CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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# M35-A2

## Abbreviated Identification of Bacteria and Yeast; Approved Guideline—Second Edition



This document provides the minimum identification criteria that can be used to rapidly identify organisms commonly isolated from clinical specimens.

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

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# Abbreviated Identification of Bacteria and Yeast; Approved Guideline—Second Edition

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## Abstract

Many microorganisms commonly isolated in human diagnostic microbiology laboratories exhibit specific morphologic or biochemical traits that can be determined rapidly upon obtaining a pure colony. When such rapidly obtained parameters allow reliable identification of the organism with a high degree of certainty, the necessity of performing more time-consuming tests is decreased, and timely patient care is enhanced.

Clinical and Laboratory Standards Institute document M35-A2—*Abbreviated Identification of Bacteria and Yeast; Approved Guideline—Second Edition* includes the minimum identification criteria that can be used to rapidly identify a limited number of organisms commonly isolated from patient specimens. Although these tests do not rule out an occasional misidentification, those errors may not have important consequences with regard to patient outcome. Those situations in which rapid test results may have limitations are described. Unless those exceptions provide clinically useful information, confirmatory identification need not be done.

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## Foreword

Microorganisms isolated in the clinical microbiology laboratory demonstrate unique biochemical and morphologic characteristics. These characteristics provide a mechanism for determination of microbial identity to the genus (and sometimes, species) level. Identification of pathogenic bacteria and yeast provides the basis for guidance of treatment with antimicrobial and antifungal agents. In the clinical microbiology laboratory, the need for accurate reporting to the clinician of microbial identification is coupled with a demand for rapid turnaround time to allow for initiation of therapy. These requirements often demand the expenditure of resources in a health care environment where laboratory personnel are faced with ever increasing financial and manpower constraints. The recognized need for finding cost-effective approaches to diagnostic microbiology obviates the use of time- and resource-consuming, comprehensive microbial identification techniques. M35 was created to supplement this effort.

This document contains instructions and flowcharts outlining the minimal characteristics required to identify the listed microorganisms to genus, and in some cases, to species, with enough reliability (≥ 95% accuracy) for clinical laboratory reports; or in some cases, to include or rule out potentially important pathogens within stated limitations. CLSI would appreciate being informed of any problems that occur as a result of using the guidelines in this document for determining patient results.

Protocols for noncommercial tests used for abbreviated identifications are listed in a separate appendix at the end of this document.

**Key Words**

Biochemical characteristics, microbiological identification

# Abbreviated Identification of Bacteria and Yeast; Approved Guideline—Second Edition

## 1 Scope

Many laboratories use commercial systems for identification of microorganisms, because the laboratories lack the confidence in or resources for performing in-house validations of alternative methods. Use of commercial panels has resulted in greater standardization and more accurate taxonomic identifications, albeit at relatively higher cost and slower turnaround time. This document provides well-documented, published studies to guide laboratories in choosing rapid, reliable, and often less expensive alternatives to commercial systems. This guideline shares the experience and expertise of practicing microbiologists for reporting bacterial and yeast identifications more rapidly than by traditional methods.

## 2 Introduction

A variety of methods can be used to identify microorganisms of clinical importance. The most well-characterized methods employ a battery of biochemical and enzymatic tests that are used after characterization of an organism based on initial Gram stain and colony morphological characteristics. Often, these methods require time and materials that add to the cost of the final identification. Even simple tests such as the bacitracin and optochin disk tests require overnight incubation. Waiting for results of these test methods may unnecessarily delay reporting of clinically important isolates, and may slow the laboratory workflow.

Although many laboratorians use rapidly determined characteristics, such as odor, immediate enzymatic reactions (spot tests), and other criteria for "presumptive" or initial identification, some of these rapid methods have not been standardized or validated. The methods described in this guideline are those believed to yield a result reliable enough for clinical decision-making, but are cost-effective, take less time, and are easier to perform than conventional methods. Several authors have examined the cost savings of using rapid methods or the overall patient care benefits (economic and general) that rapid reporting of results yields.[1-3] Tests that may be included in this category are single-tube, slide, spot, agglutination, disk, chromogenic media, fluorogenic, enzymatic, microscopic, morphologic, or plate methods that can be performed within a few hours. Proprietary multitest and molecular-based systems are not included. A critical factor in the performance of these tests is the competency and experience of the microbiologist. Initial correct interpretation of colonial and Gram stain characteristics is essential to achieving the desired results.

**Laboratory directors, managers, and supervisors are responsible for ensuring rapid methods are only used in situations in which the competency of the tester has been documented. Isolates to be tested should match the criteria required for proper identification. Inexperienced laboratorians should be under the direct supervision of an experienced technologist or use alternate methods until proficiency is achieved. It is recommended that competency testing of personnel performing the rapid tests described here include observation (or examination by distributing unknown strains) of the ability to accurately identify the colony morphology, Gram stain morphology, and smell (where this characteristic is safe to use and indicated) of the various species described in the document.**

Isolates conforming to the reactions described in the appendix will identify the named organism with >95% (based on the literature cited) accuracy, and their identification can thus be reported without qualification, with the caveats and limitations listed below. Confirmation by additional procedures is unnecessary for many of the species described in this document. It should also be emphasized that lack of a positive result in the rapid tests included here does not rule out the identification of any isolate. It simply indicates the need for further testing.

## 3 Standard and Special Precautions

Because it is often impossible to know which 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 infectious agents and thus are more comprehensive than universal precautions, which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the US Centers for Disease Control and Prevention.[4] For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to CLSI document M29.[5]

More recent appreciation of the incidence of unsuspected exposure of laboratory workers to agents of biological terrorism or agents with higher risks for transmission to workers has resulted in changes in recommendations for all culture handling in the laboratory.[6,7] Because of the danger of laboratory accidents or penetration of skin with highly pathogenic organisms, growth of colonies from cerebrospinal fluid (CSF), blood, and lymph nodes should be examined in a biological safety cabinet (BSC) while wearing protective gloves, until pathogens known to be responsible for laboratory-acquired infections (eg, *Brucella*, *Francisella*, and *Neisseria meningitidis*) have been ruled out. Any unidentified gram-negative or gram-variable rod or coccobacillus that grows on blood and chocolate only, and not on MacConkey agar, must be handled with extreme caution until highly pathogenic species, such as those listed, are ruled out. Gram stains and wet mounts should be prepared in the BSC and microbiologists should wear gloves. All procedures known to produce aerosols (such as catalase test, microdilution tray inoculation, and others) should be performed in a BSC with extra care, or avoided altogether until the highly pathogenic organisms have been ruled out. In many cases, automated identifications and susceptibilities are not reliable for those organisms, and should not be performed until the possibility of their identification as an agent of biological terrorism has been eliminated.[6,8-10]

"Sniffing" plates to determine characteristic odors of the organisms growing can be dangerous.[6,11-13] However, particularly when examining plates from noninvasively collected specimens (such as urine and sputum), the opening of plates on the open bench is a common and relatively safe practice in microbiology, once the presence of a mold colony has been ruled out. When the plate is opened but not sniffed purposely, it is still possible to detect a strong odor as described for *Pseudomonas aeruginosa*, *Burkholderia pseudomallei*, *Haemophilus influenzae*, *Eikenella*, and *S. anginosus* (*"S. milleri"*) group streptococci that can be used to aid in identification of the organisms.

## 4 Terminology

### 4.1 Definitions

**algorithm** – a set of rules for solving a problem in a finite number of steps, as for finding the greatest common divisor.

**quality control (QC)** – the operational techniques and activities that are used to fulfill requirements for quality (ISO 9000).[14]

### 4.2 Abbreviations/Acronyms

| | |
|---|---|
| aBAP | anaerobic blood agar |
| ATCC | American Type Culture Collection |
| BAP | sheep blood agar |
| BBE | Bacteroides bile esculin agar |



 

## The Quality Management System Approach

Clinical and Laboratory Standards Institute (CLSI) subscribes to a quality management system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in the most current edition of CLSI/NCCLS document HS01—*A Quality Management System Model for Health Care.* The quality management system approach applies a core set of "quality system essentials" (QSEs), basic to any organization, to all operations in any health care service's path of workflow (ie, operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The QSEs are:

| | | | |
|---|---|---|---|
| Documents & Records | Equipment | Information Management | Process Improvement |
| Organization | Purchasing & Inventory | Occurrence Management | Customer Service |
| Personnel | Process Control | Assessments—External & Internal | Facilities & Safety |

M35-A2 addresses the QSEs indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

| Documents & Records | Organization | Personnel | Equipment | Purchasing & Inventory | Process Control | Information Management | Occurrence Management | Assessments—External & Internal | Process Improvement | Customer Service | Facilities & Safety |
|---|---|---|---|---|---|---|---|---|---|---|---|
| M07 | | | | | X<br>M02<br>M07<br>M22<br>M27<br>M29 | | | | | | M29 |

Adapted from CLSI/NCCLS document HS01—*A Quality Management System Model for Health Care*.

### Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, CLSI/NCCLS document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow, which consists of three sequential processes: preexamination, examination, and postexamination. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

M35-A2 addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other documents listed in the grid, please refer to the Related CLSI Reference Materials section on the following page.

| **Preexamination** | | | | **Examination** | | | **Postexamination** | |
|---|---|---|---|---|---|---|---|---|
| Examination ordering | Sample collection | Sample transport | Sample receipt/processing | Examination | Results review and follow-up | Interpretation | Results reporting and archiving | Sample management |
| | | | | X<br>M27 | X<br>M02<br>M07<br>M27 | X<br>M02<br>M07<br>M27 | X<br>M02<br>M07<br>M27 | M27 |

Adapted from CLSI/NCCLS document HS01—*A Quality Management System Model for Health Care*.



 

## Related CLSI Reference Materials*

**M02-A9** **Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Ninth Edition (2006).** This document contains the current Clinical and Laboratory Standards Institute-recommended methods for disk susceptibility testing, criteria for quality control testing, and updated tables for interpretive zone diameters.

**M07-A7** **Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition (2006).** This document addresses reference methods for the determination of minimal inhibitory concentrations (MICs) of aerobic bacteria by broth macrodilution, broth microdilution, and agar dilution.

**M22-A3** **Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard—Third Edition (2004).** This document contains quality assurance procedures for manufacturers and users of prepared, ready-to-use microbiological culture media.

**M27-A3** **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Third Edition (2008).** This document addresses the selection and preparation of antifungal agents; implementation and interpretation of test procedures; and quality control requirements for susceptibility testing of yeasts that cause invasive fungal infections.

**M27-S3** **Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Third Informational Supplement (2008).** This document provides updated tables for CLSI antimicrobial susceptibility testing standard M27-A3.

**M29-A3** **Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline—Second Edition (2005).** 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.



* Proposed-level documents are being advanced through the Clinical and Laboratory Standards Institute consensus process; therefore, readers should refer to the most current editions.

 

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