

## CULTURE MEDIA

### I. PRINCIPLE:

Clinical specimens are processed promptly and plated to isolation media as a means to recover fungi that may be causing disease. Media and incubation temperatures are selected to allow for the growth of pathogenic and opportunistic yeasts and fungi. Routine processing of specimens and culturing will be carried out by the Clinical Microbiology Laboratory for patients within UTMB.

### II. ISOLATION MEDIA:

CAUTION - Observe universal precautions at all times!!

A variety of media are available for the primary inoculation and recovery of fungi from clinical specimens (Table 4). No one specific medium or combination of media is adequate for all specimens. Media must be carefully selected based on specimen type and fungal suspected agents. Media is dispensed into containers such as 25 x 150 mm screw cap tubes or 100 mm Petri dishes. Petri plates offer the advantage of a large surface area for isolation and dilution of inhibitory substances in the specimens, but must be poured thick with at least 25 ml of medium to resist dehydration during incubation. Because plates are vented, they are more likely to become contaminated during incubation. Plates may be placed in gas permeable bags or sealed with gas permeable tape to offset this disadvantage. Each Petri plate must be labeled on the bottom, and the lid at least must be taped at two points to prevent accidental opening of the plate. All inoculated media should be read every 2 days following incubation and twice weekly thereafter. Plates must be opened only within a biological safety cabinet to prevent contamination of the plate and exposure of personnel to potentially dangerous fungi or *Mycobacterium sp.* Media in tubes have a smaller surface area but offer maximum safety and resistance to dehydration and contamination. If the specimen is from a contaminated site, it is important to include media that contain inhibitory substances such as chloramphenicol, gentamicin, or cycloheximide. Chloramphenicol or gentamicin will inhibit most bacterial contaminants, while cycloheximide inhibits most saprobic moulds. It is important to remember that cycloheximide may also inhibit opportunistic fungi such as some species of *Aspergillus*, *Fusarium*, *Scopulariopsis*, *Pseudallescheria*, zygomycetes, some dematiaceous fungi, and yeasts such as *Cryptococcus neoformans* and some *Candida* species. Antibacterial agents may inhibit the growth of aerobic actinomycetes like *Nocardia sp.* It is important to use media with and without inhibitory agents. Specimens from normally sterile sites can be inoculated to media without inhibitory substances.

#### A. Sabouraud Dextrose Agar (SAB)

1. SAB agar (Emmons' modification contains 2% glucose and is slightly acidic (pH 6.5). It is the standard medium for recovery and maintenance of a wide variety of fungi commonly isolated in the clinical laboratory. The original SAB formulation specifies 4% glucose. Emmons' modification with less glucose is preferred as an isolation medium because some isolates, notably *Blastomyces dermatitidis* may not be recovered using the original Sabouraud formulation.
2. SAB + Chloramphenicol + Cycloheximide - Mycosel (BBL) and Mycobiotic (Difco) agars are commercially prepared media containing SAB agar, 1% glucose, chloramphenicol, and cycloheximide. These media are used for the selective recovery of dimorphic fungi and dermatophytes.

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### II. ISOLATION MEDIA: (cont'd)

#### B. Brain-Heart Infusion Agar (BHI)

BHI is an enriched medium that enhances the recovery of *Cryptococcus neoformans* from sterile specimens such as CSF. BHI is commercially available in Petri plates and 25x150 mm screw cap tubes, or it can be made in house. BHI is also used in yeast-mould conversions for *Sporothrix* and *Paracoccidioides*.

#### C. Brain-Heart Infusion Agar + Gentamicin + Chloramphenicol (16 µg/ml) + 10% sheep's blood (BHI + GC + 10% bl) is an enriched medium useful for the recovery of fungi such as *C. neoformans* from contaminated specimens. The medium is commercially available in petri plates.

#### D. Inhibitory Mould Agar (IMA)

IMA is an enriched medium with inorganic salts, chloramphenicol, and gentamicin. It is useful for inhibiting bacteria. The medium is commercially available in petri plates.

#### E. Blood Culture Media

Two plated media are commonly used in conjunction with Isolator Lysis centrifugation:

1. Inhibitory Mould Agar with chloramphenicol and gentamicin for the isolation of fungi and the inhibition of bacteria.
2. Brain-Heart Infusion Agar for fungi and bacteria which may be substituted for Chocolate Agar.

#### F. Brain-Heart Infusion Broth

BHI broth with penicillin is added to the normal battery when zygomycetes are suspected. These fungi can be very difficult to recover. The use of broth provides optimal medium/specimen contact. The aseptic addition of sterile penicillin discs will inhibit bacteria. Malt extract agar is an effective alternative to broth media for the isolation of zygomycetes. BI broth may be used routinely by some ophthalmologists for corneal scrapings.

#### G. Sheep Blood Agar Plates

This is a medium of choice of ophthalmology. The plates are received in the mycology lab having been pre-inoculated with corneal scrapings by the physician.

#### H. Sterile Bread

Sterile bread without preservatives is recommended for the recovery of zygomycetes from clinical specimens. Bread is often superior to other media used in the Clinical Mycology laboratory for recovering this group of opportunistic pathogens. A piece of bread is sterilized in a humidified Petri dish. Specimens from non-contaminated sites can be directly inoculated. Contaminated specimens should be treated with antibacterial agents before inoculation. Zygomycetes will grow rapidly, often filling the entire Petri dish within a few days.

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### II. ISOLATION MEDIA: (cont'd)

#### I. Malt Extract Agar

Malt agar is a useful alternative to bread for recovery of zygomycetes and is excellent for environmental cultures.

#### J. Yeast Extract-Phosphate Medium (YEP)

YEP medium was developed for the enhanced recovery of *Blastomyces dermatitidis* and *Histoplasma capsulatum* from contaminated specimens. The incorporation of chloramphenicol inhibits bacteria and the addition of a drop of concentrated ammonium hydroxide (approximately 58%) inhibits bacteria and yeasts.

1. Place 0.05-0.1 ml of specimen on surface of yeast extract-phosphate agar plate and streak.
2. To one side of the plate, opposite from the heavily streaked area, immediately add 1 drop (approximately 0.05 ml) of concentrated ammonium hydroxide (NH<sub>4</sub>OH) using a sterile 1.0 ml pipet. Do not streak, but allow NH<sub>4</sub>OH to diffuse through the agar.
3. Wait 24 hours; seal the plates with Scotch tape.
4. Incubate at room temperature (25-30°C) for 6 weeks before discarding as negative. Store NH<sub>4</sub>OH in screw-capped bottle at 4°C.

#### K. Dermatophyte Test Medium (DTM)

DTM is used to recover dermatophytes from heavily contaminated clinical specimens and to presumptively indicate the presence of a dermatophyte. Its most valuable application is in veterinary mycology. Dermatophytes, as well as a few other fungi and bacteria turn the medium from pink to red.

#### L. Mitchinson 7H11 Selective Agar (7H11)

7H11 is used to isolate *Mycobacteria* and *Nocardia sp.* from specimens containing mixed Flora. The medium is available commercially and can be obtained from the Mycobacteriology Laboratory refrigerator when needed.

#### M. Procedure for *Malassezia furfur* Culture from Pediatric and Nursery Specimens

1. Place 3-4 drops of specimen in a BI + GC + 10% SB or IMA plate.
2. Place 3-4 drops of olive oil on top of specimen.
3. Streak both and seal as usual.
4. Place 2-3 drops of olive oil on a BI plate for sterility check of the olive oil. One plate used per day.
5. Store and incubate the plates at 30 or 35° as the other routine cultures.

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### III. SUGGESTED READING:

1. Koneman, EW, Roberts, GDP: Practical Laboratory Mycology, 3rd Edition, Baltimore, Williams and Wilkins, 1985.
2. McGinnis, MR: Laboratory Handbook of Medical Mycology, New York, Academic Press, 1980.
3. Roberts, GC, Goodman, NL, Land, GA, Larsh, HW, McGinnis, MR: Manual of Clinical Microbiology, 4th Edition, Lennette, EH, Balows, A, Hausler, WJ, Shadomy, HJ (eds) Washington, DC, ASM, pp. 500-513, 1985.