PROCEDURE: FUNGAL CULTURES – including Aerobic Actinomycetes

I. PRINCPLE

Fungi are ubiquitous organisms that reside not only in the environment as saprobes but also in humans and animals as part of the delicate balance of organisms we refer to as normal flora.
At present there are approximately 200 species of fungi capable of causing disease in humans.
In most cases, these infections occur because of an alteration in the bacterial population or in the natural immunity of the patient, usually through medical intervention.
Infections caused by fungi vary greatly with many organisms capable of causing several types. In general, they can be broken down into four groups: superficial / cutaneous / subcutaneous / systemic.
Fungi are physiologically dissimilar to bacteria, and therefore, they are resistant to the action of antibacterial agents. They are treated with a specialized group of agents with specific antifungal activity. These drugs are often accompanied by side effects of varying severity.
Aerobic actinomycetes resemble fungi due to the formation of filaments but are in fact bacteria. They are ubiquitous in nature, and many are nonpathogenic saprobes. Certain species, most notably Nocardia can cause pulmonary, systemic and cutaneous disease.

II. AVAILABILITY

The Mycology lab is staffed from 7:30 am until 4:00 pm Monday through Friday (except on holidays). Stat calcofluor smears are available, if clinically indicated between 7:30am – 3pm, Saturday and Sunday.

III. TEST CODES

A. CXFUS – FUNGAL CULTURE
B. CXFNS – FUNGAL CULTURE- HAIR, SKIN and NAIL
C. ISFUN – BLOOD ISOLATOR FOR FUNGUS
D. CALC – CALCOFLUOR SMEAR
E. FUNID – OUTSIDE REFERRAL

IV. SPECIMEN PROCESSING AND HANDLING

A. Fungal culture is available on all specimen types except stools and nasal specimens.
B. Blood for fungal culture is accepted only when submitted in an Isolator tube and require ID approval. *Refer to Isolator 1.5 and 10 Bloods Procedure* for processing. For Pedi Isolators, please credit the ‘spin’ portion of the charge.
C. Skin and hair specimens are planted directly on appropriate media. Care should be taken to ensure good contact with the agar without causing it to crack.
D. Nails should be scraped with a scalpel or ground with a nail grinder to produce shavings or a homogenized sample. Place shavings on appropriate media, ensuring good contact. It may be necessary to push into agar for nail to stick.
E. Culture requests for *Malassezia* (or suspected through primary stain examination), require two sets of sabouraud plates to be inoculated (when quantity of specimen permits).
    1. One set should be streaked with a thin layer of olive oil (applied with a swab in a manner similar to streaking a Kirby Bauer susceptibility) before the specimen is planted.
    2. The second set is planted according to the routine planting procedure
F. The BCID-FP panel is performed on blood cultures whose Gram stains indicate budding yeasts – *Refer to BCID-FP Procedure*.
G. All fungal culture plates (original & subculture) need to be sealed with a shrink seal to avoid accidental opening and to keep media from drying out.

H. Tissue specimens where a request to rule out Mucormycetes has been made should not be ground. Instead slice or mince tissue with a sterile scalpel and place on fungal media. An additional sabouraud plate should be added and incubated at 37°.

V. EQUIPMENT AND MATERIALS

A. Shrink Seals
B. Lactophenol Cotton Blue (LPCB)
C. Class II Biological safety cabinet
D. Routine culture disposables and supplies

VI. TEST PROCEDURE

A. Incubation – duration and temperature.
   1. Routine cultures are incubated at 30°C.
   2. Length of incubation is 4 weeks or 1 week depending on specimen source.
      Note: Mouth, throat, vaginal and urine fungal cultures are primarily for the cultivation and isolation of Candida species. The length of incubation for these types of cultures is 7 days
      a. Examined daily for the first week
      b. Examined once per week (weeks 2-4)
      c. Occasionally all or part of a culture will be discarded prior to 4 weeks:
         i. Skin, nail, and hair cultures which grow a dermatophyte can be finalized when the dermatophyte work-up is complete. Bring up on rounds any other cultures that may warrant being finalized early.
         ii. Plates which are overgrown with mold can be discarded, however, the remaining plates should be incubated for the full 4 weeks

B. Yeast and yeast-like organisms workup
   1. Colonial morphology
      a. Yeasts usually require a minimum of 48 hours to grow. Occasionally very small colonies may appear after overnight incubation.
      b. Colonies are usually dull convex and white or cream in color. It is possible to have pink and tan isolates. Colonies of Cryptococcus may look mucoid.
      c. Yeast like organisms
         i. *Trichosporon sp.* Cream colored moist then becoming wrinkled, powdery or crumblike, may adhere to or crack the agar. True hyphae and pseudohyphae with blastoconidia singly or in short chains
         ii. *Blastoschizomyces capitus* smooth to wrinkled, white to cream colored. Yeastlike cells, hyphae, pseudohyphae, arthroconidia, and annelloconidia , in clusters at the end of annelids
         iii. *Geotrichum candidum* White, moist, yeastlike, later having the appearance of ground glass. True hyphae that segment into rectangular arthroconidia.
   2. Most yeast have entire edges especially when young. More mature colonies may become "star-like" as pseudohyphae form and give rise to numerous filaments surrounding the colony.
   3. Yeast Identification
      a. Primary Identification is performed by the Vitek MS (MALDI). *Refer to VITEK Mass Spec procedure* for complete instructions
      b. VITEK 2 system is used as a back-up system for identification of yeast. *Refer to VITEK 2 Procedure* for complete instructions.
      c. Yeasts are typically identified to the species level in sterile body sites and tissues. Identification to the species level may be requested in other body sites.
   4. Yeast susceptibilities

a. Susceptibilities are performed on all yeast isolated from a blood culture. The first positive blood per admission should be tested and additional positives should be referred. If referring, a MALDI should be performed to confirm id. If a patient is deceased (even if blood cultures were collected prior to patient's death) a susceptibility should not be set up. A yeast susceptibility can be referred up to one month from the original susceptibility. A physician may request a susceptibility inside of the one-month referral period.
b. Susceptibility requests on yeast isolates from all other specimen sources are done by physician request only. These requests should be brought up on Rounds.
c. Susceptibilities are performed on pure 24hour isolates grown on SAB. *Refer to Fungal MIC Procedure* for complete instructions
d. Susceptibilities on Cryptococcus isolates are sent to The South Texas Reference Laboratory at UTHC in San Antonio, TX. *Refer to Mycology Send-out Procedure.*

C. Mold workup

All molds must be examined and manipulated in the biological safety hood. Fungus is best examined when the culture first begins to grow and again a few days later.

1. Examine mold macroscopically
   a. Front - color
   b. Reverse - color
   c. Texture
   d. Topography
2. Examine mold structures microscopically with mold submerged in Lactophenol cotton blue (LPCB) for production of conidia or spores using one of the techniques described below.
   a. Teased Prep
      i. Scrape a granular area of the colony and placing the material in a drop of LPCB use a second, inoculating loop, bent needle, or applicator stick broken at an angle, to tease the mat apart to yield a thinner preparation of organism.
      ii. Place coverslip over preparation
      iii. Examine under light microscope at low and high-dry magnifications
   b. Scotch Tape Prep
      i. Prepare by placing the sticky side of a piece of clear tape to a granular area of the colony.
      ii. Place the tape over a drop of LPCB on a slide.
      iii. Put another drop of LPCB and cover with a coverslip.
      iv. Examine under light microscope at Low and high-dry magnifications
   c. Both methods will not have to be performed on each mold isolated. Scotch tape preps work well with most molds. Tease preps may be preferred when working with loosely conidiated species. i.e., Aspergillus niger
3. Dermatophyte Identification
   a. If mold appears to be a dermatophyte (isolated from skin, hair, nails, grows on mycosel and/or DTM with red color, and produces macroconidia and/or microconidia), identification is based on the following macroscopic and microscopic presentation
      i. *Microsporum* species →Macroconidia with rough walls predominate but microconidia observed
         (a) *Microsporum canis* →White colony with lemon yellow reverse and thick-walled rough macroconidia
         (b) *Microsporum gypseum complex (synonymous with Nannizzia gypsea)*→Tan, granular colony with tan reverse and many thin-walled "pickle-shaped" macroconidia

(c) *Microsporum audouini* → White colony with peach or salmon reverse; Conidia absent - set up PDA and /or potato flake agar. -- little or no conidia seen, only "beaked" chlamydoconidia

(d) *Microsporum ferrugineum* →yellow to rusty orange with cream or brownish reverse – “bamboo hyphae”, intercalary chlamydoconidia

ii. *Epidermophyton floccosum* → Only smooth walled macroconidia observed

iii. *Trichophyton* species → Microconidia predominate but macroconidia also may be observed

(a) *Trichophyton mentagrophytes* →White granular or fluffy colony with tan reverse, clusters of round grape-like conidia

(b) *Trichophyton rubrum* → White cottony colony with red reverse, single peg-shaped conidia singly along hyphae- “birds on a telephone wire”

(c) *Trichophyton verrucosum* → White compact waxy colony with cream reverse, better growth at 37°C with chains chlamydoconidia

(d) *Trichophyton tonsurans* →Yellow or tan or white powdery colony with tan reverse, varying sized and shaped microconidia

(e) *Trichophyton soudanense* →Yellow compact colony with fringed border and microscopically exhibits "reflexive branching" (Hyphae fragment and grow backwards). May be confirmed by subculture to LJ slant, where organism will grow as brown colony

(f) *Trichophyton violacium (synonymous with Trichophyton yaoundei)* →deep purplish red, waxy colony with lavender to purple reverse. Tangled,branched irregular hyphae with intercalary chlamydoconidia. Microconidia and macroconidia are not usually seen.

4. Dematiaceous mold identification

Dematiaceous = darkly pigmented on the front and on the back

b. Velvety colony - check for conidia

i. *Cladosporium species* → Black or olive velvety colony with conidia in treelike chains, showing dark spots (hila) when dislodged. Cells bearing conidia resemble shields

ii. *Cladophialophora carrionii* → branching chains of oval lemon shaped conidia NG at 42°C

iii. *Cladophialophora bantiana* → growth at 42°C, long conidial chains

iv. *Phialophora* → Only vase-shaped phialide gives rise to conidia in clusters

v. *Pleurostomophora richardsiae* (formerly *Phialophora richardsiae*→ flask shaped phialide with flared, saucer-shaped collarette, may have distinct septum at base

vi. *Phialophora verrucosa* → flared phialide

c. Mucoid colony turning velvety– check for conidia

i. *Exophiala jeanselmei complex*→ annelloconidia in clusters

ii. *Exophiala dermatitidis (Wangiella dermatitidis)*→ phialoconidia with pointed phialide; grows at 42°C. **The early yeast-like stage may be identified using MALDI-TOF.**

iii. *Aureobasidium or Hormonema species* → Black, mucoid colony with hyaline and thick-walled black arthroconidia

d. Woolly grey colonies
   i. *Alternaria species* → *W*oolly grey colony with irregularly divided macroconidia in chains
   ii. *Curvularia species* → Woolly grey colony with curved "boomerang-shaped "macroconidia
   iii. *Bipolaris* → Woolly grey colony with cylindrical macroconidia that have slightly protruding hilum
   iv. *Scedosporium sp. Complex* → Grey fluffy colony, large single or small clusters of annelloconidia. Cleistothecia of sexual state are often inhibited on mycosel.
   v. *Lomentaspora prolificans (formerly Scedosporium prolificans and Scedosporium inflatum)* → young colony cottony or moist, light grey to black. Mature colony dark grey to black and possible white mycelium. Annelides having a swollen base and elongated neck. Conidia are often olive to brown with a cut-off base and form small clusters at end of neck.
   vi. *Verruconis species*→woolly, red to brown mold with red diffusible pigment. Thread like denticles with two-celled conidia. *Verruconis gallopava* is constricted at point of conidial attachment

5. Mucormycete identification
   e. Woolly colony rapidly growing with broad aseptate or sparsely septate hyphae – growth may be better at 37°
      i. *Cunninghamella* →Single spore per sporangium
      ii. *Syncephalastrum* → Multiple spores present in a tubular sporangium (merosporangium)
      iii. *Rhizomucor* →Multiple spores present in sac-like sporangia
      iv. *Rhizopus* → sporangiophore unbranched with rhizoids opposite ("salt & pepper" colony)
      v. *Mucor* → sporangiophore branched, rhizoids absent (grey or tan colony)
      vi. *Lichtheimia corymbifera complex* (formerly *Absidia corymbifera*)→ sporangiophore branched with rhizoids internodal, septum just below sporangium which is small and compact. “martini glass shape”

6. Hyaline mold identification
   Colony lightly colored or brightly colored with limited or no growth on Mycosel agar.
   f. *Scopulariopsis* → Conidia resemble "light bulbs" with or without a rough wall originating from annelides (brown powdery colony)
   g. Conidia originate from short lateral conidiophore.
      i. *Chrysosporium* → Conidia small, arthroconidia present (white cottony colony)
      ii. *Sepedonium* → Conidia large and knobby with thick walls, (white or yellow cottony colony)
   h. Conidia originate from phialides
      i. *Aspergillus* → phialides found on vesicles
         (a) *Aspergillus niger* → Colony black, gritty with large conidial heads - phialides sit atop metulae and encircle the vesicle (radiate biseriate)
         (b) *Aspergillus fumigatus* → Colony grey green powdery with very small compact conidial head - phialides found only on the upper half of the vesicle (uniseriate columnar)
         (c) *Aspergillus flavus* → Colony bright yellow-green, gritty with large conidial head, phialides encircle the vesicle, conidiophore echinulate

(d) *Aspergillus terreus* → Colony cinnamon brown, powdery with a double row of phialides at upper 1/2 to 2/3 of vesicle
(e) *Aspergillus glaucus* → Green powdery colony with very large conidia on phialides surrounding vesicle, cleistothecia present
(f) *Aspergillus nidulans* → Green powdery colony with reddish reverse and brown conidiophore, phialides biseriate and columnar, sometimes cleistothecia and Hùlle cells

ii. *Paecilomyces variotii* →phialides arranged in penicillate fashion, phialides tapered, conidia oval (brown powdery colony)
iii. *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) → pinkish, mauve, violet colony,rough-walled metulae that support densely clustered phialides, flask or tenpin shaped.
iv. *Penicillium* →phialides arranged in penicillate fashion, phialides flask shaped, conidia round (usually green powdery colony but may vary)
v. *Verticillium* →long tapered phialides in whorls (light colored colony)
vi. *Trichoderma* → short, widely branched phialides with clusters of conidia (green powdery colony)
vii. *Fusarium* → single or poly phialides with sickle shaped conidia (cream or light purple colony)
viii. *Acremonium* → long tapered single phialides with oblong, usually 1-celled conidia (salmon colored colony)

7. Dimorphic fungi identification – Due to laboratory safety concerns, time and conversion difficulties isolates other than Sporothrix schenckii must be sent to reference lab for confirmation by DNA sequencing (PCR) or exo-antigen testing.
   Colony lightly colored with good growth on Mycosel agar
   i. Restreak organism to BAP and incubate at 37°C
      i. *Histoplasma capsulatum* → growth of small yeast at 37°C and mold with large tuberculate conidia at room temperature (tan colony becoming dark brown with age and sometimes producing a brown diffusing pigment)
      ii. *Blastomyces dermatitidis* →growth of large broad-based yeast at 37°C and mold with single tear drop shaped conidia at room temperature
      iii. *Paracoccidioides brasiliensis* →growth of large yeast with multiple buds at 37°C and mold with single tear drop shaped conidia at room temperature
      iv. *Coccidioides immitis* → growth at 25° and 37° of a white cottony mold that grows up more than spreading over entire surface of agar. Alternating thick and thin-walled barrel shaped arthroconidia with alternating empty cells
      v. *Sporothrix schenckii complex* → at 25-30° white then developing into grey, black leathery colonies with white periphery. Very narrow hypha with delicate conidia in "daisy" arrangement
      vi. *Talaromyces (Penicillium) marneffei* → at 25-30° colony is tan,powdery and velvety, later becoming reddish-yellow with possible blue-green center. Deep red soluble pigment diffuses into agar in 3-7 days. Brush- like structures with metulae bearing 4-5 phialides with round to oval conidia.

D. Actinomycete workup and identification - **see Table 1**

1. Colony is usually brittle and crunchy and composed of thin branching filaments
2. Perform partial acid-fast stain- Refer to Partial Acid-fast Stain procedure
3. A Maldi should be performed on PAF positive gram-positive branching rods. If a valid Nocardia identification is obtained the result may be reported. If Maldi identification fails, report as Nocardia species and send to the University of Texas Health Center for further id. All Nocardia isolates should be sent to the Mycobacteria/Nocardia Laboratory in Tyler Texas for susceptibility. Refer to Mycology Send-out Procedure for further instructions.

Table 1 – Identification Guide for Gram-positive Branching Rods

Organism ID	Colony	Partial Acid Fast
Nocardia asteroids complex	Coral / Orange	+
Nocardia brasiliensis	Yellow / Orange	+
Nocardia otitidiscaviarum	Tan / Orange	+
Nocardia nova	Chalky / Coral / Orange	+
Streptomyces species	Chalky / white	-
Actinomadura madurae	Red	-

VII. INTERPRETATION & REPORTING RESULTS

A. Preliminary reports should be resulted upon initial reading as:
~No Fungus Isolated to date
~Culture in Progress
~4 Weeks Required for Final Report
or when applicable (urines, throats, mouth and vaginal specimens)
~No Fungus Isolated to date
~Culture in Progress
~1 Week Required for Final Report

B. Cultures negative for yeast and mold should be finalized as:
No fungus isolated at 4 weeks
or when appropriate
No fungus isolated at 1 week

C. **NO QUANTITATION IS REPORTED FOR HAIR, SKIN, NAIL, CORNEAL(BEDSIDE) SCRAPINGS, CATHETER TIPS AND FUNGAL ISOLATORS.** All other isolates should be reported with a quantitation.

D. Yeast growing in respiratory cultures are reported relative to the mixed flora present in the specimen. They are not routinely identified to species level.
Report with quantitation using one of the following statements:
1.Respiratory flora present where only organism is yeast.
2.Mixed Respiratory flora present where predominant flora is yeast.
3.Mixed respiratory flora including yeast

E. In throat, mouth, genital, and urine specimens, when the yeast grows on Mycosel agar, isolates are reported as Yeast "**Probable Candida albicans".** Use the canned message **&CALB**.

F. Low numbers of yeast (1+) growing in a mouth/ throat or vaginal culture, especially when mixed with other predominating normal flora, should have the comment **"organisms isolated suggest mixed respiratory flora"** or **"organisms isolated suggest mixed vaginal flora"** added, along with **No Further Workup. &OISO &OISV &NFW**. Yeast present in higher quantities should have the consult required for further workup statement added. **&CONS**

G. When possible fungal culture results should be correlated with routine cultures and matching statements added when appropriate. For example, a wound culture where yeast has been included in with mixed cutaneous flora and the fungal culture grows out 1+ yeast should be reported as

1+ yeast
Organisms isolated suggest mixed cutaneous flora
No Further Workup

If the routine culture lists flora as mixed aerobic flora only, add the comment **No Further Workup** after the yeast in the fungal culture.

H. CXFNS in which a mold other than a dermatophyte is reported should have the comment '**No dermatophyte isolated at 4 weeks**" added to the final report.

I. Cultures held for 4 weeks that have yeast reported should include the comment **"No mold isolated at 4 weeks"** in the final report.

J. If a mold is reported that is an unlikely cause of the infection, an isolate comment "Possible contamination" or Probable contamination" should be added. Likely contaminants may include, *Penicillium*, *Mucor* and *Cladosporium spp*. **Molds isolated from severely immunocompromised patients- i.e., transplant, burn, cancer should always be considered significant.** Questionable cultures should be brought up on rounds.

K. Molds determined to be dermatophytes can be reported as **Mold – Dermatophyte isolated**. **No further work-up** without further speciation if a definitive identification cannot be achieved.

L. Molds growing in low numbers (1+) may in some situations represent contamination or colonization. i.e., Aspergillus in low numbers in a respiratory culture. If appropriate use the code **&FUNC** (may represent fungal contamination or colonization).

Table 1

ISOLATE COMMENT	COMMON USES	STATEMENT
&CALB	When yeast grows on Mycosel agar. Mainly used with throat, mouth, urine and vaginal cultures	Probable Candida albicans
&POSC &PROC	When reporting out molds of questionable significance Questionable cultures should be brought up on rounds.	Possible contamination Probable contamination
&FUNC	When Aspergillus, particularly fumigatus and niger, are present in low numbers (1+) in respiratory cultures (sputum and bronchial specimens)	May represent fungal contamination or colonization
&NFW	When finalizing molds where a susceptibility is not being performed. (i.e., dermatophytes). Yeasts determined to be part of commensal flora	No further workup
$CONS	Yeasts in significant quantities (i.e., >=2+ in a throat) and yeasts identified to the species level where a susceptibility is not being performed	Consult required for further workup
&OISO $OISV	Low numbers of yeast (1+) in a mouth/ throat or vaginal cx, especially when mixed with other normal flora	Organisms isolated suggest mixed oral flora Organisms isolated suggest mixed vaginal flora

VIII. NOTES

A. Yeasts and molds may be referred to the mycology lab for workup. The referral list must be checked daily.
B. Yeasts and molds from sterile body sites should always be called. Significant pathogens should also be called i.e., Cryptococcus from any body site.
C. Referred cultures and cultures finalized prior to 4 weeks are saved for one week in the 7-day save rack located at the mycology bench.
D. Mold isolates from bloods, tissues and sterile body sites and yeast that have had an MIC performed should be stocked and recorded in the Mycology save log. More fastidious organisms (Nocardia species & molds) are frozen in cryosaver vials. Yeasts can be stocked in motility. Unusual isolates should also be saved.
E. If the organism lacks conidia, it should be sub-cultured to PDA to encourage sporulation.
F. Yeasts and molds can be subbed to IMA when necessary to eliminate bacteria.
G. Mycosel agar and a blood agar plate incubated at 35°C can also be used to evaluate the significance of a mold. Molds not able to grow at body temperature may be contaminants.
H. Bring molds that cannot be identified up on rounds. Obvious contaminants (not on streak mark or on edge of plate) should not be reported.
I. Lactophenol cotton blue preps that are associated with the final id of a mold should be saved in the slide tray located on the mycology bench. The oldest tray is emptied when space is needed.
J. Significant molds from sterile body sites and tissues may need to be sent out for susceptibility testing. Physician must be notified to see if susceptibility is needed and if so, what antifungals should be tested. *Refer to Mycology Send-out procedure*.
K. Yeasts reported to the species level cannot be referred to a previous culture. An identification must be performed.
L. Bring up on rounds any yeast isolate suspected of being Candida auris. Any confirmed Candida auris isolates need to be reported to Infection control and sent to the RIDOH.

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