

# **Ministry of Health**

<b>Document Title</b>	Germ tube test SOP
<b>Document Type</b>	Procedure
Directorate/Institution	Diagnostic Laboratories Services at Directorate General of
	Specialized Medical Care (DGSMC) at Ministry of Health
	(MOH)
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## **Contents Table:**

A	.cronyms:	4
.1	Purpose	5
	. Scope	
	. Definitions	
4.	Procedure	5
5.	. Responsibilities	9
6.	. Document History and Version Control	11
7.	References:	11
8	Annexes	12

## Acknowledgment

The diagnostic laboratories services at the Directorate General of Specialized Medical Care (DGSMC) at Ministry of Health (MOH) would like to thank and appreciate the great effort of the Microbiology documents development team. Participated and contributed personnel are:

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# Acronyms:

SDA	Sabouraud Dextrose agar with Ampicillin and Gentamycin plate (SDA).
ID	Identification
IQC	Internal Quality Control
SOP	Standard operating procedure
TAT	Turnaround time

#### 1. Purpose:

This document describes the procedure for the determination of germ tube to distinguish *Candida albicans*, *Candida africana and Candida dubliniensis* from other *Candida sp*. This test is presumptive because not all isolates of *Candida albicans* will be germ tube positive.

## 2. Scope:

This document is applicable for all medical laboratories under MOH and other collaborative governmental and non-governmental health institutions.

#### 3. Definitions:

3.1 Germ tube: is defined as an appendage that is one half the width and three to four times the length of a yeast cell from which it arises.

### 4. Procedure

#### 4.1. Clinical background:

*C. albicans* is a type of yeast that is normally present in the human body, particularly in the mouth, gastrointestinal tract, and vagina. It is usually harmless and does not cause any problems. However, in certain situations, such as when the immune system is weakened or the normal balance of microorganisms in the body is disrupted, *C. albicans* can overgrow and cause infections and biofilm formation.

Candida infections can range in severity from mild to severe. They can affect different parts of the body, including the mouth (thrush), skin, and nails (cutaneous candidiasis), and the genital area (vulvovaginal candidiasis). In more severe cases, *C. albicans* can cause invasive infections, such as candidemia (a bloodstream infection) and deep tissue infections.

The germ tube test is often used in clinical settings to help diagnose *Candida* infections. It is especially useful in identifying *C. albicans* in the bloodstream, as this type of yeast is responsible for the majority of candidemia cases.

#### 4.2.Principle:

The principle of the germ tube test is based on the ability of the yeast *Candida albicans* to form germ tubes when incubated in rabbit serum. Germ tubes are thin, tubular extensions of the yeast cells that grow out from the main body of the cell. The formation

of germ tubes is a characteristic trait of *C. albicans* and is thought to be related to the yeast's ability to cause infections in the human body.

### 4.3.Pre – analytical stage:

#### 4.3.1. Sample:

- 4.3.1.1 Sample type: pure well isolated colony on solid agar medium.
- 4.3.1.2 Amount of sample required, including minimum requirements: 2-3 colonies.
- 4.3.1.3 Transportation media: Sabouraud agar plate.
- 4.3.1.4 Sample stability and storage requirements: to be processed immediately within 18-24 hours.
- 4.3.1.5 In case of delay, store the sample in 2-8 C

### 4.3.2. Material:

Reagents	Consumables/Supplies	Equipment
Sabouraud Dextrose	Microscopic slides.	Microscope
agar with Ampicillin	Cover slip 22x22.	Safety cabinet class II
and Gentamycin plate	Sterile loops	Incubators
(SDA).	Test tube glass/plastic	
Rabbit Serum	Pasture pipette	
Sterile Distilled water		

## 4.3.3. Safety precaution:

- 4.3.3.1 All specimens need to be treated as potentially infectious.
- 4.3.3.2 Standard procedures for handling of biohazard material must be followed at all times.
- 4.3.3.3 Universal Precautions must be practiced at all stages of these procedures.

#### 4.3.4. Quality control:

- 4.3.4.1 Check the expiry dates of all media, reagents and stains before use.
- 4.3.4.2 All media, reagents, kits, and stains MUST be quality controlled before use.
- 4.3.4.3 Identification tests should be run with appropriate controls.
  - ➤ Positive control Candida albicans (ATCC 10231).

- ➤ Negative control Candida species or C. tropicalis (ATCC 13803), C. glabrata (ATCC 2001).
- 4.3.4.4 Record the quality control results in the appropriate QC sheet. If any abnormal or unexpected results are obtained, a supervisor should be notified and corrective action should be taken and recorded (see annex #1).

### 4.4. Analytical stage:

- 4.4.1 Inoculate a tube containing approximately 0.5 ml serum with a few single isolated colonies.
- 4.4.2 Incubate at 37°C for 1 to 2 hours (no longer than 3 hrs).
- 4.4.3 Place 1 drop of the suspension onto a clean glass slide and place a cover slip.
- 4.4.4 Examine under low-power magnification for the presence or abscence of germ tubes.

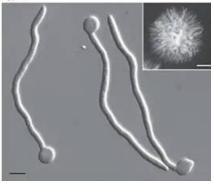
#### 4.5. Post – analytical stage:

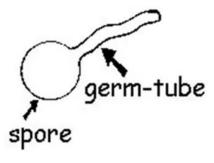
- 4.5.1. Interpretation / Results / Alerts:
  - ➤ If a minimum of five germ tubes is observed, the test is considered positive for the presence of *Candida albicans*.
  - ➤ If no germ tube is observed, the test is considered negative. No hyphal extension arises from the yeast cell or only a short one with constriction at the point of origin.





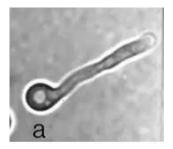




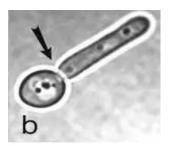


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May 2023



a. Germ tube formation Candida albicans.



b. *Candida tropicalis* blastoconiodial germination with constriction after 3 hours of incubation.

## 4.5.2. Reporting:

- 4.5.2.1 Germ Tubes = Positive = Candida albicans.
- 4.5.2.2 No germ tubes = Negative = *Candida species*.
- 4.5.2.3 If source of sample is from sterile sites (e.g. Blood, CSF, Body fluid, Tissues) inform Microbiologist immediately and refer the isolate to Central Public Health Laboratory (CPHL) for Identification and susceptibility testing.

#### 4.5.3. Limitation:

- 4.5.3.1 Too heavy inoculum may produce a false negative result due to over competition for nutrients in the serum.
- 4.5.3.2 Increased incubation period yields false positive results.
- 4.5.3.3 Cultures may be mixed and this may be difficult to detect using this method.

## 5. Responsibilities:

## 5.1.Responsible staff:

- To ensure the adherence to critical result communication procedure
- To facilitate the alternative channels once needed

## 5.2. Quality manager /officer

- To follow up the implementation of the procedure
- To monitor regularly communication of critical results and raise non-conformance with corrective action once needed.

## 5.3.All lab staff:

- To adhere to the procedure.
- To document record and release results as recommended.
- To report test failures or incident.

# 6. Document History and Version Control:

Version	Description	Review Date
1	Initial Release	May 2026

## 7. References:

Title of book/ journal/ articles/ Website	Author	Year of publication	Page
Clinical Microbiology Procedures Handbook, by ASM Press <a href="https://www.asm.org/index.php/clinical-microbiology-procedures-handbook-3rd-edition">https://www.asm.org/index.php/clinical-microbiology-procedures-handbook-3rd-edition</a>	Lynne S. Garcia & Henry D. Isenberg	2010	
Diagnostic Medical Microbiology by Mosby.	Patricia M.	2022	
UK Standards for Microbiology Investigations Introduction to the preliminary identification of medically important bacteria and fungi from culture	Public Health England (PHE) in partnership with the NHS	2021	
Diagnostic Microbiology, 9 <sup>th</sup> ed.	Baron EJ, Peterson LR, Finegold SM. Bailey & Scott's	1994	755
Growth of Candida albicans hyphae. Nature Reviews Microbiology	Sudbery, P. E.	2011	9(10), 737– 748
The germ tubes of <i>Candida albicans</i> hyphae and pseudohyphae show different patterns of septin ring localization. <i>Molecular Microbiology</i>	Sudbery, P. E.	2001	<i>41</i> (1), 19–31.
Clinical Microbiology Procedures Handbook, Fourth Edition. American Society of Microbiology.	Amy L. Leber	2016	

# **8.** Annexes #1: Daily microbiology identification test quality control sheet (Germ Tube Test):

Test Name: Germ Tube Test											Kit Manufacture Name:																		
Reagent Lot No:						Re	Reagent Exp. Date:										Reagent Open date:												
Month:																													
Positive Control result (P)																													
Negative Control result (N)																													
Quality Control (Pass/Failed)																													
Initials																													