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LAB SAFETY

Refer to [Laboratory Safety Manual](#)

Note the following when processing Mycology isolates:

Wipe off working area with Virox before and after each day's work. All mycology plates will be sealed with paraflim. If a culture is dropped or spilled, pour Virox over the contaminated area, cover with paper towels and let stand for at least 15 minutes. Wipe off the surface and deposit the contaminated material in an appropriate biohazard disposal container. Clean the surface again using 70% alcohol.

ALL work on filamentous fungus is carried out in LAMINAR AIRFLOW BIOSAFETY CABINET TYPE 2. Bio safety Level 2 procedures are recommended for personnel working with clinical specimens that may contain dimorphic fungi as well as other potential pathogenic fungi. Gloves should be worn for processing specimens and cultures.

The laboratory is NOT certified to handle Risk Group 3 pathogens and should not perform any tests on suspect Risk Group 3 organisms. This includes not opening plates or tubes and not preparing lactophenol slides, and also includes not trying to convert the mould to yeast phase. Plates should be sealed with tape and placed in biohazard bags for send out to PHOL.

Suspect Risk Group 3 Organisms Safety:

The following moulds should be treated as suspect RG3 organisms and sent to PHOL due to close resemblance to the mould forms of dimorphic fungi:



- *Malbranchea/Coccidioides,*
- *Scedosporium/Blastomyces,*
- *Sepedonium/Histoplasma,*

For laboratory technologists who are inexperienced in recognizing these uncommon isolates, send any colony reasonably suspected by virtue of clinical history, epidemiology, or colonial characteristics:

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- White mould growing on cycloheximide-containing agar after three days of incubation (*Histoplasma, Blastomyces, Coccidioides*)
- Greenish colony with red pigment in the medium (*Penicillium marneffei*)
- black, olivaceous green or brown mould from brain tissue growing slowly or moderately quickly (*Cladophialophora bantiana, Ramichloridium mackenziei*).

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DAILY ROUTINE OF THE MYCOLOGY LAB

1. New cultures received are sorted; scan to match with the daily work list, separated according to reading schedule, and length of incubation. Fungal culture plates are examined, sealed with parafilm and then placed in appropriate stacks and incubated at 28°C. Any culture medium showing fungal growth is removed for further work-up.



2. Fungal smears are stained and read once or twice daily - once before noon. All smears must be read within 24 hours except on Weekends and Holidays. Stat fungal smears are read after hours. All positive smears showing *Pneumocystis carinii* or yeast suggestive of *Blastomyces* should be checked by the charge technologist or the microbiologist. Inform Virology section on all positive *Pneumocystis carinii*. If a PCP IFA test has not been ordered, add the test into the LIS order.

3. **Screening and reading cultures:**
 - i) Sort the plates from sterile specimens and place them into a separate rack.
 - ii) Read all cultures (except special requests for dimorphic fungi) daily for the first week and two times a week (separated by at least one day) for the remaining incubation period. Work up positive specimens immediately.
 - iii) For special requests for dimorphic fungi, read cultures daily for the first two weeks and three times a week (separated by at least one day) for the remaining 4 weeks of incubation. Work up positive specimens immediately.
 - iv) LPCB (Lactophenol Cotton Blue) preparations are made at least twice a week or daily depending on volumes. Any mold referred from the bacteriology section is processed and worked up the same day (except weekends and holidays). All positive LPCB preparations are checked by the other Mycology technologist or the microbiologist.

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SPECIMEN COLLECTION AND TRANSPORTATION

See [Pre-analytical Procedure - Specimen Collection QPCMI02001](#)



PROCESSING OF SPECIMENS

See [Specimen Processing Procedure](#)

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ISOLATION AND IDENTIFICATION

I. Reading of cultures

Specimen	Incubation Period at 28°C	Comment
All specimens other than special requests for dimorphic fungi, Malassezia or environmental specimens	4 weeks	Read daily for 1st week; then 2 times per week for the remaining 3 weeks.
Special Request Dimorphic	6 weeks or Send to PHL	Read daily for 2 weeks; then 3 times per week for the remaining 4 weeks.
Special Request Malassezia	1 week	Read daily for 1 week
Environmental	7 days	Read on Day 1 and then on Day 5.

II. Identification

A) **FILAMENTOUS FUNGI**

Introduction:

Most filamentous fungi can be identified based on a combination of colonial morphology and microscopic features.

The extent to which a filamentous fungus is identified in the laboratory will depend on several factors.

The following should be used as a guide. If there is any question regarding the extent to which a filamentous fungus should be identified, consult with the microbiologist or senior mycology technologist. **Never work up a Suspect Risk Group 3 colony.**

a) Sterile site specimens:

Identify all filamentous fungi isolated including *Penicillium*. Send all to PHL for speciation. Possible culture contaminants (e.g. a single colony of *Penicillium* species or other saprophytes growing on only one of several media) should be checked with the Charge Technologist or the Microbiologist before proceeding.



b) All other specimens:

Identify all filamentous fungi isolated.

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Procedure:

Examine the culture plates as per [Reading of Cultures Schedule](#) and record the macroscopic and microscopic findings in the LIS Media Comment field.

Macroscopic Examination:

1. Colonial morphology
2. Surface pigment on non-blood containing medium
3. Reverse pigment on non-blood containing medium
4. Growth on cycloheximide containing medium

Microscopic Examination:


For **coloured filamentous fungus** (except those resembling **Suspect [Risk Group 3 colony](#)**)

1. Prepare a tease mount or scotch tape preparation of each fungus colony type from each media using Lactophenol Blue (LPCB).
2. Under the light microscope, examine the slide(s) for the presence, shape, size and attachment of conidia. Compare and match the above features with those described in a reference textbook.
3. If the filamentous fungus can be identified from the LPCB preparation, mark the identified colony (ies) with an “X” on the back of the culture plate(s) [if more than one type of fungus is identified, place number (e.g. 1, 2, 3, etc) beside the “X” which matches the number and identification entered into the LIS]. Re-incubate the original culture plates for the remaining incubation period and examine plates for additional growth. Hold at room temperature, if plates completely over grown by 4 weeks; discontinue incubation; but report the identification according the instructions in the [Reporting Section](#).
4. For *Aspergillus fumigatus* set up 50°C; if no growth report *Aspergillus fumigatus* complex.

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5. Set up a slide culture (see [Appendix VI - Slide Culture](#)) if unidentified due to overlapping conidial structures with other species.
6. If the filamentous fungus is **producing conidia** but cannot be identified, determine the significance of the isolate whether it is a probable pathogen, a possible pathogen (i.e. opportunistic fungus) or an unlikely pathogen (i.e. saprophyte), take into consideration the following:
 - Direct smear result
 - Growth on cycloheximide containing media

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- Growth at 37°C
 - Refer to the [FLOW CHARTS for IDENTIFICATION](#)
- With the help of microbiologist:
- Pathology report if available
 - Clinical data
 - See the Charge Technologist or Microbiologist for consultation if needed.
 - Send isolate to the Public Health Lab if significance/identification cannot be determined immediately.

5. If the filamentous fungus **does not produce conidia**, subculture the fungus onto the media as outlined below. Re-incubate the original plates for the remaining incubation period.

Media	Incubation
Coloured Mould:	
Potato Dextrose Agar (PDA)	O ₂ , 28°C
SAB	O ₂ , 37°C

- i) Examine the sub-cultured plates daily and record findings in the LIS Media Comment field.
- ii) If there is no growth after 7 days, forward the original culture plate to the Public Health Laboratory (PHL) for further work-up.
- iii) When sufficient growth is noted, record:

Macroscopic Examination:

- a) Colonial morphology
- b) Surface pigment
- c) Reverse pigment
- d) Growth on cycloheximide containing agar



Microscopic Examination:

- a) Prepare LPCB preparation(s) from subculture plates as required depending on colonial morphology on each plate and examine under light microscope as outlined above.
- b) If there is growth without conidia production and growth on SAB 37°C plate, send the isolate to PHL for further work-up.

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- c) If there is growth without conidia production and no growth on SAB 37°C plate, determine the significance of the isolate by taking into consideration the following:
- Direct smear result
 - Growth on cycloheximide containing media
 - Refer to the [FLOW CHARTS for IDENTIFICATION](#)
- With the help of on-call resident or microbiologist:
- Pathology report if available
 - Clinical data
 - See the Charge Technologist or Microbiologist for consultation if needed.
 - Send the isolate to PHL for further work-up if needed.
- d) Send the isolate to PHL for further work-up.
- e) If there is growth with conidia and the isolate cannot be identified, set up a slide culture (see [Appendix VI - Slide Culture](#)). If the isolate cannot be identified by slide culture, send the isolate to PHL for further work-up.



For white filamentous fungus:

1. Re-incubate the culture for another 48 hours. If the fungus remains white after 48 hours, seal and send the culture to the Public Health Laboratory for identification. DO NOT manipulate the culture. If the fungus becomes a coloured mould after 48 hours, prepare a tease mount or scotch tape preparation of each fungus colony type from each media using Lactophenol Cotton Blue (LPCB).
2. Report the identification according the instructions in the [Reporting Section](#).

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B) YEAST

If yeast is isolated from fungal media, check the bacteriology culture results. If yeast has already been identified in bacteriology, do not repeat the identification, but simply refer to the bacteriology result.

If yeast is isolated from fungal media and not in bacteriology media, identify yeast as follows:

1) Sterile sites and biopsy specimens, set up Maldi or Germ tube

a) Germ tube*: **Positive**- Report as "*Candida albicans*" "isolated".

b) Germ tube: **Negative** - Set up: Cornmeal Agar at 28°C
SAB at 28°C
Urea at 28°C
API 20C at 28°C

* Germ tube test may not be done during the afternoon. In this case skip the GT and set up cornmeal, urea SAB and API (even if we run GT the next day, it will be a day late in ID if GT turns negative).

If isolate cannot be identified by the above tests, send isolate to the Public Health Laboratory.

2) Respiratory sites isolates:

Check Bacteriology culture media to determine the amount of commensal flora. Then determine the significance and work-up of the yeast grown on fungal media as follows:

Significant growth – For sputum ($\geq 2+$ growth OR 1+ growth and predominant and if pus cells are seen on gram stain) OR for bronchoscopy specimen (amount greater than that of commensal flora), set up Maldi or Germ tube:



a) Germ tube: **Positive** - Report as "*Candida albicans*"

b) Germ tube: **Negative** - Rule out *Cryptococcus* using Urease test. If Urease is negative, report as "Yeast, not *Candida albicans* or *Cryptococcus*". If Urease is positive, confirm purity and set up: SAB at 37°C
Cornmeal Agar at 28°C
SAB at 28°C
API 20C at 28°C

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EBM at 28°C (if the isolate is not brown on original EBM)

Insignificant growth – i.e. any amount of yeast other than what has defined as significant growth.

Rule out *Cryptococcus* using Urease test. If Urease is negative, report as part of Commensal flora **without** specifically mentioning the presence of yeast. If Urease is positive, confirm purity and set up:

BA at 37°C

Cornmeal Agar at 28°C

SAB at 28°C

API 20C at 28°C

EBM at 28°C (if it was not on original EBM)

- 3) Voided urines, superficial sites, wounds and drainage fluids:
No Germ tube performed. Report as “Yeast” with quantitation. No further work-up is required.
- 4) Isolates from all other sites, set up Maldi or Germ tube:
 - a) Germ tube: **Positive** - Report as "*Candida albicans*".
 - b) Germ tube: **Negative** - Report as "Yeast, not *Candida albicans*".



If yeast is referred to Mycology from bacteriology media (i.e. Germ tube – negative), identify yeast as follows:

- 1) Sterile sites and biopsy specimens:
Set up: Cornmeal Agar at 28°C
SAB at 28°C
API 20C at 28°C
Urease at 28°C
- 2) Respiratory sites isolates (Germ tube – Negative and Urease – Positive):
Set up: BA at 37°C
Cornmeal Agar at 28°C
SAB at 28°C
API 20C at 28°C
Urease at 28°C (repeat)

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Refer to Yeast Identification Flow Chart for Identification.

The acceptance of API 20 C is >90% and must agree with cornmeal result. Refer unidentifiable isolates to the PHL for further work-up.

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If *Candida dubliniensis* is identified by the API, the isolate must be set up at 42°C for 48 hours along with *Candida albicans* as a control (*C. albicans* grows at 42°C but not *C. dubliniensis*). Report *C. dubliniensis* if there is no growth at 42°C. If it grows at higher temperature, ship the isolate to PHL for confirmation.

Any yeast or *Candida* species that are rare or unheard of, send them to PHL for confirmation even if API identifies them > 90%.

C) NOCARDIA

Nocardia species are aerobic members of the actinomycetes which are gram positive branching filamentous bacilli that fragment into rod-shaped to coccoid elements. Most clinical infections are due to *N. asteroides* and *N. brasiliensis*. Most specimens from patients with suspected Nocardiosis will be respiratory specimens (e.g. sputum, BAL, lung biopsy, etc.) although tissue (e.g. Mycetoma) and body fluid may also be submitted. For identification, proceed as follows:

1. When *Nocardia* isolation is requested or organisms suggestive of *Nocardia* are seen on gram stain, plant specimen onto Sodium Pyruvate Agar (PYRA) and incubate in O₂, 28°C for 4 weeks in Mycology. As well, the Blood Agar (BA) and Chocolate (CHOC) plates should be kept in bacteriology and incubated in O₂, 35°C for 48 hours and then send it to Mycology for the rest of the 4 weeks. Send plates to mycology for further work up. See Table 1 for nocardia work up.



Table 1. Differentiation of *Nocardia*, *Streptomyces*, Atypical Mycobacteria based on colonial and microscopic features.

	<i>Nocardia</i> species	<i>Streptomyces</i> species	Atypical Mycobacteria
Gram Stain	GPB*	GPB	GPB
Modified Kinyoun stain	Partially Acid-fast	-	+
Regular Kinyoun stain	-	-	+
Morphology	Filamentous, Branching	Filamentous, Branching	Bacillary
MacConkey (without Crystal Violet)	No Growth	-	Growth

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Strong musty smell	-	+	-
Adherence to agar	+	+	-
Report as:	Phrase 1	Phrase 2	Phrase 3

*GPB = Gram positive bacilli

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REPORTING

1. FUNGAL STAIN:

Negative Reports: "No Fungal elements seen".

NB: In LIS, status these reports as final. No verification required.

Positive Reports: as per keypad options:

- _ Fungal elements seen.
- Yeast seen (with quantitation)
- Yeast with pseudohyphae seen (with quantitation)
- Filamentous fungus seen (without quantitation); with morphologic description of organisms/structures seen (e.g. septate hyphae). Define structures. Send to PHL for further speciation for sterile site and BAL.
- Structures resembling Acanthamoeba seen.

If unable to interpret fungal elements, consult Senior Mycology Technologist.

NB: In LIS, status these reports as final. Positive smears with rare and unusual structures (fungal elements) must be checked by Senior Mycology Technologist.

2. CULTURE:

Negative Reports: "No Fungus isolated".

NB: In LIS, status these reports as final. No verification required.



Positive Reports:

- When reporting a fungus culture result, DO NOT quantitate filamentous fungi.
- If fungus has already been identified and **reported under bacteriology result**, then enter one of the following phrases as appropriate in the **Test Comment Field**:

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- a) "Please see Culture and Sensitivity report for fungus isolate(s)". (Use this phrase when no additional fungi are isolated on the fungal media).
- b) "Please see Culture and Sensitivity report for additional fungus isolate(s)". (Use this phrase when additional fungi are isolated on the fungal media)
Add the appropriate phrases:
 - a) "(Organism name)"
 - b) "(Organism name); normally non-pathogenic".
e.g. *Penicillium species*: Report: normally non-pathogenic except:
 - o Direct microscopy positive for fungal element.
 - o Sterile site.
 Isolate send to PHL for further identification.
 - c) "(Organism name or description); cannot rule out contamination".
 - d) "Non - sporulating fungus, normally non-pathogenic".
 - e) "Filamentous fungus; further identification to follow".
 - f) "(Organism name); Confirmation to follow".

NB: In the LIS, status the report as interim (^I).

- For organisms **isolated in fungal media only:**

Yeast:

All sites except sterile sites and BAL, report yeast with quantitation

For sputum,

Significant growth: *Candida albicans*

"Organism name"

Cryptococcus neoformans

Insignificant growth: "Yeast isolate; normally commensal flora"

Filamentous Fungi:

If the filamentous fungus is deemed to be **significant**, and when the work-up and identification of the isolate is complete, use one of the following phrases as appropriate:



- a) "(Organism name)"
- b) "(Organism name); normally non-pathogenic".
- c) "(Organism name or description); cannot rule out contamination".
- d) "Non-sporulating fungus, normally non-pathogenic".
- e) "Filamentous fungus; further identification to follow".

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f) "(Organism name); Confirmation to follow".

NB: In LIS, status the report as interim (if further incubation required) **or** final (if no further incubation required).

If the filamentous fungus is deemed to be **insignificant**:

"(Organism name); likely not significant"

When a fungus isolate has been forwarded to the Public Health Laboratory (or other reference laboratory) for further identification or confirmation, use one of the following phrases as appropriate:

- a) "Filamentous fungus; further identification to follow".
- b) "(Organism name); Confirmation to follow".

NB: In LIS, status the report as interim (^I).

- When reporting a fungus culture result, please note the following:

When the Public Health Laboratory (PHL) report becomes available, proceed as follows:

- i) Replace the isolate name or description in the **Isolate Field** with the PHL result.
- ii) In the **Isolate Comment Field**, remove the statement "Further identification to follow" or "Confirmation to follow" and enter the following phrase (from the keypad) and add the PHL report number: "Public Health Laboratory Report No. _____".
- iii) Status the report as final (^F).



- ***Nocardia***

- a) "*Nocardia* species; further identification to follow".
- b) "Branching Gram positive bacilli; further identification to follow".
- c) "Mycobacterium species isolated."
- d) Forward isolates to the Public Health Laboratory (PHL) for further identification.

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STAINING METHODS

Acid Fast Stain for Nocardia (Modified Kinyoun)

Calcofluor Stain

Fungi-Fluor TM Stain

India Ink



Lactophenol Cotton Blue (LPCB)

See: Technical Manual

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ISOLATOR 10 BLOOD CULTURE SYSTEM FOR DIMORPHIC FUNGI

I. Introduction

The Isolator 10 blood culture system should be used for the isolation and detection of dimorphic fungi such as Histoplasma and Blastomyces.

If BacT/Alert bottles are received with a request for dimorphic fungi, notify the ward / ordering physician that they must use the Isolator 10 collection tubes. The BacT/Alert bottles should only be processed as per routine blood cultures.

II. Collection and Transport

Using aseptic technique, collect 10 ml of blood into a clean, sterile syringe. Transfer the blood into an Isolator 10 microbial tube. Transport to the laboratory immediately for processing. If a delay in transport or processing is anticipated, the tubes can be held for 24 hours at room temperature.

III. Reagents / Materials / Media

Refer to Appendix I.

IV. Procedure



A. Processing of Isolator 10 Microbial Tubes:

1. Centrifuge specimen at 4000 rpm for 30 minutes.
2. Disinfect the stopper using 10% PVP iodine or tincture of iodine. Allow to dry completely.
3. Place cap over stopper. Grasp only the sides of the cap.
4. Position cap under press and pull down handle and release.
5. Collapse bulb of supernatant pipette completely before inserting stem into the tube.

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6. Insert stem into tube and release bulb to withdraw supernatant fluid. Discard the supernatant.
NB: The use of a safety hood is mandatory for steps 6 to 9.
7. Vortex the tube for at least 10 seconds at the highest setting.
8. Collapse bulb of concentrate pipette completely and then insert stem into tube. Slowly withdraw all concentrate.
9. Dispense concentrate in a straight line along the surface of the agar. Keep inoculum away from the edge of the plate.

Media	Incubation
	O ₂ at 28°C
Inhibitory Mold Agar (IMA)	x 4 weeks
Esculin Base Medium (EBM)	x 4 weeks
Brain Heart Infusion Agar with 5% Sheep Blood, Gentamicin, Chloramphenicol, Cyclohexamide (BHIM) x 2	x 4 weeks

10. Using the tip of the pipette, streak the plates. Use 15-20 passes perpendicular to the original inoculum line.
11. Forward plates to Mycology for incubation and processing.

B. Interpretation of Fungal Culture Plates:

Refer to Mycology Manual.

V. **Reporting Results**

Refer to Mycology Manual.



VI. **Reference**

1. Isolator 10 Product Insert.

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Julius Kane, Richard Summerbell, Lynne Sigler, Sigmund Kraiden and Geoffrey Land. Laboratory Handbook of DERMATOPHYTES. A Clinical Guide and Laboratory Manual of Dermatophytes and other Filamentous Fungi from Skin, Hair and Nails. 1997, Star Publication.

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J.W. Rippon. Medical Mycology. The Pathogenic Fungi and the Pathogenic Actinomycetes. Third Edition, 1988, W.B. Saunders.

E.W. Koneman et al. Color Atlas and Textbook of Diagnostic Microbiology. 5th Edition, 1997, J.B. Lippincott.

G.J. Hageage Jr. et al. Use of Calcofluor White in Clinical Mycology. Laboratory Medicine 15:109-112, 1984.

J. Barenfanger. Identification of Yeasts and Other Fungi from Direct Microscopic Examination of Clinical Specimens. Clin. Micro. Newsletter 12:9-15, Jan. 15, 1990.

Davis H. Larone. The Identification of Dematiaceous Fungi. Clin. Micro. Newsletter 11:145-150, Oct. 1, 1989.

K.J. Kwon-Chung, J.E. Bennett. Medical Mycology. 1992, Lea & Febiger.



Bailey & Scott's Diagnostic Microbiology. Eleventh Edition by Betty A. Forbes, Daniel F. Sahn & Alice S. Weissfeld. 2002 (Mosby).

Medically Important Fungi. A Guide to Identification, 4th. Edition by Davise H. Larone. 2002 (ASM Press).

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Atlas of Clinical Fungi, 2nd. Edition by Dr. G.S. de Hoog, J. Guarro, J. Gene & M.J. Figueras. CBS Utrecht, the Netherlands 2000.



www.doctorfungus.com

<http://microbiology.mtsinai.on.ca/mig/index.shtml>

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APPENDIX I - CONVERSION (Converting Mycelial Phase of Dimorphic Mould to a Yeast Phase)

I. Purpose

To be used only for suspected *Sporothrix schenckii* and *Blastomyces dermatitidis* when full function Mycology Lab is in place. Send all other suspected dimorphic fungi to the Public Health Laboratory for identification and/or confirmation.

II. Procedure

1. Transfer a large inoculum of the filamentous culture onto the surface of a fresh, moist slant of Brain Heart Infusion Agar (BHIA). If the slant is dry, add 0.5 ml sterile distilled water before inoculation.
2. Incubate culture at 37⁰C.
3. Examine daily for the presence of yeast-like, creamy colonies.

NB: It is not necessary for the whole culture to convert. *S. schenckii* usually converts rapidly to the yeast phase without the need for further 37⁰C subcultures.

4. Confirm the presence of any yeast forms by LPCB tease prep (Refer to Lactophenol Analine Blue (LPCB) procedure in the Staining Methods).



III. Reference

Koneman, E.W., et al. 1997. Color Atlas and Textbook of Diagnostic Microbiology, 5th edition. J.B. Lippincott.

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APPENDIX II - CORNMEAL TWEEN-80/OXGALL AGAR

I. Purpose

To be used for yeast morphology when the germ tube is negative, but further identification is required as outlined in the section: Isolation and Identification. Cornmeal Tween-80 provides excellent diagnostic morphological features for yeast identification, but produces chlamydospores more slowly than oxgall agar.

II. Procedure



A. Cornmeal Tween 80:

1. Using a sterile wire loop, inoculate a small portion of a yeast colony by making two parallel streaks a few mm apart on the surface of a cornmeal agar plate. Do not cut into the agar.
2. Streak over the lines in a "zigzag" fashion (Dolmau technique).
3. Place a clean coverslip over the streaked area and press gently.
4. Include controls *T. glabrata* (negative) and *C. parapsilosis* (positive).
5. Incubate at 25-28°C for 48 hours.
6. Using the light microscope examine under low power and high dry objectives for the presence of hyphae, pseudohyphae, blastoconidia, chlamydospore and/ or arthroconidia. The plate may need to be reincubated if the morphology is not fully developed (eg. arthroconidia formation).

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B. Oxgall:

Oxgall agar is specifically used to show chlamyospores. Oxgall does not always give the classic diagnostic morphology of yeast as is seen with the Cornmeal Tween-80 agar.

1. Using a sterile spade shaped spatula, inoculate lightly by making 2 to 3 parallel cuts approximately 1/2 inch apart at 45° angle. Avoid cutting the agar through to the bottom.
2. Apply a clean coverslip on the inoculated area and press gently.
3. Include controls *C. albicans* (positive) and *C. tropicalis* (negative).
4. Incubate at 28°C for up to 48 hours.
5. Examine the areas where the agar is cut under low and high dry objectives using the light microscope.
6. Observe for the presence of hyphae, pseudohyphae, blastoconidia, chlamyospores and arthroconidia.
7. Refer to Table 1. "Identification of yeast" in the section, Isolation and Identification for interpretation.

Note: Yeast producing chlamyospores on cornmeal and/ or oxgall are reported as *Candida albicans*. Yeast not producing chlamyospores or pseudohyphae may require further testing.



III. References

1. J.B. Fisher, Julius Kane, "Production of Chlamyospores by *Candida albicans* cultivated on dilute Oxgall Agar". Mycopath. 35:223-229, 1968.

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APPENDIX III - DETERMINING CYCLOHEXIMIDE RESISTANCE OF AN ISOLATE

I. Purpose

To rule out or help confirm the presence of a possible dimorphic fungus or dermatophyte. Normally used in the identification of white moulds.

The following pathogenic fungi are resistant to Cycloheximide:

Blastomyces dermatitidis
Histoplasma capsulatum
Coccidioides immitis
Sporothrix schenckii
Paracoccidioides brasiliensis
Trichophyton sp.
Microsporum sp.
Epidermophyton floccosum

Cycloheximide inhibition rules out the above fungi. Resistance to cycloheximide **may** indicate one of the above pathogens.

II. Procedure

1. Subculture the isolate to Potato Dextrose Agar (PDA) and Mycosel Agar (MYC) and incubate at 28°C (or RT). MYC contains cycloheximide.
2. Observe periodically for 7-10 days or until good growth on one or both media.


III. Interpretation

- i) PDA+/ MYC + : Fungus **MAY** be one of the above listed pathogens.
- ii) PDA +/- MYC - : Fungus is **NOT** one of the ABOVE listed pathogens.

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iii) PDA -/ MYC - : Test invalid, repeat.

NB: *Penicillium marneffei* (dimorphic fungus) is inhibited by cycloheximide.

IV. Reference

M.R. McGinnis. Laboratory Handbook of Medical Mycology 1980, Academic Press.

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APPENDIX IV - FLUORESCENT MICROSCOPE (INSTRUCTIONS)



1. Record date and time in UV Record Book when turning on the fluorescent microscope.

Note: If UV light is recently turned off, WAIT FOR ONE HOUR before turning it on again.
2. Switch on the microscope
3. Allow the microscope to warm up for 5 minutes.
4. Turn filter setting to 2 (G filter) (Leitz Ortholux Microscope) -- (right side of Grey structure above objectives. Use filter #3 for bright-field microscopy).
5. Pull out the small black ring (rod) on the left side above the revolving nose piece to let UV light pass through the objective; push it in to prevent the slide from fading when not examining the preparation.
6. When finished, turn off the "ON" button.
1. Enter the time and calculate the total time used in the UV Record Book.
8. Fluorescent bulbs are good for a maximum of 200 hours. Notify the senior technologist that the bulb should be changed when the maximum time has been reached.

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APPENDIX V - GERM TUBE TEST

I. Introduction

This is a rapid test for the presumptive identification of *C. albicans*.

II. Reagents / Materials / Media

Bovine serum - A small volume to be used as a working solution may be stored at 2 to 8°C. Stock solution can be dispensed into small tubes and stored at -20°C.

Clean glass microscope slides

Glass coverslips

Glass tubes (13 x 100 mm)

Pasteur pipettes

III. Procedure

1. Put 3 drops of serum into a small glass tube.
2. Using a Pasteur pipette, touch a colony of yeast and gently emulsify it in the serum. The pipette can be left in the tube.
3. Incubate at 35°C to 37°C for up to 3 hours but no longer.
4. Transfer a drop of the serum to a slide for examination.
5. Coverslip and examine microscopically using x 40 objective.

IV. Interpretation

Germ tubes are appendages half the width and 3 to 4 times the length of the yeast cell from which they arise. There is no constriction between the yeast cell and the germination tube.


Positive test: presence of short lateral filaments (germ tubes) one piece structure

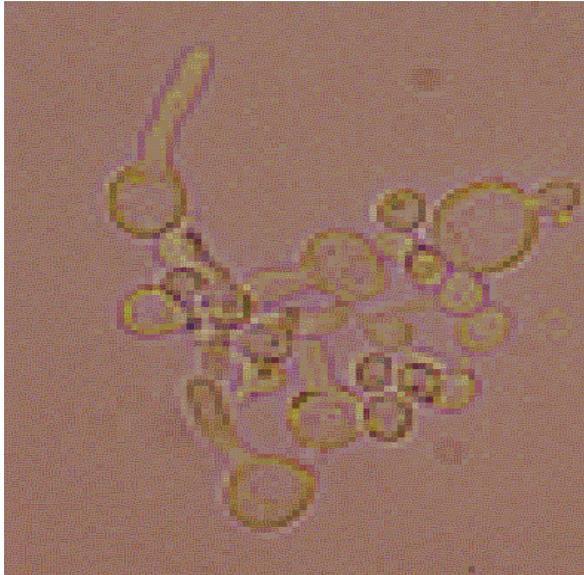
Negative test: yeast cells only (or with pseudohyphae) always two pieces

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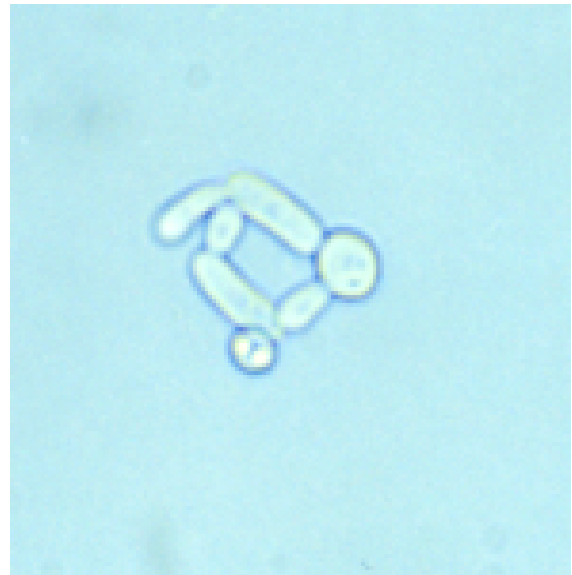
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Positive Germ Tube
(Parallel sides; Non-septate)



Negative Germ Tube
(Constriction at point of attachment)

Note: *C. tropicalis* may form pseudohyphae (usually after 3 hours incubation) which may be falsely interpreted as germ tube positive.

IV. Quality Control

Set up known controls daily:

C. albicans: positive
C. tropicalis: negative



V. References

1. Haley LD and Callaway CS, Laboratory Methods in Medical Mycology, CDC Publication No. 79-8361, 1979. p119.

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APPENDIX VII - STOCK CULTURES -WATER CULTURE TECHNIQUE

I. Purpose

To maintain stock cultures of fungi.

II. Procedure

1. Rub a sterile moistened swab over the surface of an actively sporulating fungal colony (or, using a spatula, scrape off the mycelial growth of the fungus above the agar).
2. Wash the swab (or spatula) off into a screw cap bijou bottle containing approximately 4 ml of sterile distilled water.
3. Tighten the cap and store at room temperature.
4. Add sterile distilled water periodically to prevent evaporation.
5. Subculture at least once a year to maintain viability of the stock cultures. Twirling the culture to resuspend the conidia, subculture using a sterile pipette.
6. Record stock cultures in the appropriate stock book in Mycology with the date the stock was made.



III. Reference

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

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APPENDIX VIII - API 20 CAUX - Yeast Identification System

I. Principle

The API 20C AUX strip consists of 20 microtubes containing dehydrated substrates in which 19 assimilation tests are performed. After inoculation and incubation, the reactions are interpreted by comparison to growth controls and use of the Identification Table provided with each kit.

II. Material

API 20C AUX Strip
 Incubation tray
 C Medium
 Pasteur pipettes (or Plastic pipettes)
 Suspension medium
 RAT medium



III. Procedure

1. Create a humid atmosphere within an incubation tray by distributing 5 ml of distilled water into the bottom of the tray.
2. Use a cotton swab to suspend a portion of the yeast colony in the suspension medium equal to a 2 McFarland standard.
3. Place 1 drop of yeast suspension into RAT Medium.
4. Transfer 100 µl (3 drops) of RAT Medium suspension into an ampoule of C medium.
5. Using a pasteur pipette, fill the capsules with the suspension in C medium.
6. Put lid on and incubate at 28°C x 48-72 hours.
7. After 24, 48 (and 72 hrs if needed) check for growth.
8. Record results onto the supplied report form and compare results with the Identification Table to identify yeast.
9. Record results in the LIS.

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IV. Quality Control



Control strains are set up for each new lot number of strips.

Use the follow isolates:

1. *C. laurentii* ATCC 18803
2. *C. guilliermondii* ATCC 6260
3. *C. glabrata* ATCC 15126

V. References

1. API 20C AUX package insert #20210.

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APPENDIX IX - MEDIA / REAGENTS

See: Microbiology and Mycology Media

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APPENDIX X – Flow Charts for Identification and Reporting

Flow Chart 1 - [Mycology Fungi Smear and Culture Reading](#)

Flow Chart 2 - Direct Microscopy

Flow Chart 3 - Zygomycetes

Flow Chart 4 - Dematiaceous

Flow Chart 5 - Hyalohyphomycetes

Flow Chart 6 - White Mould

Flow Chart 7 - Aspergillus

Flow Chart 8 – Dimorphic Fungi

Flow Chart 9 - Actinomyces



Flow Chart 10 - Yeast

Flow Chart 11 - Dermatophytes

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Record of Edited Revisions



Manual Section Name: Mycology Manual

Page Number / Item	Date of Revision	Signature of Approval
Annual Review	May 23, 2002	Dr. T. Mazzulli
Annual Review	May 12, 2003	Dr. T. Mazzulli
Specimen collection and specimen processing move to separate manuals	October 19, 2004	Dr. T. Mazzulli
Filamentous fungus workup – revised	October 19, 2004	Dr. T. Mazzulli
Yeast workup – revised	October 19, 2004	Dr. T. Mazzulli
Reporting – revised	October 19, 2004	Dr. T. Mazzulli
Flow charts for identification – added	October 19, 2004	Dr. T. Mazzulli
Annual Review	October 19, 2004	Dr. T. Mazzulli
Annual Review	May 12, 2005	Dr. T. Mazzulli
Annual Review	July 23, 2006	Dr. T. Mazzulli
Handling white mould pages 8, 32	July 16, 2007	Dr. T. Mazzulli
Replace BEAA with BHIM on bloods	July 16, 2007	Dr. T. Mazzulli
Annual Review	July 16, 2007	Dr. T. Mazzulli
Annual Review	July 31, 2008	Dr. T. Mazzulli
Annual Review	July 31, 2009	Dr. T. Mazzulli
Annual Review	July 31, 2010	Dr. T. Mazzulli
Updated Workflow	November 10, 2010	Dr. T. Mazzulli
Annual Review	July 31, 2011	Dr. T. Mazzulli
Annual Review	July 31, 2012	Dr. T. Mazzulli
Appendix X added quality control for media listed	November 13, 2012	Dr. T. Mazzulli
Annual Review	July 31, 2013	Dr. T. Mazzulli
Appendix VI – slide culture (Obsoleted)	July 25, 2014	Dr. T. Mazzulli
Annual Review	July 25, 2014	Dr. T. Mazzulli
Obsoleted GMS and PAS	July 25, 2014	Dr. T. Mazzulli
Updated MSH/UHN Logo	September 5, 2014	Dr. T. Mazzulli
New flow chart added on and BHI with 10% sheep blood Plus CCG and albumin	September 5, 2014	Dr. T. Mazzulli
Penicillium species; Direct microscopy positive for	December 5, 2014	Dr. T. Mazzulli

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fungus element and sterile site send to PHL for further speciation.		
For Aspergillus fumigatus set up 50°C; if no growth report Aspergillus fumigatus complex.	December 5, 2014	Dr. T. Mazzulli
Lactophenol Aniline Blue (LPAB) changed to Lactophenol Blue (LPCB)	December 5, 2014	Dr. T. Mazzulli
Annual Review	February 1, 2015	Dr. T. Mazzulli
Mycology staining procedures moved to technical manual	March 11, 2015	Dr. T. Mazzulli
Replaced text in appendix IX (media/reagents) with link to Media Manual	October 1, 2015	Dr. T. Mazzulli
Annual Review	February 1, 2016	Dr. T. Mazzulli
Addition in Laboratory Safety section avoid any work up of Suspect Risk Group 3 Organisms Safety: Removed from Filamentous Fungus ID: “Pathogenic dimorphic fungi such as <i>Blastomyces</i> , <i>Histoplasma</i> , <i>Sporothrix</i> , can be presumptively can often be presumptively identified by the presence of their characteristic conidia seen on Lactophenol Cotton Blue (LPCB) preparations of culture isolates.” Added: Never work up a Suspect <u>Risk Group 3</u> colony.	July 9, 2016	Dr. T. Mazzulli
Annual Review	February 1, 2017	Dr. T. Mazzulli

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