₄) ₂ SO ₄	0.50g
MgSO ₄ .7H ₂ O.....	0.20g
CaCl ₂ .2H ₂ O	0.05g
Trace Elements Solution SL-4 see med 310	10.0ml

Distilled Water 1.00L

Adjust pH to 6.9

Rehydrate and cultivate lyophilised cells in complex medium (e.g. TSA). After this reactivation, cultivate in Mineral Medium (1556) with the appropriate carbon source.

1557 JB MEDIUM WITH 0.2% GLUCOSE

Tryptone (BD 211705) 5.0g
Yeast extract 15.0g
K₂HPO₄ 3.0g
Glucose 2.0g
Distilled water 1.0L

Adjust pH to 7.3-7.5

Autoclave at 121°C for 15 mins.

1558 AG MEDIUM

Glucose 1.0g
Glycerol 1.5g
Peptone 5.0g
Yeast Extract 5.0g
Malt extract 2.0g
CaCO₃ 7.0g
Distilled Water 1.00L

Agar may be added to solidify media at a final concentration of 1.5%

Adjust pH to 6.8

1559 PYG MEDIUM

Peptone 5.0g
Yeast extract 5.0g
Glucose 5.0g
Difco Bacto Agar 20.0g

Adjust pH to 7.0

1560 MARINE MEDIUM WITH IOTA CARAGEENAN

Solution A:

NaCl 25.0g
MgSO₄x7H₂O 5.0g
CaCl₂x2H₂O 0.2g
KCl 0.1g

NaNO₃ 2.0g
Casamino acids 2.5g
Iota-carageenan 2.5g
Distilled water 1.00L

Solution B:

0.2M Na₂HPO₄ 60.0ml

Solution C:

0.3% FeSO₄ x7H₂O 10.0ml

For solid medium, add 15.0g agar to Solution A.

Autoclave each solution separately at 121°C for 15 minutes.

Add solutions B and C aseptically to solution A.

The final pH is approximately 7.2

1561 HORIKOSHI-I MEDIUM

Solution 1:

Glucose 10.0g
Polypeptone 5.0g
Yeast Extract 5.0g
K₂HPO₄ 1.0g
MgSO₄x7H₂O 0.2g
Agar 15.0g
Distilled Water 900.0ml

Solution 2:

NaCO₃ 10.0g
Distilled Water 100ml

Autoclave the two solutions separately ? at 121°C for 15 minutes.

Aseptically combine the two solutions, and check the final pH is approximately 10.0.

1562 YSG MEDIUM

Yeast Extract 2.0g
Soluble Starch 2.0g
Glucose 1.0g
Distilled Water 1.0L

Adjust pH to 3.7 with 2N H₂SO₄.

1563 CHOPPED MEAT MEDIUM WITH CARBOHYDRATES

Oxoid CM1 (cooked meat medium) prepare as per manufacturer's instructions.

Supplement with the following, adding the cysteine hydrochloride last:

Yeast Extract 5.0g

K ₂ HPO ₄	5.0g
Resazurin	1.0mg
Glucose	4.0g
Cellobiose.....	1.0g
Maltose.....	1.0g
Soluble Starch	1.0g
Cysteine hydrochloride.....	0.5g
Distilled Water	1.0

**1564 MODIFIED PAYNE, SEGHAL, AND GIBBONS.
RECIPE TO FOLLOW**

**1565 REINFORCED CLOSTRIDIAL MEDIUM WITH
ADDITIONAL 0.05% CYSTEINE**

To Medium 264 aseptically add 0.05% cysteine.

1566 NUTRIENT AGAR (MEDIUM 201) +75mg/L AMPICILLIN.

After autoclaving and cooling to 50°C, aseptically add the appropriate volume of a concentrated filter sterilised solution of Ampicillin to give a final concentration of 75mg per litre. Mix gently and dispense.

1567 HALF STRENGTH BACTO MARINE MEDIUM 2216

Prepare the medium as per the manufacturer's instructions but use half the stated quantity of medium per litre i.e. 18.7g/L.
The medium may be solidified by adding 15.0g/L agar

Final pH 7.6.

1568 BEER MEDIUM.

Prepare MRS medium (medium 20) in normally hopped beer eg bitter, instead of distilled water.

Adjust pH to 5.2.

1569 NUTRIENT BROTH (OXOID CM1).

Prepare as per manufacturer's instructions.

1570 NUTRIENT BROTH 2 (OXOID CM67).

Prepare as per manufacturer's instructions.

1571 TENFOLD DILUTION TSA (CM131)

Prepare as per manufacturer's instructions but use one tenth amount of medium per litre i.e. 4.0g/L

1572 MRS +5µg/ml CHLORAMPHENICOL

To MRS medium aseptically add 5µg/ml chloramphenicol

1573 ALKALIMONAS KOLLAGENIMARINA MEDIUM

Solution A

Bacto tryptone (Difco).....	1.0g
Yeast extract	5.0g
NaCl	10.0g
MgSO ₄ .7H ₂ O.....	2.0g
K ₂ HPO ₄	0.2g
Agar.....	15.0g
Distilled water	500ml

Solution B

NaHCO ₃	10.0g
Distilled water	500ml

Make up as above to give double strength solutions.

Autoclave solution A and solution B separately at 121°C for 15 minutes.

After autoclaving aseptically combine the two solutions to give a single strength medium.

Adjust pH to 9.0

1574 CYSTEINE HEART MEDIUM WITH 5% SHEEP BLOOD

Solution A – Basic agar mix

Solution B – Haemoglobin solution

Solution C – Sheep blood

Solution A

Bacto Heart Infusion Broth (BD 238400)	25.0g
Glucose (BDH 100176)	10.0g
L-Cysteine (Sigma C-7880)	1.0g
Agar (Oxoid LP11)	15.0g
Distilled water	900ml

Dissolve the ingredients minus agar in the water.

Adjust the pH to 6.8±0.2.

Add the agar and bring to the boil (constantly stirring).

Solution B

Soluble haemoglobin powder (OxoidL53)	2.0g
Distilled water	100ml

Mix carefully and heat gently to dissolve.

Solution C

Sheep blood	50ml
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Bring to room temperature.

Autoclave solution A and B for 15 minutes at 121°C
Cool to approx 48-50°C in a water bath

Aseptically combine solution A and B and add 50ml sheep
blood,(solution C)
Mix carefully and dispense.

1575 REINFORCED CLOSTRIDIAL MEDIUM WITH 1% SODIUM CARBONATE

Lab M Reinforced Clostridial Medium 38.0g
Na₂CO₃ 10.0g
Agar 15.0g
R.O. water 1.0L
Sodium Carbonate to be sterilised separately and added aseptically to
main medium post autoclaving.

Autoclave at 121°C/15 mins.

Final pH 10_±0.2 (at 25°C)

1576 TRYPTONE SOY MEDIUM WITH IRON SULPHATE

Tryptone soya agar or broth (Oxoid) with 0.25mM Fe₂SO₄.H₂O added.
Autoclave at 121°C/15 mins.

1577 BLOOD AGAR BASE NO 2(CM0271) +5% BLOOD

CM0271 Blood agar base no 2 (Oxoid) with 5% blood added
Make as per manufacturer's instructions.
Autoclave at 121°C/15 mins.
Cool to 45-50°C
Aseptically add 5% blood.

1578 BACILLUS POLYGONUMI MEDIUM

Peptone 8.0g
Yeast Extract 3.0g
K₂HPO₄ 1.0g
Na₂CO₃ 10.0g
Distilled water 1.0L

Autoclave at 121°C/15mins.

1579 BLOOD AGAR BASE (CM55) = 2% NaCl+ 10% BLOOD

CM55 Oxoid with 2% NaCl and 10% blood added
Make as per manufacturer's instructions.
Autoclave at 121°C/15 mins
Cool to 45-50°C.
Aseptically add 10% blood.

1580 L-PHASE MEDIUM (LPM)

Sucrose200.0g
Glucose5.0g
Yeast Extract5.0g
Peptone5.0g
Mg SO₄.7H₂O0.1g
Agar7.5g
Distilled water 1.0L

Autoclave 10mins@115lbs

1581 MODIFIED MRS MEDIUM

Oxoid MRS broth (CM0359) with 1% Maltose and 0.05% fresh Yeast
Extract added, pre autoclaving.

Autoclave at 121°C/15 mins.

Aseptically add sterile Cysteine HCl to give 0.05% per litre MRS.

Final pH 6.2_±0.2

1582 ACETOBACTER YEAST GLUCOSE MEDIUM

Glucose 100g
Yeast Extract 10g
CaCO₃ 20g
Agar 25g
Distilled water 1.0L

Dissolve the CaCO₃ separately in a portion of the total volume of
distilled water and autoclave both solutions at 121C/15 min. After
sterilization aseptically mix the solutions and dispense.

1583 R2A MEDIUM +10mg/l MnSO₄

Oxoid R2A agar with 10mg/l MnSO₄ added pre autoclaving

1584 LENTIBACILLUS HALOPHILUS MEDIUM

Casamino acids (Difco)5.0g
Yeast Extract (Difco).....5.0g
Sodium glutamate..... 1.0g
Trisodium citrate3.0g
MgSO₄.7H₂O.....20.0g
KCl2.0g
NaCl200.0g
FeCL₂.4H₂O36.0g
MnCl₂.4H₂O0.36g
Agar20.0g
Distilled water 1.0L

Adjust pH to 7.0-7.2.

Autoclave at 121°C/15 mins.

**1585 INORGANIC SALTS-STARCH MEDIUM (ISP4
(Becton Dickinson & Co ISP Medium 4)**

Soluble Starch	10.0g
K ₂ HPO ₄	1.0g
MgSO ₄ .7H ₂ O	1.0g
NaCl	1.0g
(NH ₄) ₂ SO ₄	2.0g
CaCO ₃	2.0g
Trace Salts solution (see below)	1.0ml
Agar	20.0g
Distilled water	1.0L

Unadjusted pH will be 7.0-7.4

Autoclave at 121°C/15mins

Trace salts solution:

FeSO ₄ .7H ₂ O	0.1g
MnCl ₂ .4H ₂ O	0.1g
ZnSO ₄ .7H ₂ O	0.1g
Distilled water	100.0ml

1586 MIDDLEBROOK 7H10 MEDIUM

Na ₂ HPO ₄	1.5g
KH ₂ PO ₄	1.5g
(NH ₄) ₂ SO ₄	0.5g
L-Glutamic acid	0.5g
Sodium citrate	0.4g
Ferric ammonium citrate	0.04g
MgSO ₄ .7H ₂ O	0.025g
ZnSO ₄ .7H ₂ O	1.0mg
CuSO ₄ .5H ₂ O	1.0mg
Pyridoxine	1.0mg
Biotin	0.5mg
CaCl ₂ .2H ₂ O	0.5mg
Malachite green	0.25mg
Middlebrook OADC enrichment	100ml
Glycerol	5.0ml
Agar	15.0g
Distilled water	1.0L

Adjust pH to 6.6+ 0.2.

Add glycerol to 900ml of distilled water and add all remaining components except Middlebrook OADC enrichment.

Mix thoroughly. Gently heat and bring to boiling.

Autoclave at 121°C /15 mins

Cool to 50-55°C

Aseptically add 100ml sterile Middlebrook OADC enrichment.

Mix thoroughly.

Pour into sterile petri dishes or distribute into sterile tubes.

1587 ANCYLOBACTER-SPIROSOMA MEDIUM

Peptone	1.0g
Yeast extract	1.0g
Glucose	1.0g
Agar	15.0g
Distilled water	1.0L

Adjust pH to 6.8-7.0

Autoclave at 121°C/15mins.

1588 SAN FRANCISCO MEDIUM

Tryptone	10.0g
Meat extract	2.0g
Yeast extract	7.0g
Glucose	7.0g
Fructose	7.0g
Maltose	7.0g
Sodium gluconate	2.0g
Sodium acetate. 3H ₂ O	5.0g
Citric acid diammonium salt	5.0g
KH ₂ PO ₄ .3H ₂ O	2.5g
MgSO ₄ .7H ₂ O	0.2g
MnSO ₄ .4H ₂ O	50mg
FeSO ₄ .7H ₂ O	10mg
Cysteine HCl	0.5g
Tween 80	1g
Fresh baker's yeast	21g
Rye or wheat bran	50g
Distilled water	1.0L

Adjust pH to 5.4

Autoclave at 121°C/15 mins.

1589 AMYCOLATOPSIS HALOTOLERANS MEDIUM

Glucose	10.0g
L-Asparagine	1.0g
K ₂ HPO ₄	0.5g
Yeast extract	2.0g
Agar	15.0g
Distilled water	1.0L

Adjust pH to 7.3

1590 V8 JUICE MEDIUM

V8 canned vegetable juice (Campbell)	200ml
CaCO ₃	3.0g
Agar	15.0g
Distilled water	1.0L

Adjust pH to 7.2

Filter V8 juice through 4 layers of cheesecloth or centrifuge (3,000 rpm, 10min) to eliminate fibres.

1591 MEDIUM 83 + 0.2% METHANOL

As per medium 83 but replace trimethylamine with methanol, to a final concentration of 0.2%.

1592 PEPTONE MEAT EXTRACT MEDIUM

Peptone 2.5g
Meat extract 1.5g
Agar 15.0g
Distilled water..... 1.0L

Dissolve the components separately in the distilled water.
Adjust pH to 8.0.
Autoclave at 121°C/15 mins.

1593 M.R.S. MEDIUM (LAB M)

Mixed peptones..... 10.0g
Yeast extract 5.0g
Meat extract 10.0g
Glucose 20.0g
Potassium phosphate..... 2.0g
Sodium acetate..... 5.0g
Magnesium sulphate 0.2g
Manganese sulphate..... 0.05g
Tween 80 1.08g
Ammonium citrate 2.0g
Agar 15.0g
Distilled water 1.0L

Add 55g of powder to 1.0L distilled water. Allow to soak for 10 minutes, swirl to mix and warm to dissolve.
Dispense into final containers.
Autoclave at 121°C /15 mins.
Final pH is 6.4± 0.2.

1594 MBM MEDIUM

MgSO₄, 20.83ml of 2.4M solution (50mM final concentration)
KCl, 5.0ml of 2.0M solution (10mM final concentration)
NH₄Cl, 19.0ml of 1.0M solution (19 mM final concentration)
Na₂SO₄, 200ul of 0.5M solution (0.1 mM final concentration)
FeCl₂·6H₂O, 135ul of 50mg/ml solution (0.025mM final concentration)
Na₂EDTA, 139ul of 67mg/ml solution (0.025 mM final concentration)
K₂HPO₄·3H₂O 660ul of 0.5M solution (0.33 mM final concentration)
Micronutrients supplements 1.0ml solution
Tris HCL (pH 7.6) 50ml of 1.0M solution(50 mM final concentration)
NaCl 29.22g/L

Adjust pH to 7.6 with HCl or NaOH.
Make up to 964 ml then autoclave at 121°/20 mins.

After autoclaving aseptically add 36ml of sterile 10% glucose stock solution to the medium. (Final concentration 20mM).
Mix thoroughly and dispense into sterile containers.

1595 NEW OXOID R2A AGAR

Yeast extract..... 0.5g
Proteose peptone 0.5g
Casein hydrolysate 0.5g
Glucose 0.5g
Starch 0.5g
Di-potassium phosphate 0.3g
Magnesium sulphate..... 0.024g
Sodium pyruvate 0.3g
Agar..... 15.0g
Distilled water 1.0L

Suspend 18.1g in 1litre of distilled water.
Bring to the boil to dissolve completely.
Mix and sterilise by autoclaving.
Autoclave at 121°C for 15 mins.
Final pH 7.2 ± 0.2

1596 YEAST MALT pH 9

As per medium 29 .
Adjust final pH to 9.0 ± 0.2.

1597 MARINE MEDIUM 2216 + 8% NaCl.

As per medium 211 with % NaCl added pre autoclaving.

1598 METHYLOVIRGULA MEDIUM

KH₂PO₄..... 0.1g
(NH₄)₂SO₄..... 0.1g
MgSO₄·7H₂O..... 0.05g
CaCl₂·2H₂O..... 0.01g
NaCl 0.02g
Yeast Extract 0.1g
Methanol 1ml
Trace elements (soln 4 med 131)..... 1ml
Distilled water 1L
Agar..... 2%

pH 5-5.8
Autoclave at 121°C for 15 mins.

1599 SABAROUND DEXTROSE MEDIUM

Mycological peptone 10.0g
 Glucose 40.0g
 Agar 15.0g
 Distilled water 1L

pH 5.6± 0.2

Autoclave at 121°C for 15 mins.

1600 UNIVERSAL MEDIUM FOR YEASTS

Yeast extract 3.0g
 Malt extract 3.0g
 Peptone from soya beans 5.0g
 Glucose 10.0g
 Agar 15.0g
 Distilled water 1L

Autoclave at 121°C for 15 mins.

1601 BLOOD AGAR BASE (CM55) +7% DEFIBRINATED HORSE BLOOD + 0.1% CYSTEINE

Medium 52 with 7% sterile defibrinated horse blood and 0.1% sterile cysteine added aseptically after autoclaving and cooling to 50°C.

1602 R2A MEDIUM + SALTS

Medium 1595 with the following salts added per litre:

NaCl 20g
 MgCl₂ 3g
 KCl 0.5g
 CaCl₂ 0.2g

Autoclave at 121°C for 15 mins.

pH 7.0

1603 AMPHIBACILLUS MEDIUM

Glucose 10.00g
 K₂HPO₄ 1.00g
 NH₄NO₃ 2.0g
 MgSO₄×7H₂O 0.20g
 MnSO₄×7H₂O 0.005g
 FeSO₄×7H₂O 0.005g
 CaCl₂×2H₂O 0.10g
 Yeast extract 3.00g
 Polypeptone (BBL) 0.30g
 Agar 15.00g
 Distilled water 1L.

After sterilisation add sterile 1M Na-sesquicarbonate solution (1ml in 10ml) to achieve a pH of 9.7.

Na sesquicarbonate solution:

NaHCO₃ 4.20g
 Na₂CO₂ (anhydrous) 5.30g
 Distilled water 100.00ml

1604 LURIA BERTANI +50µg/L KANAMYCIN

Medium 113 with 50µg/L kanamycin added aseptically after autoclaving and cooling to 50°C.

1605 BHI + 1% TWEEN 80 MEDIUM

BHI with 1% Tween 80 added prior to autoclaving at 121°C for 15 minutes.

1606 CLOSTRIDIUM ALGINATE MEDIUM

K₂HPO₄ 2.0g
 Peptone 1.0g
 Yeast extract 1.0g
 Sodium alginate 10.0g
 Agar 15.0g
 Seawater 1.0L

Heat seawater with K₂HPO₄ and filter twice.

Add other ingredients and adjust pH to 7.0-7.5.

For semi-solid medium reduce agar to 5.0g

Autoclave medium at 121°C for 15 minutes.

1607 PLATE COUNT AGAR

Tryptone 5.0g
 Yeast extract 2.5g
 Dextrose 1.0g
 Agar 15.0g
 Distilled water 1.0L

Adjust pH to 7.0.

1608 PYA MEDIUM 2

Solution 1

Peptone 8.0g
 Yeast extract 3.0g
 K₂HPO₄ 1.0g
 Agar 15.0g
 Distilled water 900ml

Solution 2

Na₂CO₃ 10.0g
 Distilled water 100ml

Solutions 1 and 2 should be autoclaved separately then aseptically mixed together and used for plates or slopes.

Final pH will be around 10.

1609 TSA + 2% SEA SALTS

TSA with 2% sea salts added prior to autoclaving.

1610 AUREOBACTERIUM TERREGENS MEDIUM

Casamino acids 2.0g
 Yeast extract 1.0g

Glucose 1.0g
(NH₄) citrate 1.0g
K₂HPO₄ 2.0g
MgSO₄ x 7H₂O 0.50g
FeCl₃ x 6H₂O 10.0mg
Distilled water..... 1Litre

Adjust pH to 7.0.

After sterilisation, aseptically add 1ml/L of a 10% solution of acetylacetone in ethanol.

1611 PLUTON MEDIUM MODIFIED (ANAEROBIC)

Glucose 10.00g
Starch..... 2.00g
Peptone 2.50g
Yeast Extract 2.50g
Malt Extract 5.00g
Tryptone 2.00g
1M K₂HPO₄ 50ml
Distilled water..... 950ml

Dispense 10ml volumes into crimp top vials under a nitrogen blanket.

Autoclave at 121°C for 15 minutes.

Aseptically add 0.1ml 2.5% Cysteine HCl to each 10ml vial before use.

1612 GLYCEROL PEPTONE MEDIUM

Glycerol 10.00ml
Peptone 10.00g
Distilled water 1 litre
Agar 15.00g

Autoclave at 121°C for 15 minutes.

1613 R2A MEDIUM WITH SEA WATER

Oxoid R2A agar prepared with artificial sea water in place of distilled water.