MYCOLOGY CULTURE EVALUATION

I. Principle

Mycology plates are interpreted frequently to optimize the recognition and identification of yeasts and molds.

II. Specimen

Inoculated mycology culture plates

III. Materials

A. Glass slides
B. 22 X 22 mm coverslips
C. Scotch tape
D. Light Microscope with 10X and 40X objectives
E. LPCB (lactophenol cotton blue) stain, store at room temperature until the expiration date.
F. Sterile water, store at room temperature until the expiration date.
G. Wooden applicator sticks

IV. Schedule

A. Blood Isolators
    1. Examine the SAB and BHIA with gent and chloro Monday, Wednesday, and Friday.
    2. Refer to the **"Fungal Blood Cultures: DuPont Isolator System Method"** procedure for the workup of positive cultures.
B. Routine Mycology cultures
    1. Additional Friday duties
        a. Interpret the mycology cultures (day 1through week 4).
        b. Perform all of the workups on new and old isolates.
    2. Additional Monday duties
        a. Interpret all of the mycology cultures (day 1 through week 4).
        b. Perform all of the workups on new and old isolates.
        c. Move the cultures ahead 1 week to the next spot in the incubator.
        d. Finalize the reports on the 4 week old cultures under MNG (Micro No Growth) if negative, and discard the media into appropriate receptacles.
        e. Review the Incomplete log.
    3. Additional monthly duties
        a. Subculture all of the stock yeast and mold isolates on the last Friday of every month.
C. Cultures referred from Bacteriology and Mycobacteriology
    1. Perform identifications on these isolates to the genus and species level.
    2. Report in Mysis, and charge appropriately.

V. Procedure

A. Remove the positive plates from the 30° incubator, and place them on the Mycology cart.
B. Review each culture, and bring positive cultures up on rounds.
C. Plates with yeast-like or bacteria-like growth: perform a wet prep examination on all of the different colony types.
    1. Wet prep procedure

a. Place a drop of water on an appropriately labeled glass slide.
b. Touch a suspected colony with an applicator stick, and inoculate it into the drop of water. Apply a cover slip.
c. Examine the smear under the 10X and 40X objectives for bacteria/yeast.
d. If yeast is observed, refer to the Yeast Identification Procedure.

D. Yeasts for antifungal susceptibility testing
1. Refer to the *Candida* Disk Diffusion Procedure for routine testing for fluconazole and voriconazole.
2. If other antifungal agents are requested, the antibiotics should be specified by the requesting physician.
3. Subculture the yeast onto 2 SAB slants.
4. Give 1 SAB slant and the test request to PAML or SHMC sendouts to be sent to ARUP for MIC testing.
5. Store the other SAB slant at 2-8°C in the Microbiology.

E. *Nocardia* for Susceptibility Testing
1. This testing is only performed on physician request. The physician must request what antibiotics should be tested.
2. Subculture the organism to 2 SAB slants.
3. Submit 1 SAB slant with the request to the PAML or SHMC sendout department.
4. *Nocardia* isolates are sent for susceptibility testing to ARUP.
5. Store the second SAB slant in the mycology lab until the results are final.

F. Plates with mold:
1. Refer to Figure 1 for mold isolate workup.
2. Determine the specimen type.
a. Sterile body fluids and nonsterile sites except for hair/skin/nails with one colony of mold or one colony of several molds: bring it up on rounds before performing the lactophenol cotton blue preparation.
b. Sterile body fluids with numerous colonies of a similar mold on one or both plates: perform the lactophenol cotton blue scotch tape preparation (refer to **"Lactophenol Cotton Blue Preparations"** procedure), and bring it up on rounds.
c. Nonsterile body fluids with numerous colonies of a similar mold on one or both plates: perform a lactophenol cotton blue scotch tape preparation, and bring it up on rounds.
d. Hair/skin/nails: perform a lactophenol cotton blue preparation on all mold colonies.
i. If the mold shows sterile hyphae on the LPCB prep, subculture the isolate to potato flake agar.
3. Bring up all significant mold isolates on rounds.
4. Mold isolates should be identified, if necessary, using Figures 2-7.
5. Record all of the isolate's characteristics in the Misys computer.
a. Indicate the color of both the surface and the reverse of the colony.
b. Describe any typical microscopic morphologic characteristics observed.

G. Notify Rounds of any significant bacterial isolates on sterile body sites.
H. Follow the appropriate procedures for the identification of yeasts and molds.

VI. Result Reporting

A. Report "No fungus isolated" on all final mycology cultures that exhibit no fungal growth after the appropriate length of incubation.

B. Send a preliminary report of "*Candida albicans* or yeast, not *Candida albicans*" on cultures demonstrating yeast.
C. Report preliminary results on any mold isolated.
D. Send out a preliminary report with an organism identification if the culture work-up is completed. Send out final reports when the plate incubation is complete. Report the genus and species of the fungus isolated.
   1. Example: *Candida albicans*
      Aspergillus fumigatus
E. Critical values should be called to the patient care unit and/or physician of record. (Refer to **"Critical Values - Division of Microbiology"**).

VII. Reference

A. Isenberg, HD, et al. (ed.). 1992. Clinical Microbiology Procedure Handbook, Vol 1. p 6.6.1-6.8.1. American Society for Microbiology, Washington, DC.

Figure 1

Mold Isolate Workup

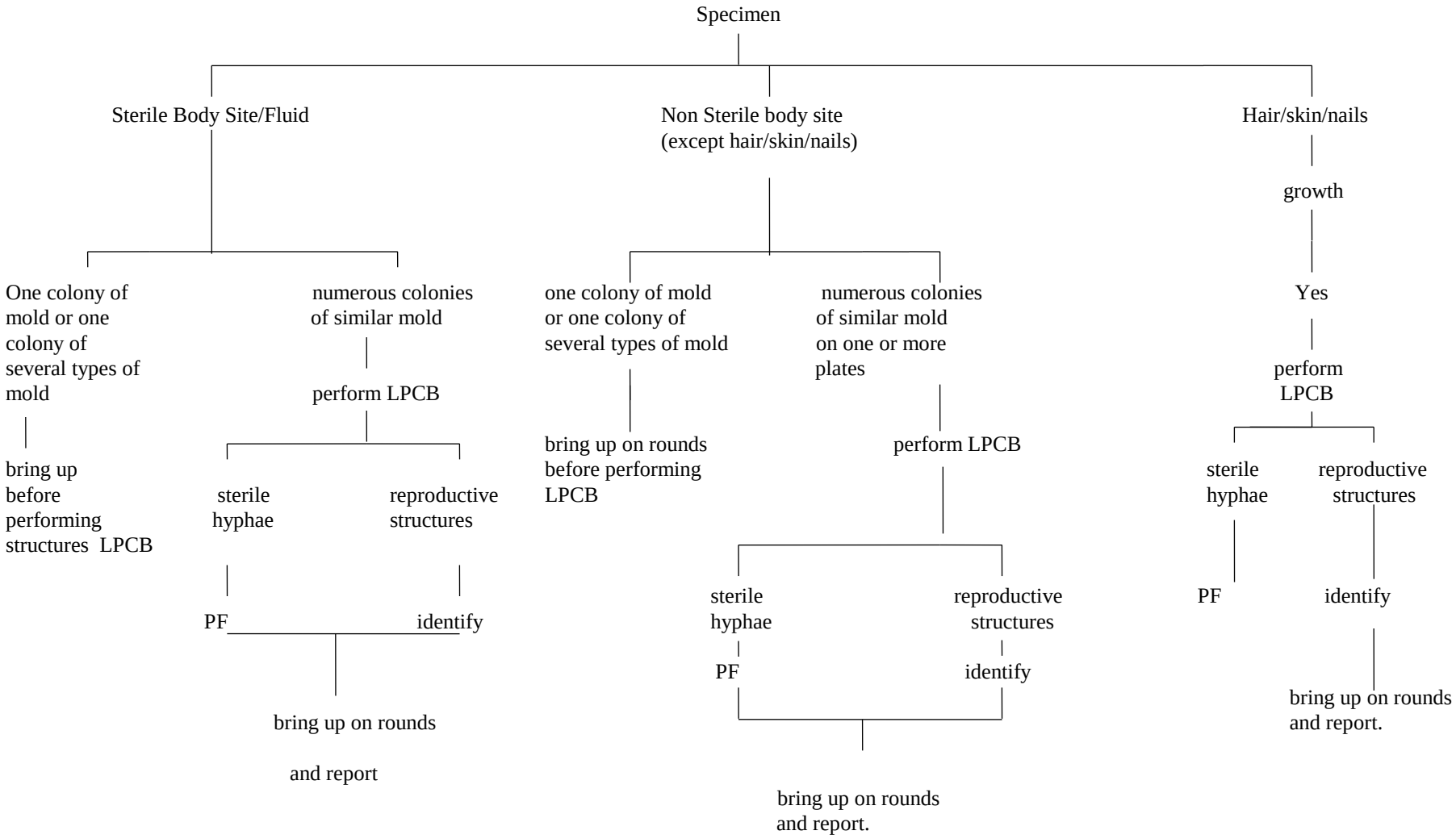


Figure 2

Guide to Identification of Common Molds

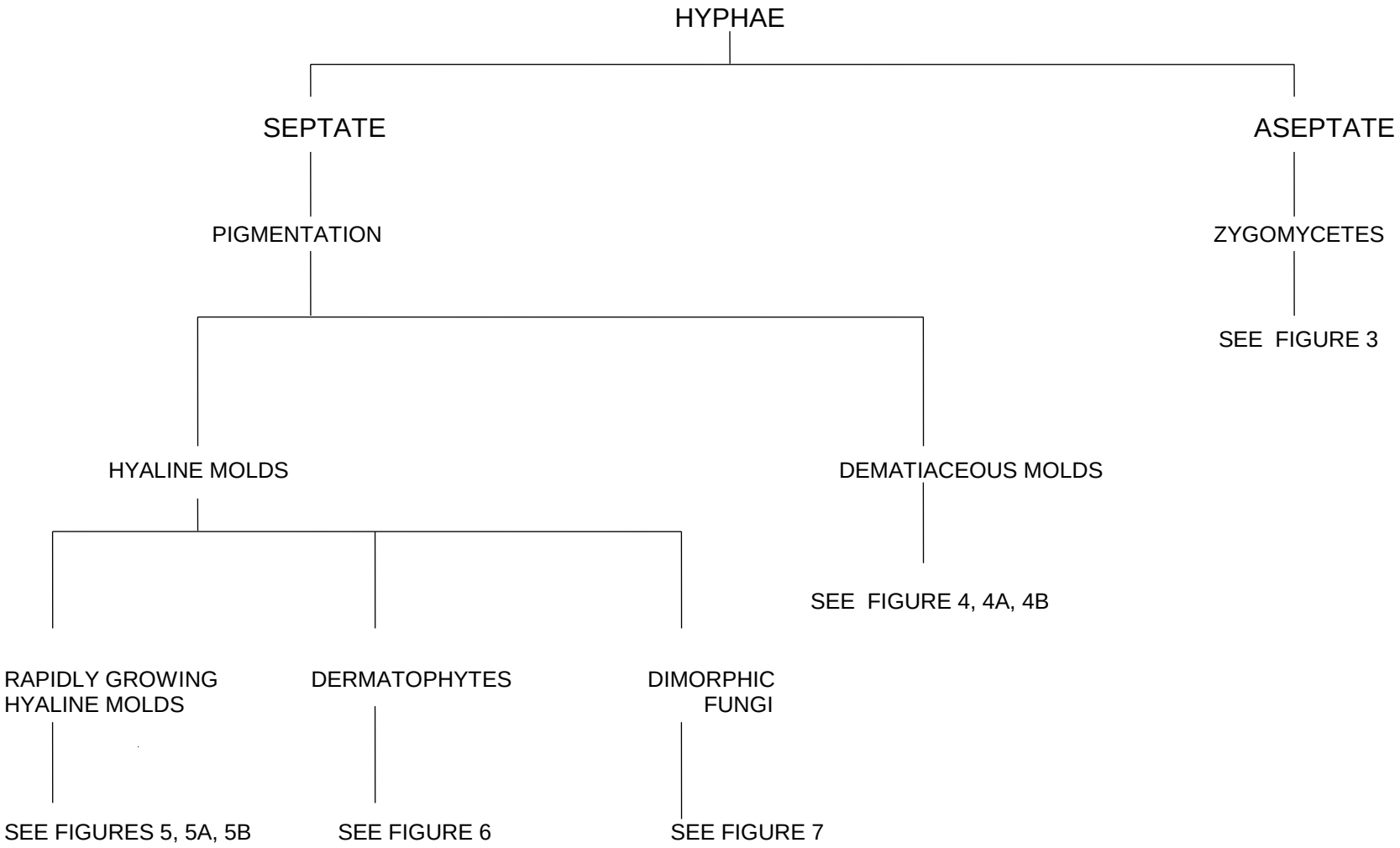
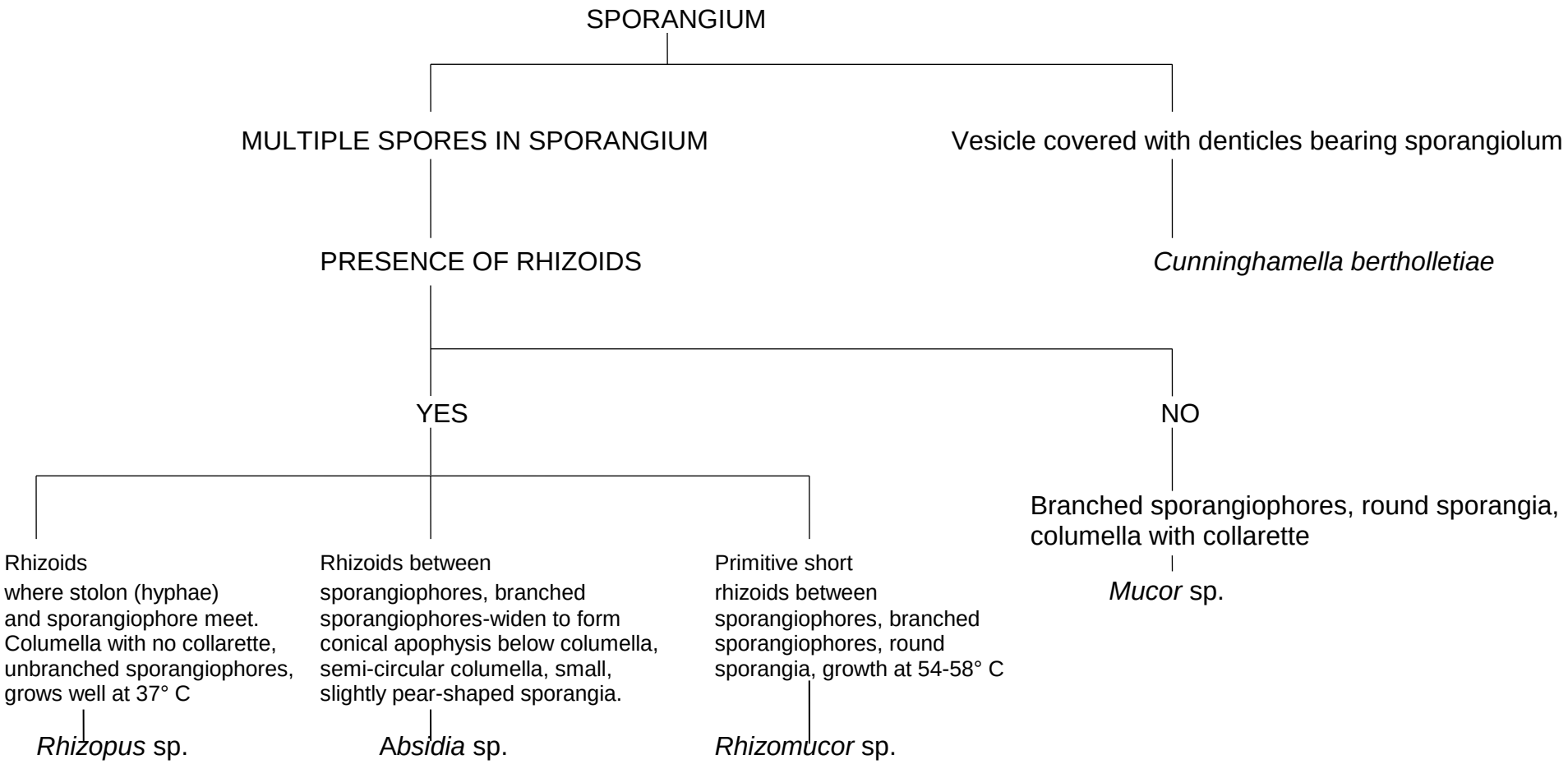


Figure 3

ZYGOMYCETES
Wooly, White/gray, Rapidly Growing Colony with Broad Aseptate or Sparsely Septate Hyphae



OTHER POTENTIAL PATHOGENS: *Conidiobolus coronatus, Basidiobolus sp., Syncephalastrum racemosum, Cokeromyces recurvatus, Saksenaea vasiformis, Apophysomyces elegans*

Figure 4

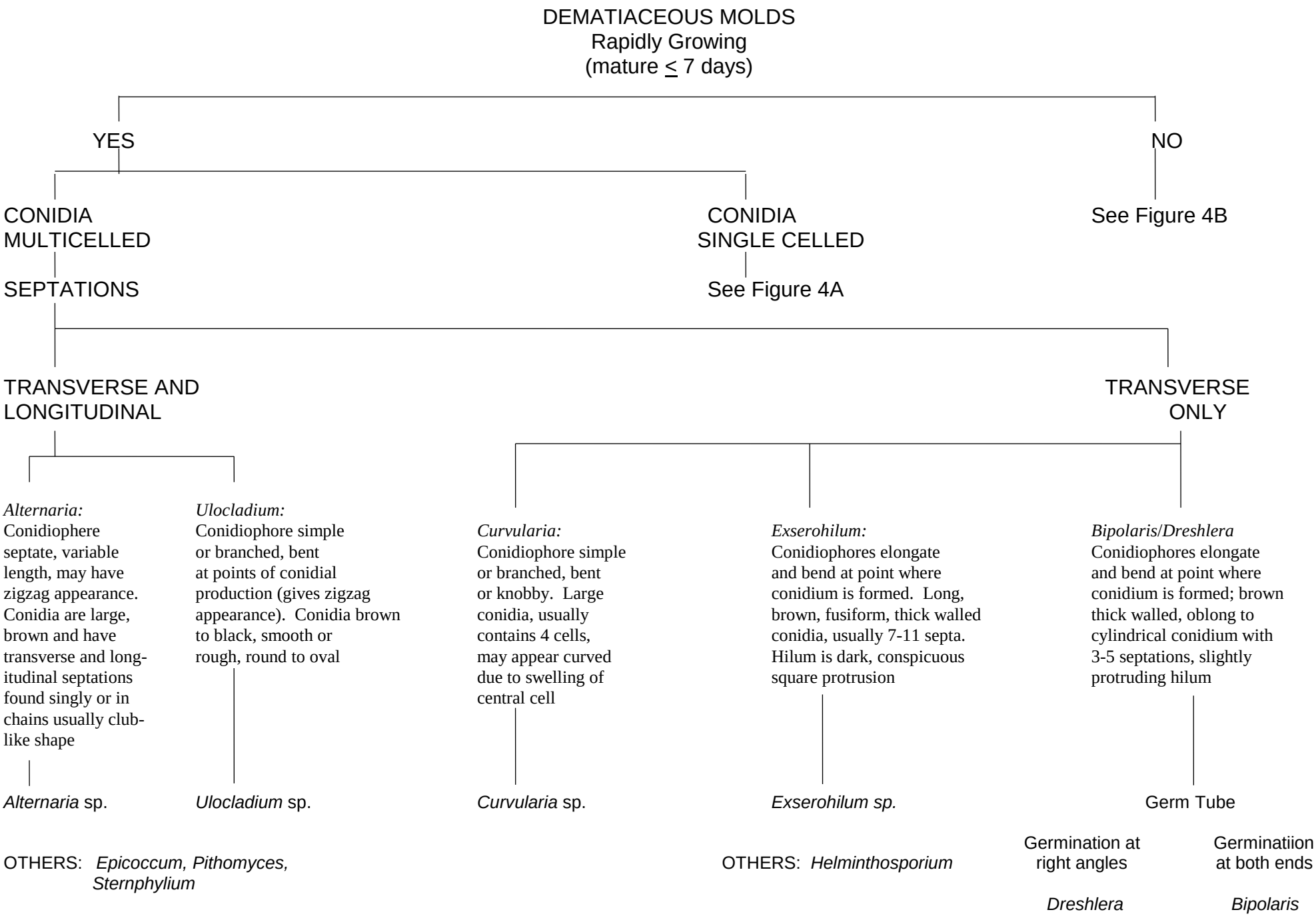

DEMATIACEOUS MOLDS
Rapidly Growing
(mature ≤ 7 days)
YES
NO
CONIDIA MULTICELLED
CONIDIA SINGLE CELLED
See Figure 4B
SEPTATIONS
See Figure 4A
TRANSVERSE AND LONGITUDINAL
TRANSVERSE ONLY
Alternaria: Conidiophere septate, variable length, may have zigzag appearance. Conidia are large, brown and have transverse and long-itudinal septations found singly or in chains usually club-like shape
Ulocladium: Conidiophore simple or branched, bent at points of conidial production (gives zigzag appearance). Conidia brown to black, smooth or rough, round to oval
Curvularia: Conidiophore simple or branched, bent or knobby. Large conidia, usually contains 4 cells, may appear curved due to swelling of central cell
Exserohilum: Conidiophores elongate and bend at point where conidium is formed. Long, brown, fusiform, thick walled conidia, usually 7-11 septa. Hilum is dark, conspicuous square protrusion
Bipolaris/Dreshlera Conidiophores elongate and bend at point where conidium is formed; brown thick walled, oblong to cylindrical conidium with 3-5 septations, slightly protruding hilum
Alternaria sp.
Ulocladium sp.
Curvularia sp.
Exserohilum sp.
Germ Tube
OTHERS: Epicoccum, Pithomyces, Sternphylium
OTHERS: Helminthosporium
Germination at right angles
Dreshlera
Germinatiion at both ends
Bipolaris

Figure 4A

RAPIDLY GROWING DEMATIACEOUS MOLDS WITH SINGLE CELLED CONIDIA

Single Celled Conidia

Cladosporium:
Dark, branched conidiophores produce 2 or more conidial chains. Brown, oval, smooth conidia that form branching treelike chains that are easily dislodged. Dark spots (hila) at point of attachment

Cladosporium sp.

Aureobasidium:
Young colony: unicellular, budding yeast like cells. Develops 2 types of hyphae:

1. hyaline, delicate, thin walled, produces conidia from hyphae wall at fertile point
2. thick walled, dark, closely septated, some cells forming short tube that produce conidia. Hyaline, oval conidia continue to multiply by budding.

Aureobasidium pullulans

Scedosporium:
annelides (conidia bearing cells) with swollen base and elongated neck. Olive to brown, one celled, smooth, ovoid conidia with a narrowed, truncated base. Form small clusters at apex of annelide.

Scedosporium prolificans

Pseudallescheria boydii:
(*Scedosporium apiospermum)*
Simple long or short conidiophores bearing conidia singly or in small groups. Unicellular, oval conidia with larger end toward apex, appear cut off at the base.

CLEISTOTHECIA
(sexual stage) at 35°C

PRESENT

Pseudallescheria boydii

ABSENT

Scedosporium apiospermum

Figure 4B

SLOW GROWING
DEMATIACEOUS MOLDS

Slow Growing

Fonsecaea pedrosoi:

4 types of conidiation may be seen:

1. Fonsecaea type: erect, septate conidiophores, distal end develops swollen denticles that bear primary single-celled ovoid conidia. Denticles on primary conidia support secondary single celled conidia.
2. Rhinocladiella type: erect septate, condiophores, swollen denticles bear ovoid conidia at the tip and along side of conidiophore. Usually only primary conidia develop.
3. Cladosporium type: erect conidia give rise to large primary shield shaped conidia that in turn produce short, branching chains of oval conidia having small dark hila.
4. Phialophora type: vase shaped phialides with terminal cuplike collarettes. Round to oval conidia accumulate at apex of phialide

Fonsecaea pedrosoi

Phialophora verrucosa:

Vase shaped phialides with flared cuplike collarette. Round to oval conidia accumulate at apex of phialide.

Phialophora verrucosa

Cladosporium carrionii:

Lateral and terminal conidiophores. Conidiophores produce long, branching chains of brown smooth walled pointed conidia. Conidia are easily dispursed and have dark scars of attachment.

Cladosporium carrionii

Figure 5
RAPIDLY GROWING HYALINE MOLDS

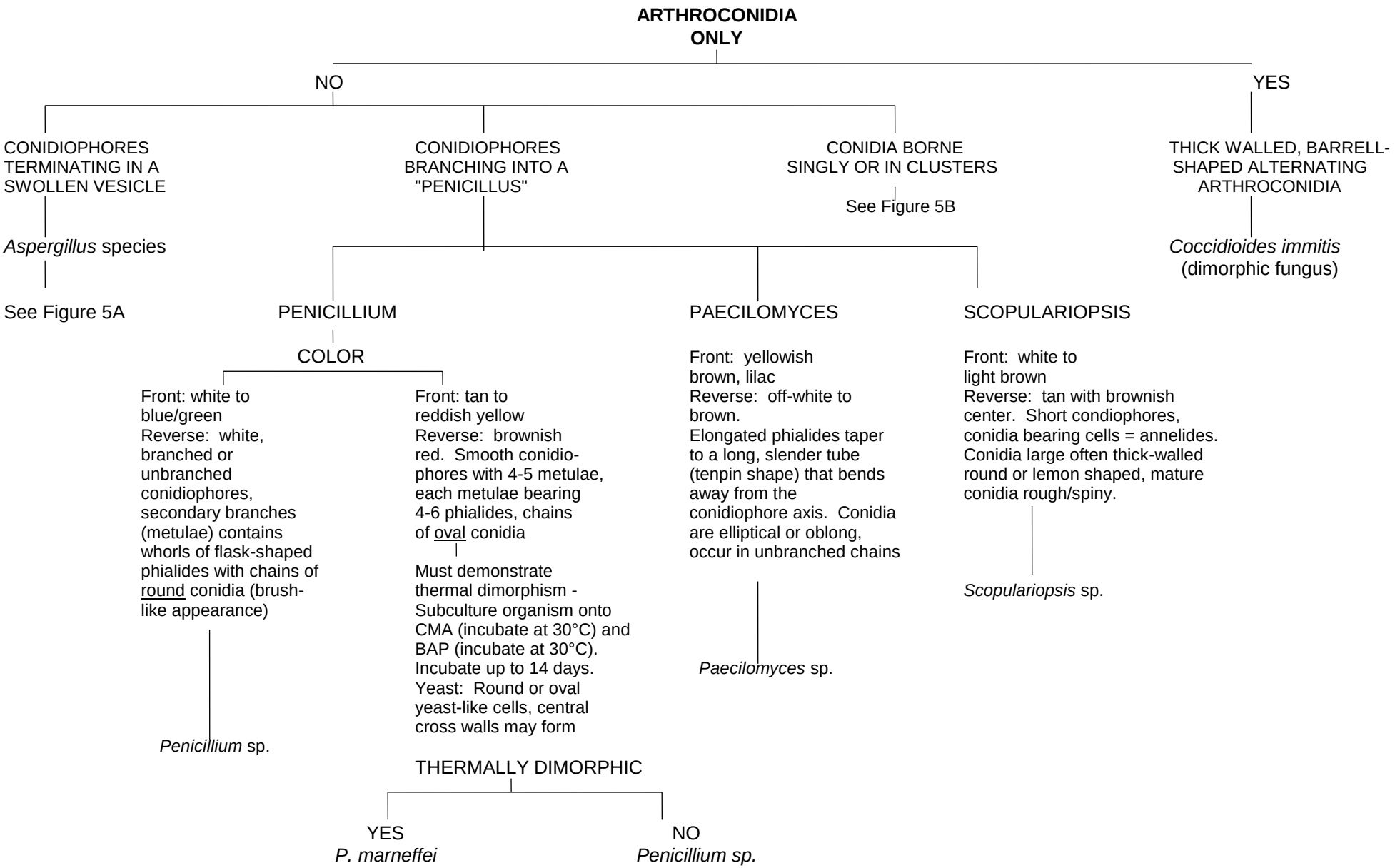


Figure 5A

ASPERGILLUS SPECIES

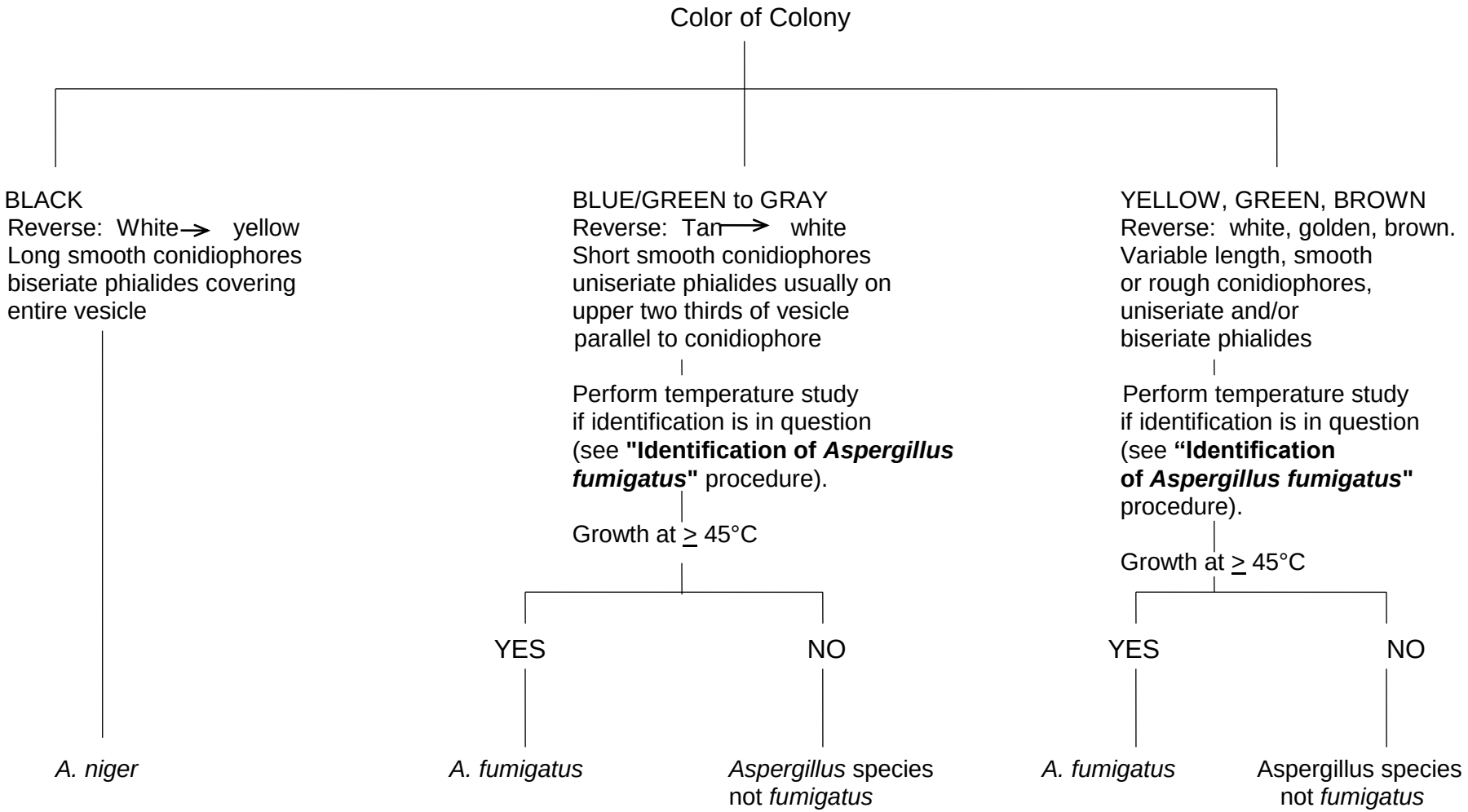


Figure 5B

RAPIDLY GROWING HYALINE MOLDS WITH CONIDIA BORNE SINGLY OR IN CLUSTERS

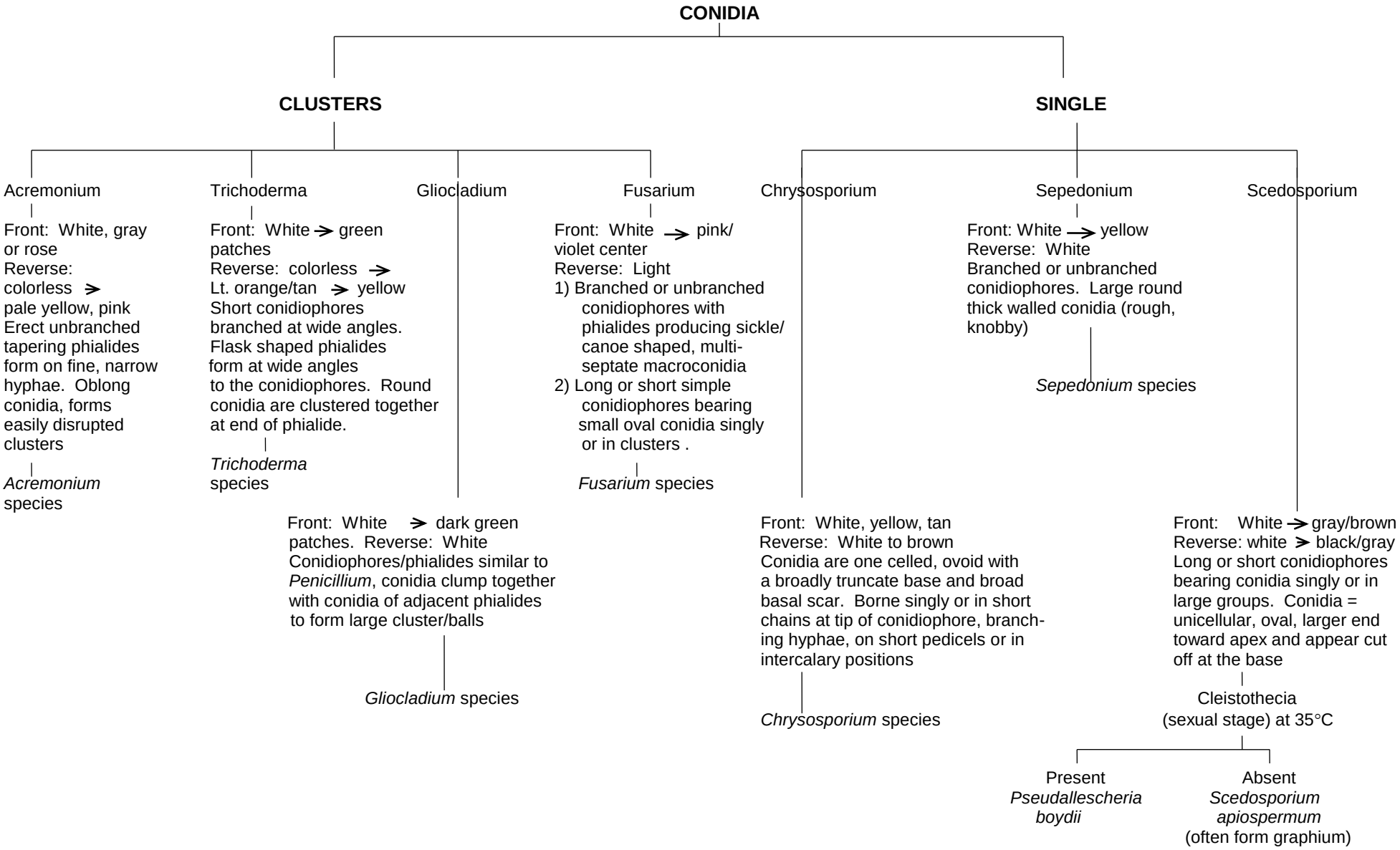
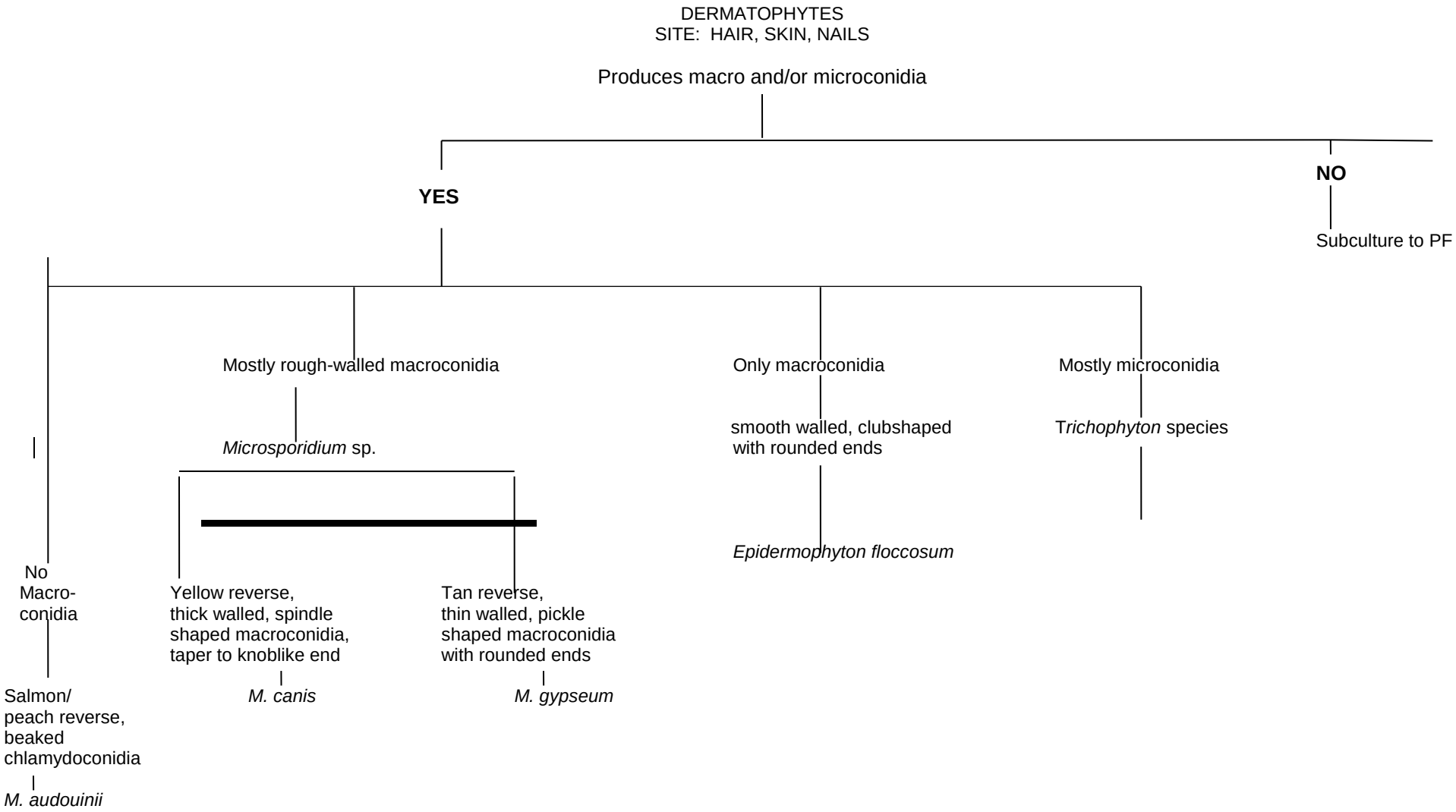


Figure 6

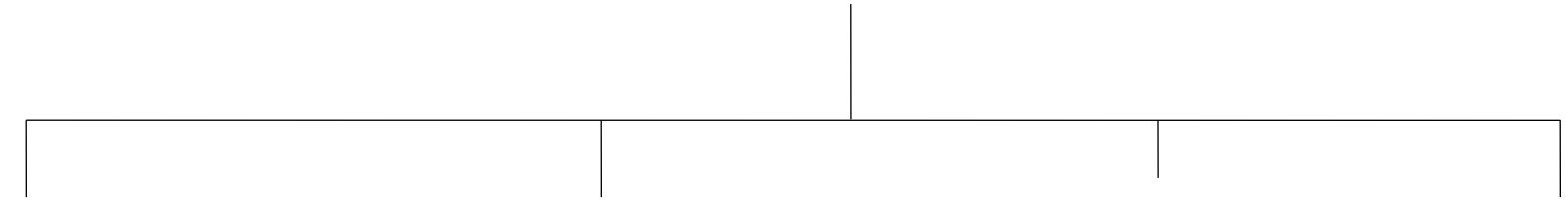


*Other hair, skin, nail potential pathogens include: *Bipolaris* (skin), *Exserohium* (skin), *Scopulariopsis* (nail), *Acremonium* (nail), *Fusarium* (skin, nail), *Chrysosporium* (nail)

Figure 7

Dimorphic Fungi

Yeast in Tissue (35°C)
Mold at 30°C



Yeast: cream-tan colony.
Round, oval, fusiform budding cells of varying sizes-cigar bodies
Mold: White to brown colony becoming brown/black with slender, tapering conidiophores rising at right angles. Apex of conidiophore slightly swollen bears many pear-shaped to round conidia on threadlike denticles forming rosette-like cluster.

Sporothrix schenckii

Yeast: White colony.
Small round or oval budding yeast

Mold: White to brown colony
Conidiophores with large, thick walled, round to pear-shaped tuberculate macroconidia

Histoplasma capsulatum

Yeast: cream-tan colony.
Broad, budding yeast with thick wall

Mold: First yeastlike then cottony-white to tan/brown. Conidiophores with round or pear-shaped conidia. Thick walled chlamydoconidia in older cultures.

Blastomyces dermatitidis

Yeast: cream-tan colony.
Large round thick-walled cells with single and multiple buds. Buds attached by narrow connections.

Mold: White colony turns brown with age. Septate hyphae with intercalary and terminal chlamydospores, few microconidia may form along hyphae.

Paracoccidioides brasiliensis

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Revisions & Updates: updated procedure to delete use of corn meal for subculturing molds. Deleted references for staining procedures no longer in use. Changed reference laboratory for send out testing to ARUP.