HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER
MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank (Microbiology)		
Document:	Internal Policy and Procedures		
Title:	Procedure for Fungal Culture		
Applies To:	All Laboratory Staff		
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1. PURPOSE:

1.1 To establish system and responsibilities for processing fungal culture.

2. DEFINITONS:

2.1 **Candida albicans** is a common coloniser of human skin and mucous membranes; an average of 25% to 30% of individual's harbour C. albicans in their oral cavity; approximately 50% of the population harbour this organism in their gastrointestinal tract and about 30% of females may have vaginal colonization (Vaginal carriage is particularly prevalent during pregnancy).
Except for strains of C. stellatoidea/ C. dubliniensis; generally considered phenotypic variants of C. albicans, and rare strains of C. tropicalis, only C. albicans forms germ tubes under standard test conditions.

2.2 **Candida Species other than Candida albicans** are normal flora of cutaneous and muco-cutaneous surfaces and are only rarely incriminated agents of clinical disease. Nevertheless, several Candida species have been incriminated as agents of virtually every form of candidiasis. The more common ones are C. tropicalis, C. glabrata, C. parapsilosis, C. krusei, C. guilliermondii, C. kefyr, and C. lusitaniae.

3. POLICY:

3.1 Specimens should be collected and transported in a properly labelled, sealed, sterile container.
3.2 If a delay in transport or processing is anticipated, store all specimens except CSF, hair, skin and nails at 4°C. CSF, hair, skin and nail specimens should be kept at room temperature.
3.3 Because C. albicans is the species of yeast most frequently isolated from clinical specimens, initial laboratory studies should be directed to its identification before additional, costly tests are performed.
3.4 For its identification, the rapid germ tube test is used in the laboratory.
3.5 Yeasts isolated from sites where they occur as part of the normal, commensal flora like oropharynx, GIT, throat, sputum, bronchial washing, stool and skin are of questionable significance & identified as C. albicans or 'Yeast other than C. albicans' based on the germ tube test.
3.6 Careful clinical correlations are necessary before accepting a Candida species isolate as being clinically significant and in need of a definitive identification.
3.7 The repeated isolation of yeasts from a series of clinical specimens from the same patient usually Indicates infection with the organism recovered and identification of the isolate is necessary.
3.8 Identification to species level, along with antifungal susceptibility testing (AST) is **performed if suspected to be pathogenic**

4. PROCEDURE:

4.1 **Processing of specimens:**

4.1.1 KOH examination (if available).

4.1.2 Culture:

Media	Incubation
Sabouraud Agar (SD)	O2, 20-30°C x 3 weeks.
Sabouraud Agar (SD)	O2, 35+2 °C x 3 weeks.

N.B.: Most fungi of clinical significance can grow on blood agar.

4.1.3 If fungal culture of sterile body fluids is requested, specimens should be centrifuged at 3500 rpm x 20 min prior to inoculating them using sterile centrifuge tubes.

4.1.4 The supernatant should be removed leaving sufficient volume to re-suspend the sediment and inoculate the appropriate number of culture media.

4.1.5 The plates should be wrapped by parafilm.

4.2 **Reading of culture &Interpretation of results:**

4.2.1 Reading of cultures:

4.2.1.1 CSF, blood and lung biopsy (and special request) cultures are read daily for the first 2 weeks and then two times a week for the remaining incubation period.

4.2.1.2 All other cultures are read three times a week for the first two weeks, and then two times a week for the remaining incubation period.

4.2.1.3 A colony with **a mucoid appearance and consistency** suggests capsule formation and may provide an initial clue to the identification of **Cryptococcus neoformans.**

4.2.1.4 Direct Mounts (wet mounts):

4.2.1.4.1 Emulsify a small portion of the yeast colony with a drop of sterile normal saline on a clean slide.

4.2.1.4.2 Apply cover slip and examine under high power objective.

4.2.1.4.3 Look for presence of yeast cells. Note the size, shape, presence of budding or pseudo hyphae.

4.2.1.5 **The Germ Tube Test:**

4.2.1.5.1 Take a small portion of an isolated colony of the yeast and suspend in a test tube containing 0.5 ml of human plasma.

4.2.1.5.2 The tube is incubated at 35°C for no longer than 2 hours. The test may not be valid if examined after 2 hours.

4.2.1.5.3 A drop of the yeast-plasma suspension is placed on a microscope slide and overlaid with a cover slip.

4.2.1.5.4 Examine microscopically for the presence of germ tubes.

4.2.1.5.5 The true germ tube of C. albicans has no constriction at the point of origin.

4.2.1.5.6 A constricted germ tube represents pseudo-hyphae formation derived from a budding process of the blast-conidia.

4.2.1.5.7 Both constricted and non-constricted "germ tubes" may be seen in the germ tube test for C. albicans; however, as the isolate is most likely to be C. albicans, it should be as such. See **Appendix 7.1**

4.2.2 Identification of filamentous fungi (MOULDS):

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

4.2.2.2 Prepare a wet mount of any fungus that is growing using normal saline.

4.2.2.3 Under the light microscope, examine the slide(s) for the presence, shape, size and attachment of conidia.

4.2.2.4 Compare and match the above features with those described in a reference textbook and record your observations in the register.

4.2.2.5 Report the identification of any isolate.

4.2.3 Identification of YEAST:

4.2.3.1 All yeast isolates except from voided urine (if insignificant count), sputum (if growth is few), superficial sites, wounds, and drainage fluid will be screened using a Germ tube.

4.2.3.2 Depending on the result of the Germ tube, proceed as follows:

4.2.3.3 Sterile site, body fluid and biopsy isolates:

4.2.3.3.1 Germ tube positive: Report as "C .albicans isolated" without quantitation.
4.2.3.3.2 Germ tube negative: report as: "Yeast isolated, further identification to follow" with quantitation.
4.2.3.3.3 Follow up with complete ID/AST report by Vitek 2 system.
4.2.3.3.4 Negative urease test rules out Cryptococcus.

4.2.3.4 Sputum, bronchoscopy and other respiratory specimen isolates:
4.2.3.4.1 If Growth is few, no Germ tube is performed. Report as part of Commensal flora without specifically commenting on the presence of yeast.
4.2.3.4.2 Growth (Moderate /heavy):
4.2.3.4.2.1 Germ tube positive: Report as "Candida albicans" with quantitation.
4.2.3.4.2.2 Germ tube negative: Rule out Cryptococcus by urease test.
4.2.3.4.2.3 If organism is not Cryptococcus (urease negative), then report as "Yeast, not Candida albicans or Cryptococcus" with quantitation.

4.2.3.5 All other isolates:
4.2.3.5.1 Germ tube Positive: Report as "Candida albicans".
4.2.3.5.2 Germ tube Negative: Report as "Yeast, not Candida albicans".

4.3 **Quality control for germ tube test:**
4.3.1 Use fresh frozen plasma from Blood Bank.
4.3.2 Thaw and dispense 0.5 ml in sterile tubes & store at -70℃.
4.3.3 Expiry date will be considered as one year from Blood Bank collection date.
4.3.4 Thaw plasma prior to use:
4.3.5 Positive control: Candida albicans ATCC 14053 (expected result--- Germ tube formation).
4.3.6 Negative control: Candida tropicalis ATCC 66029 (expected result--- No germ tube formation).

5. MATERIAL AND EQUIPMENT:

5.1 Media: Sabouraud Dextrose Agar (SAB), Sheep Blood agar
5.2 Germ Tube reagent: human plasma obtained from Blood Bank (screened for HIV, HB sAg, HCV etc.)
5.3 28-30°C Incubator
5.4 Vitek 2 system ID & AST cards
5.5 Glass slides, cover slips, inoculating loops
5.6 Light microscope

6. RESPONSIBILITIES:

6.1 The assigned technician/ technologist for microbiology lab.
6.2 The C. Pathology Specialist/ Consultant.

7. APPENDICES:

7.1 Candida Albicans' germ tube production.

8. REFERENCES:

8.1 Procedure Manual, Toronto Medical laboratories / Mount Sinai Hospital department of microbiology.
8.2 Larone, D. (2002). Medically Important Fungi, A guide to identification. 4th Edition. ASM Press. Washington DC, USA.
8.3 Bailey & Scott's Diagnostic Microbiology. Feingold & Baron; 12th. Ed.2007, C.V. Mosby Co. p. 301.
8.4 Henry D. Isenberg. Clinical Microbiology Procedures Handbook. 2010. American Society for Microbiology. Washington D.C., USA.
8.5 Murray, Baron, Jorgensen, Landry and Pfaller (2007). Manual of Clinical Microbiology. 9th Edition. ASM Press. Washington DC, USA.

9. APPROVALS:

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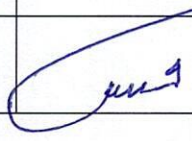
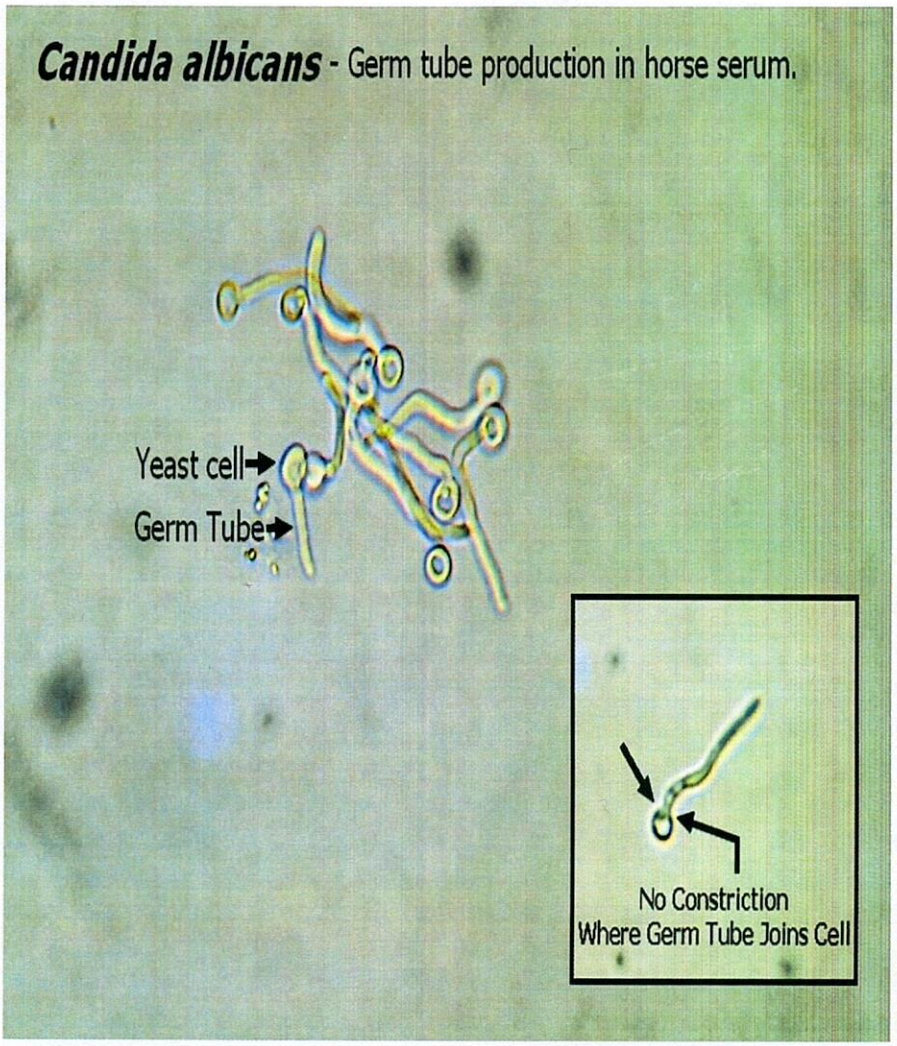
Approved by:	Mr. Fahad Hazam Alshammari	Hospital Director		January 20, 2025


Candida albicans - Germ tube production in horse serum.
Yeast cell
Germ Tube
No Constriction
Where Germ Tube Joins Cell