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BHI Agar for Etest (BBL)

1. Suspend 52 g of BHI agar (BBL) in 1 L of deionized water
2. Mix thoroughly
3. Adjust pH
4. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder
5. Autoclave at 121^{o}C for 15 minutes
6. Before use agitate gently to distribute the precipitate uniformly throughout the media
7. Pour plates

Final pH 7.4 ± 0.2
Store at 4^{o}C

Casein Agar Plates

1. Dissolve 25 g skim milk (DIFCO) in 250 ml deionized water by heating (use a 500 ml flask). DO NOT BOIL. Adjust pH.
2. Autoclave skim milk at 115^{o}C for 10 minutes
3. Dissolve 5 g Agar (Bacto-Difco) in 250 ml deionized water, adjust pH and heat to boiling (use a 1 L flask).
4. Autoclave Agar at 121^{o}C for 15 minutes
5. Cool in a water bath to 45^{o}C
6. Mix the 2 solutions and pour plates (20 ml)

Final pH 7.0 ± 0.2
Store plates at 4^{o}C

Cetrimide Agar

1. Suspend 45.3 g of the Difco Cetrimide Agar Base powder in 1 L of deionized water containing 10 ml glycerol
2. Mix thoroughly and adjust pH
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder
4. Autoclave at 121^{o}C for 15 minutes
5. Cool and pour plates

Final pH 7.2 ± 0.2

Store at 4°C

Enterococcus agar with 6mg/L vancomycin

1. Suspend 42g of the Enterococcus Agar in 1 L of deionized water
2. Mix thoroughly
3. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder
4. DO NOT AUTOCLAVE
5. Cool in a water bath to 50°C
6. Add 6.0 ml of Vancomycin solution (1000µg/ml)
7. Mix well and pour plates

Final pH 7.2 ± 0.2
Store plates at 4°C
Store Vancomycin solution at -20°C

MacConkey (With salt and crystal violet)

1. Suspend 51.5g of MacConkey #3 (OXOID) in 1 L of deionized water
2. Mix thoroughly
3. Heat to boil to dissolve completely
4. Autoclave at 121°C for 15 minutes
5. Pour plates

Final pH 7.1 ± 0.2
Store plates at 4°C

MacConkey with Colistin (With salt and crystal violet)

1. Suspend 51.5g of MacConkey #3 (OXOID) in 1 L of deionized water
2. Mix thoroughly
3. Heat to boil to dissolve completely
4. Autoclave at 121°C for 15 minutes
5. Cool in a water bath to 50°C
6. Add 0.75 ml (Coli-Mycin M 10,000 µg/ml (=300,000 U) and mix thoroughly
7. Pour plates

Final pH 7.1 ± 0.2
Store plates at 4°C

Preparation of Colistin Solution

150 mg = 150,000 µg/ml
Reconstitute using 3 ml sterile deionized water and then dilute 1:5 (= 10,000 µg/ml)
Store at -70°C

OCBL Agar

1. Suspend :

OF Base (Difco)	9.4 g
Bacto Agar	15.0 g
Deionized water (RO pure)	900 ml

2. Mix thoroughly to dissolve the powder
3. Autoclave at 121°C for 15 minutes
4. Cool to 50°C
5. Then add:

Lactose (10% filter sterilized)	100 ml
Colistin (Coli-Mycin M 10,000 µg/ml (=300,000 U)	1.0 ml
Bacitracin (1000 U)	0.2 ml

6. Mix well and pour plates

Final pH 6.8 ± 0.2
Store at 4°C

Preparation of solutions

Colistin preparation

150 mg = 150,000 µg/ml
Reconstitute using 3 ml sterile deionized water and then dilute 1:5 (= 10,000 µg/ml)
Store at -70°C

Bacitracin preparation

Reconstitute using sterile deionized water
Store at -70°C

Olive Oil

Dispense olive oil into 13X100 tubes (2ml per tube)
Sterilize in hot air oven 2 hours at 160^{o}C

Pyruvate Agar for Nocardia

Deionized water	500 ml
Sodium pyruvate (Pyruvic acid sodium salt) (SIGMA – P2256 Store at 4^{o}C)	2.5 g
Yeast extract	0.25g
Bromcresol Purple Indicator (1.6g in 100ml of 95% ethanol Store at 4^{o}C)	0.5ml

1. Mix thoroughly
2. Adjust pH to 6.9
3. Add Agar (Bacto) 10.0g
4. Mix thoroughly and heat to dissolve
5. Autoclave at 121^{o}C for 15 minutes
6. Cool and pour plates

Final pH 6.8 ± 0.2
Store at 4^{o}C

Soybean-Casein Digest Medium Extra Strength (ESD14)

1. Suspend 50g of the Tryptone Soya Medium powder in 1 L of deionized water
2. Mix thoroughly
3. Adjust pH
4. Dispense 15 ml amounts into large screw cap tubes (20 X 150) (flat bottom, white cap)
5. Autoclave at 121^{o}C for 15 minutes

Final pH 7.3 ± 0.2
Store at room temperature

Soybean-Casein Digest Medium Single Strength (SD14)

1. Suspend 30g of the Tryptone Soya Medium powder in 1 L of deionized water
2. Mix thoroughly
3. Adjust pH
4. Dispense 15 ml amounts into large screw cap tubes (20 X 150) (flat bottom, white cap)
5. Autoclave at 121^{o}C for 15 minutes

Final pH 7.3 ± 0.2
Store at room temperature

Thioglycollate Broth (BD BBL 211720) Extra Strength (ETH14)

1. Suspend 50g of the Thioglycollate powder in 1 L of deionized water
2. Mix thoroughly
3. Allow to cool and adjust pH
4. Heat to boil for 1 minute
5. Dispense 15 ml amounts into large screw cap tubes (20 X 150) (round bottom, black cap)
6. Autoclave at 118^{o}C for 15 minutes

Final pH 7.0 ± 0.2
Store at room temperature

Thioglycollate Broth (BD BBL 211720) Single Strength (TH14)

1. Suspend 30g of the Thioglycollate powder in 1 L of deionized water
2. Mix thoroughly
3. Allow to cool and adjust pH
4. Heat to boil for 1 minute
5. Dispense 15 ml amounts into large screw cap tubes (20 X 150) (round bottom, black cap)
6. Autoclave at 118^{o}C for 15 minutes

Final pH 7.0 ± 0.2
Store at room temperature

TCBS for Vibrio

1. Suspend 89 g of TCBS Agar (Difco) in 1 L of deionized water
2. Mix thoroughly
3. Adjust pH
4. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder
5. Cool in a water bath to 50^{o}C
6. Pour plates

Final pH 8.6 ± 0.2
Store plates at 4^{o}C

Tube Coagulase

Rabbit plasma is rehydrated aseptically with sterile deionized water according to manufacturer's instructions on the label.
Mix well and dispense 0.5 ml aseptically into 13X100 sterile blue capped tubes

Store at -20^{o}C

Trypticase Agar Slants (2ml)

1. Suspend 40 g of Trypticase Soy Agar (BBL) in 1 L of deionized water
2. Mix thoroughly
3. Adjust pH
4. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder
5. Dispense 2 ml in Bijou bottles
6. Autoclave at 121^{o}C for 15 minutes. DO NOT OVERHEAT.
7. Slant tubes with no butt to solidify

Final pH 7.3 ± 0.2
Store at 4^{o}C

Tryptone water (for MUG test)

1. Suspend 1 g of Neopeptone in 100 ml deionized water.
2. Dissolve completely
3. Autoclave at 121^{o}C for 15 minutes.
4. Cool
5. Dispense aseptically0.3 ml in clear plastic 10X70 snap top tubes

   Store tubes at 4^{o}C

Mycology Media / Reagents

Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM)

Commercially prepared by Bio-Media lab

Columbia agar base
5% sheep blood agar
Gentamicin
Vancomycin
Chloramphenicol
Cyclohexamide

Purpose: Primary isolation media for fungi especially *Histoplasma capsulatum*.

Quality Control

Organisms	Incubation	Results
Trichophyton mentagrophytes ATCC 9533	28^{o}C (1-3 days)	Good Growth

Candida albicans ATCC 10231	28°C (1-3 days)	Good Growth
Escherichia coli ATCC 25922	28°C (1-3 days)	Inhibited

Brain Heart Infusion Agar (BHIA)

Brain Heart Infusion Agar Powder 47 g. (Oxoid CM375)
Dist. H_2O 1000 ml.

Mix and boil to dissolve completely.
Distribute 10 ml. into 25-ml. UGB bottles.
Autoclave 15 minutes at 15 psi and 121^{0}C.
Cool on a slant.
Store at room temperature.
Final pH approximately 7.4

Purpose: Isolation of opportunistic and dimorphic fungi from uncontaminated specimens.

Also to convert some dimorphic fungi from the mycelial phase to the yeast phase when incubated at 37^{o}C with extra moisture added to the tube.

Quality Control:

Organisms	Incubation	Results
Trichophyton mentagrophytes ATCC 9533	28^{o}C (1-3 days)	Good Growth white cream yellow

Cornmeal Tween 80 Agar (Oxoid)

Cornmeal	40 gm
Agar	20 gm
Tween 80 (polysorbate 80)	10 ml
Distilled water	1,000 ml

1. Mix cornmeal well with 500 ml of water; heat to 65^0C for 1 hour.
2. Filter through gauze and then paper until clear. Restore to original volume.
3. Adjust to pH 6.6-6.8.
4. Add agar dissolved in 500 ml of sterile water.
5. Add Tween 80.
6. Autoclave for 15 minutes.
7. Dispense into sterile screw cap bottles (175 mls) to be melted and poured into petri dishes (15 ml/dish) as needed.

Purpose: For yeast morphology.

Quality Control:

Organisms	Incubation	Results
Candida albicans ATCC 10231	22°C for 48hrs	Growth, white and with chlamydospores
Candida krusei ATCC 6258	22°C for 48hrs	Growth, white/cream and no chlamydospores

Reference

Davise H. Larone. Medically Important Fungi. A Guide to Identification. 1995, ASM Press.

ESCULIN BASE MEDIUM (EBM) pH 7.1

Dist. H_2O	1000 mL.
Bacto Agar (Difco)	15 g.
Dextrose (BBL)	5 g.
Bacto-Peptone (Difco)	10 g.
Esculin (Difco/BDH)	0.5 g.
Difco Yeast Extract	1.0 g.

Mix thoroughly to dissolve.
Autoclave at 121^{o}C/ 15 minutes

Cool to 45^{o}-50^{o}C, aseptically remove 5.0-ml. agar, then add:

2.5 ml. Gentamicin sulphate = 25,000 µg/litre
2.5 ml. Chloramphenicol = 10,000 µg/litre

Mix well and pour plates.
Store in fridge.

Gentamicin Sulphate Stock Solution (10,000 µg/ml)

Vial contains 2.0 ml. (40 mg/ml) = 80,000 µg

Transfer contents of vial and make up to a volume of 8 ml. using phosphate buffer pH 8.0 (= 10,000 µg/ml). Distribute 3 ml. amounts into bijou bottles. Store at -20^{o}C.

Chloramphenicol Stock Solution (4,000 µg/ml)

Purpose

Differential medium for isolation of Cryptococcus neoformans and also isolation medium for other fungi from contaminated specimens. Also provides presumptive identification of *C. neoformans*.

Principle

C. neoformans produces phenol oxidase enzyme that breaks down the substrate esculin, resulting in the production of a melanin-like pigment and the development of dark brown colonies. It takes about 48-72 hours for colonies to become brown. Other yeast colonies are cream to beige.

Rare strains of *C. neoformans* fail to produce pigmented colonies; also rarely yeasts other than *C. neoformans* produce dark colonies.

When EBM is used (set up as a test to determine brown pigment by *Cryptococcus neoformans*), it **must never be set up at 35°C** but no more than 28°C.

Quality Control

Organisms	**Incubation**	**Results**
Cryptococcus neoformans ATCC 76484	25°C	Growth + Brown pigment
Candida glabrata ATCC 2001	25°C	Growth + No pigment
Escherichia coli ATCC 25922	25°C	No Growth

References

S.C. Edberg et al. Esculin - Based Medium for Isolation and Identification of *Cryptococcus neoformans*. J. Clin. Micro. 12:332-335, 1980.

Inhibitory Mold Agar (IMA)

Tryptone	3.0 g.
Beef extract	2.0 g.
Yeast extract	5.0 g.
Dextrose	5.0 g.
Starch (soluble)	2.0 g.
Dextrin	1.0 g.
Chloramphenicol	0.125 g.
Salt A	10.0 ml.
Salt C	20.0 ml.
Agar	17.0 g.
Distilled water	970.0 ml.

Salt A:	
NaH_2PO_4	25.0 g.
Na_2HPO_4	25.0 g.
$NaH_2PO_4H_2O$	28.71 g.
H_2O	250.0 ml.

Salt C:	
$MgSO_4$ - $7H_2O$	10.0 g.
$FeSO_4$ - $7H_2O$	0.5 g.
NaCl	0.5 g.
$MnSO_4$ - $7H_2O$	2.0 g.
H_2O	250.0 ml.

Manufacturer: Que-lab Inc. Dehydrated medium.

Materials are dissolved in water that is brought to a boil to suspend the agar. After cooling, pH is adjusted to 6.7. Autoclave 15 psi/15 minutes. Chloramphenicol is first dissolved in 2 ml of alcohol (95%) and added to boiling media. Pour into sterile, plastic petri dishes (35 ml/plate). For isolation and subculture of fungi.

Quality Control

Organisms	Incubation	Results
Trichophyton mentagrophytes ATCC 9533	28°C (1 - 3 days)	Good Growth

Candida albicans ATCC 10231	28°C (1 - 3 days)	Good Growth
Escherichia coli ATCC 25922	28°C (1 - 3 days)	Inhibited

Lactophenol Aniline Blue Stain (LPAB)

Distilled water	20.0 ml.
Lactic acid	20.0 ml.
Phenol crystals	20.0 g.
Aniline blue	0.05 g.
Glycerol	40.0 ml.

Dissolve phenol in the lactic acid, glycerol, and water by gently heating. Then add aniline blue.

Purpose: Used for wet mount preparations of fungal cultures.

Mycosel Agar

Dehydrated Mycosel Agar	36 g.
Distilled Water	1000 ml.

Mix thoroughly. Heat with frequent agitation until medium boils, not longer. Dispense 10 ml. amounts into 25-ml UGB bottles. Autoclave 15 min/ 118^{o}C. Cool on a slant. Store at room temperature. Final pH 6.9 ± 0.2.

Formula per litre

Papaic Digest of Soybean Meal	10.0 g.
Dextrose	10.0 g.
Agar	15.5 g.
Cycloheximide	0.4 g.
Chloramphenicol	0.05 g.

Note: This medium must <u>not</u> be incubated at 35^{o}C since the antibiotics at the higher temperature inhibit pathogenic fungi.

Purpose

a) to isolate pathogenic fungi (especially dermatophytes) from contaminated specimens (it inhibits bacteria and most saprophytic fungi).

b) to determine Cycloheximide resistance of fresh isolates as a screening test for pathogenic fungi.

Quality Control

Organisms	**Incubation**	**Results**
Trichophyton mentagrophytes ATCC 9533	28^{o}C (1 - 3 days)	Good Growth
Candida albicans ATCC 10231	28^{o}C (1 - 3 days)	Good Growth
Escherichia coli ATCC 25922	28^{o}C (1 - 3 days)	Inhibited

Oxgall Agar

Bacto oxgall powder	20 (10) g. (Difco 0128-02)
Bacto agar powder	18 (15) g. (Difco 0140-01)
Dist. H_2O	1000 ml.

Mix well. Final pH 7.3 ± 0.2.
Autoclave 121°C/15 minutes.
Cool to 50°C.
Pour plates.

Purpose

For the rapid production of chlamydospore by *Candida albicans* within 24 to 48 hours.

Quality Control

Organisms	**Incubation**	**Results**
Candida albicans ATCC 10231	25°C	Chlamydospore
Candida tropicalis ATCC 13803	25°C	No Chlamydospore

Reference

J.B. Fischer, J. Kane "Production of Chlamydospores by Candida Albicans Cultivated on Dilute Oxgall Agar" Mycopath 35:223-229, 1968.

Potato Dextrose Agar (PDA)

Slopes:

Potato dextrose agar (CM 139)	39 g.
Distilled Water	1000 ml.

Mix well, bring to a boil to dissolve. Cool to 50^0C (check pH with ATC - pH ± 5.6). Dispense 10 ml amounts into pre-sterilised UGB bottles. Autoclave (with loose caps) at 118^0C/10 minutes. Cool in a slanted position. Tighten the caps. Label the bottles "PDA". Store at 4^0C.

Plates:

Potato dextrose agar	39.0 g.
Dist. H_2O	1000.0 ml.

Mix well, bring to a boil. Cool to 50^oC (check pH with ATC - pH ± 5.6). Autoclave at 121^oC/15 minutes. Cool. Pour plates (label "PDA"). Shrink wrap plates individually. Store at 4^oC.

Purpose

Sporulation medium for fungi (can also be used in slide culture).

Quality Control:

Organisms	Incubation	Results
Trichophyton mentagrophytes ATCC 9533	28^oC (1 - 3 days)	Good Growth

Sodium Pyruvate Agar (NPA) For Nocardia

Distilled Water	500 ml.
Sodium Pyruvate (Pyruvic acid. Sodium salt) SIGMA - P2256	2.5 g.
Yeast Extract	0.25 g.
Indicator (1.6 g bromocresol purple in 100 ml of 95% ethanol)	0.50 ml.

Mix well, pH to 6.8. Then add:

Agar (BACTO)	10 g.

Mix thoroughly, heat to dissolve. Sterilize 121^0C/15 min. Cool, pour plates. Store in refrigerator. (Final pH 6.8)

Purpose

Isolation medium for Nocardia from contaminated specimens. Growth of other bacteria is usually suppressed.

Quality Control:

Organisms	**Incubation**	**Results**
Nocardia brasiliensis ATCC 19296	28°C (1 - 3 days)	Good Growth
Escherichia coli ATCC 25922	28°C (1 - 3 days)	Inhibited

References

Saksun, J.M., Kane J. and Schaacter, R.K. 1978. Mycetoma caused by Nocardia madurae. CMAJ 119:911-914

Sabouraud Agar Modified (DIFCO)

Purpose

To provide a better and less inhibitory medium than the original formula for isolation and subculture of fungi.

Sabouraud Agar (Modified)	50 g.
Distilled Water	1000 ml.

Mix well pH 7.5

Heat to boiling to dissolve.

For slopes: Dispense 10 ml. amounts into UGB bottles. Autoclave 121°C/15 minutes. Slope. Store at RT. Final pH 7.0 at 25°C.

For plates: Autoclave flask 121°C/15 min. Cool. Pour plates. Store in plastic bags at 4°C.

Formula per litre

Bacto-neopeptone	10 g.
Bacto-Dextrose	20 g.
Bacto-Agar	20 g.

Principle

The modified Sabouraud contains 2% rather than 4% dextrose and has a near neutral pH i.e. 7.0 as compared to 5.6. Other antibiotic containing media can now replace the very low pH in the original formula for suppressing bacterial contaminants.

Quality Control

Organisms	Incubation	Results
Trichophyton mentagrophytes ATCC 9533	25°C	Good Growth
Candida albicans ATCC 10231	25°C	Good Growth

Escherichia coli ATCC 25922	25°C	Inhibited

Sabouraud Gentamicin (50 Mg/L) Agar Slopes

Sabouraud Agar (Difco-modified-0747-01)	50 g.
Dist. H_2O	1000 ml.

Mix well, bring to a boil to dissolve. Cool to 50°C (check pH with ATC - pH ± 7.0). Add 1.25 ml Gentamicin (1 vial = 80 mgm/2 ml.). Mix well. Dispense 10-ml amounts into pre-sterilised UGB bottles. Autoclave 118°C/10 minutes. Cool in a slanted position. Tighten the caps. Label the bottles "SAB G". Store at 4°C.

Purpose

To isolate fungi especially yeast from contaminated specimens.

Quality Control

Organisms	Incubation	Results
Trichophyton mentagrophytes ATCC 9533	25°C	Good Growth
Candida albicans ATCC 10231	25°C	Good Growth
Escherichia coli ATCC 25922	25°C	Inhibited

Urea Agar Slopes

Urea agar base* (Gibco M52600)	29 g.
Distilled Water	100 ml.

Sterilize by filtration.

Agar	15 g.
Distilled Water	900 ml.

Autoclave at 121^{0}C for 15 minutes.

Cool to 50^{0}C. Then add filtered urea agar base solution. Mix thoroughly and dispense 2.5 ml amounts aseptically into sterile disposable tubes (13 x 100) with white caps. Allow medium to cool in slanted position so that a deep butt is formed.

*Urea agar base is kept in the refrigerator.

Quality Control

Organisms	**Incubation**	**Results**
Cryptococcus neoformans ATCC 76484	25^{o}C	Positive – pink
Escherichia coli ATCC 25922	25^{o}C	Negative – no pink

10% Potassium Hydroxide

Potassium hydroxide (KOH)	10g
Glycerol	20ml
Distilled Water	80 ml

Dissolve the potassium hydroxide in distilled water, then add glycerol. Mix well. Filter sterilize. Store in sterile amber bottle. Keep for 3 months.

Purpose

To digest or clear organic material e.g. tissue cells in a specimen in order to allow fungal structures to be more easily demonstrated.

Principle

Fungi are unaffected by KOH. Glycerol prolongs shelf-life by preventing crystallization and preserves the slides for a few days.

Record of Edited Revisions

Manual Section Name: Microbiology and Mycology Media Preparation Manual

Page Number / Item	Date of Revision	Signature of Approval
Annual Review	August 15, 2009	Dr. T. Mazzulli
Annual Review	August 15, 2010	Dr. T. Mazzulli
Annual Review	August 15, 2011	Dr. T. Mazzulli
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Mycology Media added on	September 4, 2014	Dr. T. Mazzulli
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Annual Review	September 18, 2016	Dr. T. Mazzulli
Annual Review	September 18, 2017	Dr. T. Mazzulli
Annual Review	April 16, 2018	Dr. T. Mazzulli