ISOLATION AND IDENTIFICATION

I. Reading of cultures

Specimen	Incubation Period (Weeks) at 28°C	Comment
Special Request Dimorphic	6 weeks	Read daily for 2 weeks; then 3 times per week for the remaining 4 weeks.
Isolator Blood Cultures Tissues Sterile Fluids Respiratory Tract Specimens	4 weeks	Read daily for 1 week; then 3 times per week for the remaining 3 weeks.
BAL (Routine Lung Transplant)	2 weeks	Read daily for 1 week; then 3 times per week for the remaining 1 week.
Environmental	5 days	Read on Day 1 and then on Day 5.
Special Request Malassezia	1 week	Read daily for 1 week
Other specimens	3 weeks	Read daily for 1 week; then 3 times per week for the remaining 2 weeks.

II. Identification

A) FILAMENTOUS FUNGI

Introduction:

Most filamentous fungi can be identified based on a combination of colonial morphology and microscopic features. Pathogenic dimorphic fungi such as *Blastomyces*, *Histoplasma*, *Sporothrix*, etc., can often be presumptively identified by the presence of their characteristic conidia seen on Lactophenol Aniline Blue (LPAB) preparations of culture isolates.
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.

a) Sterile site specimens:
Identify all filamentous fungi isolated. 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 Senior Technologist or the Microbiologist before proceeding.

b) All other specimens:
Identify all filamentous fungi isolated.

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
1. Prepare a tease mount or scotch tape preparation of each fungus colony type from each media using Lactophenol Aniline Blue (LPAB).

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 LPAB 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.

   Report the identification according the instructions in the Reporting Section.

4. 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
   - Pathology report if available
   - Clinical data
   - Growth on cycloheximide containing media
   - Growth at 37^{o}C

- Refer to the Identification Flow Charts.

See the Senior Technologist or Microbiologist for consultation if needed.

5. Set up a slide culture (see **Appendix VI - Slide Culture**) if full identification is needed.

6. 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
White Mould:		
Potato Dextrose Agar (PDA)	O_2,	28°C
Mycosel Agar	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 LPAB 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.
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
- Pathology report if available
- Clinical data
- Growth on cycloheximide containing media
- Growth at 37°C
- Refer to the Identification Flow Charts.

See the Senior Technologist or Microbiologist for consultation if needed. Send the isolate to PHL for further work-up if needed.

d) If the isolate cannot be identified by slide culture, 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.

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:

a) Germ tube: **Positive** - Report as "*Candida albicans*" "isolated".
b) Germ tube: **Negative** - Set up: Cornmeal Agar at 28°C
SAB at 28°C
API 20C at 28°C

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 (≥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):
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: 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)

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:
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 – positive)**, 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

2) Respiratory sites isolates (Germ tube – Positive 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)

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.

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 (eg. Mycetoma) and body fluid may also be submitted. For identification, procede as follows:

1. When Nocardia isolation is requested or organisms suggestive of Norcardia are seen on gram stain, plant specimen onto Sodium Pyruvate Agar (PYRA) and incubate in O_2, 28^oC for 4 weeks in Mycology. As well, the Blood Agar (BA) and Chocolate (CHOC) plates should be kept in bacteriology and incubated in O_2, 35^oC 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.

	***Nocardia* Species**	***Streptomyces* 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
Strong musty smell	-	+	-
Adherence to agar	+	+	-
Report as:	Phrase 1	Phrase 2	Phrase 3

*GPB = Gram positive bacilli