SELECTED MEDIA[1]

Cereal Agar (CER)

Mixed Cereal baby food (Pablum or Heinz)	25 g
Agar	5 g
Water	250 ml

Purpose
Used for promoting sporulation in many fungi including dark molds, dermatophytes and other hyphomycetes and for maintaining stock cultures. Not commercially available.
Preparation
Add cold water to dry ingredients in a 1 l flask. Mix well and autoclave at ⁰ 121C/20 minutes.
Cautions: 1) Because this medium is thick and the mixed cereal often contains spore-bearing bacilli, CER should be prepared in small amounts using a large container and autoclaved for the full time. 2) Use mixed cereal not oatmeal cereal. 3) For routine culture, this formulation is more suitable than a dilute form of the medium with added salts (Medium B:5, see Padhye et al. 1973). 4) Antibiotics may be added but are not recommended if the medium is to be used for maintenance of stock cultures.
Reference
Padhye, A.A. et al. 1973. Ascocarp production by *Nannizzia* and *Arthroderma* on keratinous and non-keratinous media. Sabouraudia 11:109-114. (Medium A:4, page 110).

Potato Dextrose Agar (PDA)

Potato starch	4.0 g
Glucose	20.0 g
Agar	15.0 g
Distilled water	1.0 L

Purpose
A general purpose sporulation medium available commercially (Difco).
Preparation
Suspend 39 g of powdered medium in 1 L water. Autoclave at 121C /15 minutes.

Oatmeal-salts Agar (OAT)

Magnesium sulphate	1.0 g
Monopotassium phosphate	1.5 g
Sodium nitrate	1.0 g
Oatmeal flakes (slow cooking type)	10.0 g
Agar	18.0 g
Distilled water	1.0 L

Purpose
Also known as Weitzman-Silva Hutner Agar. A medium to promote the development of the meiotic states of Onygenales and other ascomycetes.
Preparation
Pulse oatmeal in blender a few times to break flakes into smaller pieces. Babyfood oatmeal may be used.
Caution: 1) This medium boils over easily. Divide recipe into 2 one litre flasks. Adjust pH to 5.6. Autoclave 121C/15 min.
Reference
Weitzman, I. and Silva-Hutner, M. 1967. Non-keratinous agar media as substrates for the ascigerous state in certain members of the Gymnoascaceae pathogenic for man and animals. Sabouraudia 5:335-340.
Padhye, A.A. et al. 1973. Ascocarp production by *Nannizzia* and *Arthroderma* on keratinous and non-keratinous media. Sabouraudia 11:109-114. (Oatmeal salts agar medium E p. 111)

Cornmeal Agar (CMA)

Cornmeal (yellow)	50 g
Agar	15 g
Distilled water	1 L

Purpose
A nutritionally deficient medium used to promote sporulation, especially in ascomycetes and some dematiaceous hyphomycetes
Preparation
Mix cornmeal in 500 ml water. Heat for one hour in a 52 C waterbath or autoclave for 10 minutes at 121 C. Filter through cheesecloth (filtrate does not need to be clear) . Discard cornmeal residue. Bring volume of filtrate up to 1000 ml. Divide filtrate evenly into two

[1] The media recipes included here are those in common use at UAMH for enhancing sporulation. Recipes for these media as well as additional media and some techniques are described also in Kane, J., R.C. Summerbell, L. Sigler, S. Krajden, G. Land. 1997. *Laboratory handbook of dermatophytes. A clinical guide and laboratory manual of dermatophytes and other filamentous fungi from skin, hair and nails.* Star Publishing Co., Belmont, CA.

flasks. Add 7.5 g of agar to each flask. Boil to dissolve agar. Autoclave 121C/15 minutes. Note: Also available commercially.
Reference
McGinnis, M. 1980. Laboratory handbook of medical mycology. Academic Press, Harcourt Brace Jovanovich, New York.

V-8 Juice Agar (V-8)

V-8 juice	100.0 ml
Calcium carbonate	2.0 g
Agar	18.0 g
Distilled water	880.0 ml

Purpose
To promote sporulation in dark-colored fungi, yeasts and some coelomycetes and ascomycetes.
Procedure
Dissolve and sterilize 121C/15 minutes. Caution: Use fresh V-8 juice and store plates at 5C in dark.
Reference
Uchida, J.Y. et al. 1986. Basidiospore formation by Ceratobasidium sp. on agar. Mycologia 78:587-592.

Takashio's Agar (TAK)

Sabouraud dextrose broth (Difco)	3.0 g
Magnesium sulphate	1.0 g
Monopotassium phosphate	1.0 g
Agar	20.0 g
Distilled water	1.0 L

Purpose
To promote the sporulation of Onygenales and other ascomycetes.
Procedure
Dissolve and autoclave 121C/15 minutes.
Reference
Takashio, M. 1972. Sexual reproduction of some *Arthroderma* and *Nannizzia* on diluted sabouraud agar with or without salts. Mykosen 15:11-17.

Modified Leonian's Agar

Maltose	6.25 g
Malt extract	6.25 g
Monopotassium phosphate	1.25 g
Yeast extract	1.0 g
Magnesium sulphate	0.625 g
Peptone (Bacto)	0.625 g
Agar	20.0 g
Distilled water	1.0 L

Purpose
To promote sporulation in ascomycetes & hyphomycetes.
Preparation
Mix ingredients and heat to dissolve agar. Autoclave at 121C/15 min.. Dispense into plates or tubes.
Reference
Malloch, D. 1981. Moulds. Their isolation, cultivation and identification. Univ. of Toronto Press. p. 26.

Mycosel Agar (MYC)

Glucose	10.0 g
Phytone peptone	10.0 g
Agar	15.5 g
Cycloheximide	0.4 g
Chloramphenicol	0.05 g
Distilled water	1.0 L

Available commercially from BBL. A similar medium is also available as Mycobiotic agar (Difco).
Purpose
A selective medium for isolation of most pathogenic fungi that are tolerant of cycloheximide.
Preparation
Mix, heat with agitation until boiling. Sterilize 15 min. @ 118 C. Caution: Avoid overheating.
Reference
Georg, L.K. et al. 1954. Use of cycloheximide in the selective isolation of fungi pathogenic to man. J. Lab. Clin. Med. 44:422-428.

Phytone Yeast Extract Agar (PYE)

Glucose	40 g
Phytone (BBL)	10 g
Yeast extract	0 5 g
Agar	17 g
Water	1 L
Chloramphenicol stock soln. (25 mg/ml)	2 ml
Streptomycin stock soln.(5 mg/ml)	6 ml

Purpose
Commercially available. Similar to Sabouraud glucose agar but supports better growth of some dermatophytes.
Preparation
Dissolve and heat to boiling with agitation. Autoclave 121C/15 minutes. Cool and aseptically add antibiotics to give final concentrations of chloramphenicol 50 ug/ml and streptomycin 30 ug/ml. *Caution*: Caution: Avoid overheating. *Note*: This modification of Sabouraud glucose agar incorporates soy based

peptone and yeast extract. The medium may be made selective by addition of cycloheximide (similar medium available commercially as Mycosel agar).

Reference

Carmichael, J.W. 1962. *Chrysosporium* and some other aleuriosporic hyphomycetes. Can. J. Bot. 40:1139 (1137-1173).

Sabouraud Glucose Agar (SAB)

Glucose	40.0 g
Bacto Peptone or Neopeptone (Difco)	10.0 g
Difco agar	5.0 g
Distilled water	1 L

Also known as Sabouraud agar, Sabouraud dextrose agar or peptone glucose agar. Available commercially. Follow manufacturer's instructions.

Purpose

Widely used for growth of fungi but many fungi do not sporulate well on this medium. For use as a primary isolation medium, antibacterial antibiotics are usually added.

Preparation of plain medium

Mix ingredients and adjust pH to 7.0. Autoclave at 121C/15 min. Notes: Peptone source may vary. Some fungi demonstrate different colonial morphologies when grown on modifications of Sabouraud agar containing different peptones.

Preparation of medium with antibiotics

To 1 litre of medium, add:

Chloramphenicol (25 mg/ml)	4 ml
Cycloheximide (in 2 ml acetone)	0.1 g
Gentamicin (10 mg/ml)	5 ml

*Reference*s

Odds, F.C. 1992. Sabouraud('s) agar. J. Med. Vet. Mycol. 29:355-359..

Tap Water Agar (TWA)

Agar	15 g
Tap water	1 L

Purpose

Used as basal support medium in slide culture method and for stimulation of sporulation especially of darkly pigmented fungi.

Preparation

Mix well. Autoclave 121C/15 min. Pour 20 ml amounts into 100 mm or 10 -12 ml into 60 mm petri dishes.

Reference

Harris, J. 1986. Modified methods for fungal slide culture. J. Clin. Microbiol. 9:460-461.

Modified Melin Norkrans Medium

glucose	5.0 g
malt extract	2.0 g
yeast extract	1.0 g
potassium phosphate monobasic [KH2PO4]	0.5 g
ammonium phosphate dibasic [(NH4)2HPO4] (10% soln)	2.5 ml
magnesium sulphate [MgSO4]	0.15 g
calcium chloride [CaCl2] (1% soln)	5 ml
sodium chloride [NaCl] (1% soln)	2.5 ml
ferric chloride [FeCl3] (1% soln)	1.2 ml
agar	15 g
water	1 L

Preparation

Mix well using magnetic stir bar. Autoclave 121C /30 min. Cool to 55C and pour plates, keeping medium well mixed.

References:

Hutchison, L.J., Mycotaxon 42:389 (387-504) 1991; Marx, D.H., Phytopathology 59: 153-163 1969.

Morel Growth Agar - MGA

sucrose	6.5 g
mannose	3.4 g
yeast extract	0.14 g
calcium carbonate [$CaCO_3$]	10.0 g
agar	15 g
water	1000 ml

Preparation

Mix ingredients and autoclave 121C for 20 minutes. Note: The calcium carbonate does not dissolve completely, so plates will look cloudy. $CaCO_3$ is not required, but causes the fungus to grow faster.

Reference:

Winder, R.S. Mycological Research 110:612-623, 2006.

ANTIBIOTIC SOLUTIONS

Chloramphenicol Stock Solution

Chloramphenicol, pure 250 mg capsules	2
Ethyl alcohol	10 ml
Sterile distilled water	10 ml

Purpose
For inhibition of bacteria in culture media.
Procedure
Dissolve contents of capsules in alcohol. Add water to achieve stock concentration of 25 mg/ml. Add 2 ml to 1 l of medium to achieve final concentration of 50 µg/ml; add 4 ml to 1 l to obtain a final concentration of 100 µg/ml. Note: Use pure chloramphenicol, not Cm-succinate or Cm-palmitate.

Gentamicin

Purpose
For inhibition of Gram negative bacteria.
Procedure
Use injectable gentamicin (Garamycin) to achieve final concentration of 50 µg/ml. Formulation for SAB is based on pediatric strength of 10 mg/ml gentamicin dispensed in 2 ml vials. The formulation for adult strength injectable Garamycin is 60 mg/ml or 80 mg/ml.

Streptomycin

Streptomycin - injectable 0.5 g/ml	1 ml
Sterile distilled water	99 ml

Purpose
For inhibition of bacteria.
Procedure
Dilute 1:100 by adding 1 ml of injectable streptomycin to 99 ml sterile distilled water to obtain a stock concentration of 5 mg/ml. Aliquot into 7 ml volumes. Label with concentration and date prepared. Store at -20 C (or -70 C). Add 6 ml to 1 l of medium to achieve a final concentration of 30 µg/ml.

Cycloheximide (Actidione)

Cycloheximide	0.1 g
Acetone	2 ml

Purpose
Fungicidal, for inhibition of many saprophytic fungi. Many keratinophilic fungi resembling dermatophytes are not inhibited by cycloheximide.
Preparation
Dissolve cycloheximide in acetone. Add to hot medium while stirring.

Tetracycline Stock Solution

Tetracycline, pure 250 mg capsules	2
Sterile distilled water	20 ml

Purpose
For inhibition of bacteria in culture media.
Procedure
Dissolve contents of capsules in water to achieve stock concentration of 25 mg/ml. Add 2 ml to 1000 ml of medium to achieve final concentration of 50 µg/ml; add 4 ml to 1000 ml to obtain a final concentration of 100 µg/ml.