PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Germ Tube Procedure

Department of Microbiology

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1.0 Purpose

Candida albicans can be rapidly differentiated from other species of *Candida* by performing a germ tube test.

2.0 Clinical Significance

The extent to which yeast isolates need to be identified depends primarily on the body site from which they are isolated and the clinical relevancy of full identification. The most common yeasts associated with infection are *Candida* species. For many specimen types, it is sufficient to report *Candida albicans* or *Candida* not *albicans* based on the microscopic morphology and the results of the germ tube test.

3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiarized and trained to identify yeast isolates. Testing includes but is not limited to: morphologic recognition, confirmatory testing, and Quality Control testing of reagents.

4.0 Safety - Personal Protective Equipment

Performance of this procedure will expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

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The reagents and/or chemicals that are used in this procedure may be hazardous to your health if handled incorrectly. Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Bloodborne pathogens
- Airborne pathogens

To perform this procedure, you must use:

- Gloves – must be worn when handling human plasma.
- Laboratory Coat – must be worn when handling cultures and reagents.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

5.0 Specimen Requirements

Yeast colonies should be well isolated when performing screening and confirmatory testing. Yeast may be differentiated by colony or microscopic morphology. Sabouraud dextrose agar is the best medium to isolate yeasts for germ tube production; sheep blood agar is an acceptable substitute. Isolates should be 24 to 48 h old. *C. albicans* frequently produces colonies with spiking around the edge of the colony when grown on blood agar in CO_2. These colonies should not be used to perform germ tube tests because hyphal or pseudohyphal cells will be present in the inoculum.

6.0 Materials

6.1 General

- Heat block
- Microscope slides
- Coverslips
- Transfer pipettes

6.2 Reagents

- Germ Tube Plasma (human plasma obtained from blood bank). Store aliquots at -20 ± 2°C until needed. Thaw at room temperature.

7.0 Procedure

1. Retrieve an aliquot of germ tube reagent from the freezer, and allow it to thaw at room temperature.
2. Lightly touch a yeast colony with a wooden applicator stick.
3. Suspend yeast cells in tube of germ tube reagent labeled with the culture accession number.
4. Incubate at 35 ± 2°C for 2 h.
5. Place a drop of the suspension on a microscope slide.
6. Place a coverslip over the suspension.
7. Examine under high power (X400) for the presence or absence of germ tubes.

8.0 Interpretation of Results

A germ tube appears as a short lateral extension from the yeast cell and does not have a constriction where it meets the cell or any constriction along the tube. A minimum of 5 cells with germ tubes should be observed before an isolate is called positive.

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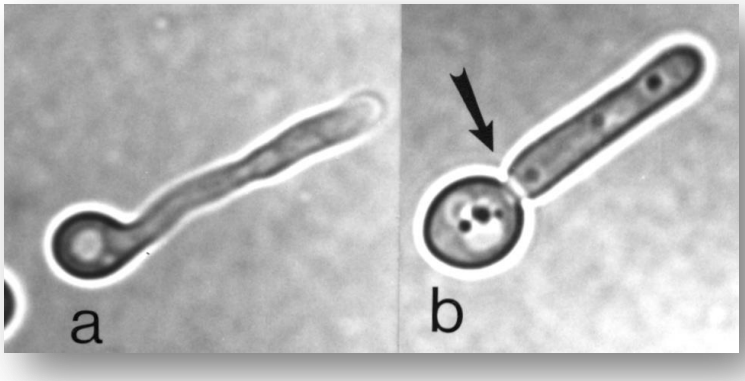

(a) Germ tube formation of *Candida albicans;* (b) blastoconidial germination with constriction (arrow) of *Candida tropicalis* not seen with true germ tubes of *C. albicans. Candida tropicalis* can produce hyphal initials, but the blastoconidia are larger than those of *C. albicans,* and there is a definite constriction where the hyphal initial joins the blastoconidium.

9.0 Quality Control

Each new lot of reagent should be tested using positive and negative control strains. Quality control should be performed weekly thereafter.

- *Candida albicans* ATCC 24433: Positive
- *Candida tropicalis* ATCC 750: Negative

QC results should be documented in the LIS QC module. Notify supervisor or technical specialist if QC testing fails to produce the expected results.

10.0 Limitations

1. If the germ tube suspension is incubated longer than 2 h, other types of *Candida* may start producing germ tubes, resulting in a false positive result.
2. Medium must be sterile, and the isolate must be pure. Germ tube production may be inhibited or delayed in contaminated reagent.
3. A heavy inoculum can cause false-negative results.
4. Some *C. tropicalis* isolates produce pseudohyphae that require careful observation to discriminate them from germ tubes produced by *C. albicans*.
5. *C. dubliniensis* is also germ tube positive.
6. Not all *C. albicans* will produce germ tubes

11.0 References

1. Versalovic, J, K. C. Carroll, G. Funke, J. H. Jorgensen, M. L. Landry, D. W. Warnock. 2011. Manual of Clinical Microbiology, 10th ed., Vol. 1, ASM Press, Washington, D.C.
2. Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.

12.0 Document Control History

Reviewed by director (AR): 5/19/2000, 04/22/2014

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Revisions: 04/22/2014 Separated Germ Tube Procedure from previous Yeast Identification Procedure.

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