

# 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.

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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

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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.

Clinical and Laboratory Standards Institute (CLSI). *Principles and Procedures for Detection and Culture of Fungi in Clinical Specimens*. 2nd ed. CLSI guideline M54 (ISBN 978-1-68440-098-0 [Print]; ISBN 978-1-68440-099-7 [Electronic]). Clinical and Laboratory Standards Institute, 2021.

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## Suggested Citation

CLSI. *Principles and Procedures for Detection and Culture of Fungi in Clinical Specimens*. 2nd ed. CLSI guideline M54. Clinical and Laboratory Standards Institute; 2021.

Previous Edition:  
October 2012

M54-Ed2

ISBN 978-1-68440-098-0 (Print)

ISBN 978-1-68440-099-7 (Electronic)

ISSN 1558-6502 (Print)

ISSN 2162-2914 (Electronic)

Volume 41, Number 1

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Acknowledgment

CLSI, the Consensus Council, and the Document Development Committee on Detecting Fungi gratefully acknowledge the following volunteers for their important contributions to the revision of this guideline:

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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**

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# 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

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## 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,<sup>1</sup> M38,<sup>2</sup> M44,<sup>3</sup> M51,<sup>4</sup> M60,<sup>5</sup> and M61<sup>6</sup>) 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<sup>7</sup> and MM18<sup>8</sup>).

### 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.<sup>9</sup> 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.<sup>10</sup> For detailed information on biosafety practices specific to the mycology laboratory, see Subchapter 6.1.