CLINICAL AND LABORATORY STANDARDS INSTITUTE®

2nd Edition

# M54

## Principles and Procedures for Detection and Culture of Fungi in Clinical Specimens



This guideline includes protocols, quality control parameters, and interpretive criteria for culturing fungi and for detecting and identifying fungi in direct examinations.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

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# Principles and Procedures for Detection and Culture of Fungi in Clinical Specimens

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## Abstract

Clinical and Laboratory Standards Institute guideline M54—*Principles and Procedures for Detection and Culture of Fungi in Clinical Specimens* describes recommended processes for plating and examining fungal cultures as well as principles and procedures for the direct detection of fungi in clinical specimens, including criteria for performing and interpreting direct microscopic examinations. Safety considerations unique to mycology laboratories and a discussion of appropriate levels of laboratory service (eg, when to refer samples to more experienced laboratories) are highlighted. Specimen collection, transport, and processing recommendations, including rejection criteria, are provided to guide the collection of high-quality specimens for direct examinations and fungal cultures. Fungal stains and interpretive criteria appropriate for detecting and characterizing fungal elements in direct microscopic examinations are emphasized as critical components for rapid detection of fungi. Descriptions of serological and antigen-based testing and molecular assays are also provided. Media selection, incubation conditions, and other growth requirements for fungal cultures are provided with suggested culture examination schedules, interpretations for growth on positive cultures, and reporting criteria.

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# Foreword

The ability of the laboratorian to detect and characterize fungal elements directly from patient specimens can rapidly provide substantial information about possible pathogens. Therefore, characterization of yeasts and yeast-like organisms based on size and other microscopic characteristics is emphasized together with, where possible, differentiation of molds in direct examinations. Current antibody and antigen tests, as well as molecular assays for detection of fungi, are also described. Stains commonly used for direct examinations in the mycology laboratory are presented along with information on the utility of histopathology stains that may come to the attention of the mycologist.

## Overview of Changes

This guideline replaces the previous edition of the approved guideline, M54-A, published in 2012. Several changes were made in this edition, including:

- Updating information, including current fungal taxonomy and nomenclature
- Expanding tables and consolidating text
- Adding new subchapters on antibody, antigen, and molecular testing
- Adding new figures, including new photographs

**NOTE:** The content of this guideline is supported by the CLSI consensus process and does not necessarily reflect the views of any single individual or organization.


**KEY WORDS**

Culture
Direct examination
Fungi
Mold
Molecular
Mycology
Safety
Serology
Specimen collection
Stock cultures
Yeast

# Chapter 1

# Introduction

## This chapter includes:

- Guideline's scope and applicable exclusions
- Standard precautions information
- Terminology information, including:
  - Terms and definitions used in the guideline
  - Abbreviations and acronyms used in the guideline

# Principles and Procedures for Detection and Culture of Fungi in Clinical Specimens

## 1 Introduction

### 1.1 Scope

This guideline provides recommendations to laboratories on procedures for collecting, processing, and handling fungal specimens and interpreting direct stain examinations and culture results. In addition, methods for direct or indirect detection from patient specimens, such as antigen, antibody, and molecular testing, are included. Because the relative importance of any fungus isolated from a patient specimen depends on the pathogenic potential of the fungus and the clinical setting in which it is isolated, these issues as well as factors to consider regarding the isolate's clinical significance are discussed.

Fungal taxonomy has been updated in this edition of M54. Direct molecular methods to detect fungi in clinical specimens are also discussed, such as the use of magnetic resonance to detect *Candida* spp. in blood and the use of PCR to detect *Pneumocystis jirovecii* in respiratory specimens, other fungal pathogens, and the emerging pathogen *Candida auris*. This guideline considers individualized quality control plan (IQCP) issues related to fungal media and includes a table listing the differential diagnosis of various yeasts and yeast-like organisms on direct examination. Antigen and antibody detection of fungi and the extent of identification needed to provide clinical and therapeutic guidance are also discussed.

The intended users of this guideline are laboratorians who process specimens for fungal culture, perform fungal direct microscopic examinations, and/or perform antibody, antigen, or molecular testing for fungi. Antifungal susceptibility testing methods (see CLSI documents M27,[1] M38,[2] M44,[3] M51,[4] M60,[5] and M61[6]) are not discussed in this guideline. Although *Nocardia* spp. and other aerobic actinomycetes can be encountered growing on mycology media, methods for detecting these organisms are not discussed in this guideline. Additionally, definitive fungal identification from culture growth (eg, examination of cellulose tape preparation, matrix-assisted laser desorption/ionization time of flight mass spectrometry [MALDI-TOF MS], or DNA sequencing) is outside this guideline's scope (see CLSI documents M58[7] and MM18[8]).

### 1.2 Standard Precautions

Because it is often impossible to know what isolates or specimens might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all known infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of bloodborne pathogens. Published guidelines are available that discuss the daily operations of diagnostic medicine in humans and animals while encouraging a culture of safety in the laboratory.[9] For specific precautions for preventing the laboratory transmission of all known infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all known infectious diseases, refer to CLSI document M29.[10] For detailed information on biosafety practices specific to the mycology laboratory, see Subchapter 6.1.

## 3.3 Specimen Types

For detailed information on acceptable clinical specimen types for fungal culture, refer to Table 2.

**Table 2. Specimen Collection, Transport, and Processing Chart**

| Specimen Site and/or Type | Collection Guidelines | Collection Container | Unacceptable Specimen Collection Method and/or Type | Specimen Processing Method | Notes |
|---|---|---|---|---|---|
| Abscesses and wounds | • The surface exudate should be removed with preservative-free, sterile saline or 70% ethanol.<br>• Aseptic aspiration from undrained abscesses should be performed using a needle and syringe.<br>• Tissue from the deepest part of the lesion plus the advancing margin should be included. | Sterile tube | Swabs | < 2 mL direct inoculation; > 2 mL concentrate. Excessively mucoid specimens can be processed with a mucolytic agent before plating.[a] | If grains or granules are present:<br>• The color should be documented.<br>• They should be removed and washed in sterile saline.<br>• A granule should be crushed between two glass slides and examined microscopically.<br>• If fungal elements are present, individual sterile, washed granules should be processed by crushing and plating directly. |
| Blood | • The skin should be thoroughly cleansed using standard blood culture collection protocols.<br>• The maximum amount of blood recommended for the collection container being used should be collected. | • A special mycological blood culture medium bottle or lysis centrifugation tube should be used.[18-24]<br>• Routine blood culture bottles may be appropriate for some yeasts. | • Expired media bottles or tubes<br>• Blood cultures should not be refrigerated after specimen collection. | The manufacturer's instructions should be followed. | Direct examination should not be performed. Refer to CLSI document M47.[25] |

### 4.1.1 Small-sized Yeasts (2 to 5 µm)

The yeasts and yeast-like organisms in the small-sized category that may be seen by direct examination include but are not limited to *C. auris, C. glabrata, H. capsulatum, P. jirovecii, Malassezia* spp., and *S. schenckii* species complex. The nonbudding endospores of *Coccidioides* spp., which may appear individually when the spherule has ruptured, are also in this size range. A fungus encountered primarily in Southeast Asia that also has forms in this size range is *T. marneffei.*

- ***C. auris:*** *C. auris* is similar in size to *C. glabrata* but can be morphologically distinguished from other larger-sized *Candida* spp. by its size and inability to form filaments *in vitro* (see Figure 2). *C. auris* very rarely forms pseudohyphae *in vivo*.[14]

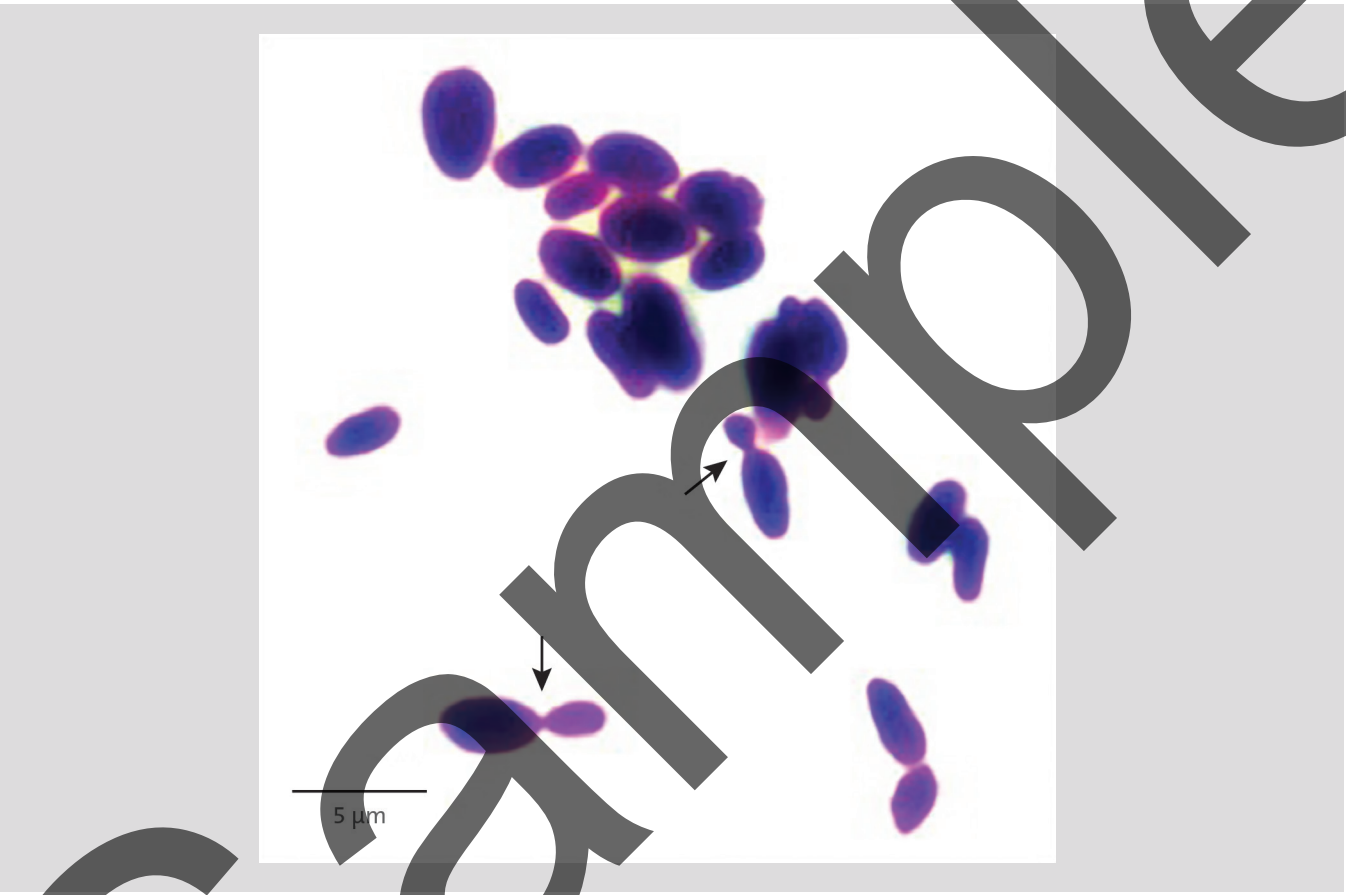


**Figure 2. Gram Stain of a *C. auris* Culture.** Arrows point to narrow-budding yeast with no pseudohyphae.

# Related CLSI Reference Materials[a]

**EP23™** **Laboratory Quality Control Based on Risk Management. 1st ed., 2011.** This document provides guidance based on risk management for laboratories to develop quality control plans tailored to the particular combination of measuring system, laboratory setting, and clinical application of the test.

**M22** **Quality Control for Commercially Prepared Microbiological Culture Media. 3rd ed., 2004.** This document contains quality assurance procedures for manufacturers and users of prepared, ready-to-use microbiological culture media.

**M27** **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. 4th ed., 2017.** This standard covers antifungal agent selection and preparation, test procedure implementation and interpretation, and quality control requirements for susceptibility testing of yeasts that cause invasive fungal infections.

**M29** **Protection of Laboratory Workers From Occupationally Acquired Infections. 4th ed., 2014.** Based on US regulations, this document provides guidance on the risk of transmission of infectious agents by aerosols, droplets, blood, and body substances in a laboratory setting; specific precautions for preventing the laboratory transmission of microbial infection from laboratory instruments and materials; and recommendations for the management of exposure to infectious agents.

**M38** **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi. 3rd ed., 2017.** This standard includes antifungal agent selection, preparation of antifungal stock solutions and dilutions for testing, test procedure implementation and interpretation, and quality control requirements for susceptibility testing of filamentous fungi (moulds) that cause invasive and cutaneous fungal infections.

**M44** **Method for Antifungal Disk Diffusion Susceptibility Testing of Yeasts. 3rd ed., 2018.** This guideline provides an established methodology for disk diffusion testing of *Candida* spp., along with recommendations for results interpretation and quality control testing.

**M47** **Principles and Procedures for Blood Cultures. 1st ed., 2007.** This document provides recommendations for the collection, transport, and processing of blood cultures as well as guidance for the recovery of pathogens from blood specimens taken from patients who are suspected of having bacteremia or fungemia.

**M51** **Method for Antifungal Disk Diffusion Susceptibility Testing of Nondermatophyte Filamentous Fungi. 1st ed., 2010.** This document describes the guidelines for antifungal susceptibility testing by the disk diffusion method of nondermatophyte filamentous fungi (moulds) that cause invasive disease.



[a] CLSI documents are continually reviewed and revised through the CLSI consensus process; therefore, readers should refer to the most current editions.

## Related CLSI Reference Materials (Continued)

**M58** **Methods for the Identification of Cultured Microorganisms Using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry. 1st ed., 2017.** This guideline includes performance, reporting, and quality assurance recommendations for the identification of cultured microorganisms by medical laboratory professionals using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Recommendations for end-user verification and workflow integration are also included.

**M60** **Performance Standards for Antifungal Susceptibility Testing of Yeasts. 2nd ed., 2020.** This document provides updated minimal inhibitory concentration, zone diameter, and quality control tables for the Clinical and Laboratory Standards Institute antifungal susceptibility testing documents M27 and M44.

**M61** **Performance Standards for Antifungal Susceptibility Testing of Filamentous Fungi. 2nd ed., 2020.** This document provides minimal inhibitory concentration breakpoints and quality control tables for the Clinical and Laboratory Standards Institute antifungal susceptibility testing documents M38 and M51.

**MM18** **Interpretive Criteria for Identification of Bacteria and Fungi by Targeted DNA Sequencing. 2nd ed., 2018.** This guideline includes information on sequencing DNA targets of cultured isolates, provides a quantitative metric for perceiving microbial diversity, and can serve as the basis to identify microorganisms. By establishing interpretive criteria for microorganism identification by targeted DNA sequencing, this guideline provides structure to laboratories that identify microorganisms for medical use.

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