CLINICAL AND LABORATORY STANDARDS INSTITUTE®

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M56-A

Principles and Procedures for Detection of Anaerobes in Clinical Specimens; Approved Guideline

This document presents standardized, cost-effective, and efficient best practice processes for anaerobe bacteriology to assist clinical laboratories in selecting those methods that lead to improved patient care.

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

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Principles and Procedures for Detection of Anaerobes in Clinical Specimens; Approved Guideline

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Abstract

Clinical and Laboratory Standards Institute document M56-A—*Principles and Procedures for Detection of Anaerobes in Clinical Specimens; Approved Guideline* provides procedures for performing testing and providing accurate, reliable, and useful results to laboratories with differing levels of expertise in anaerobe bacteriology. Preexamination requirements for specimen selection, collection, transport, timely processing, and examination procedures are discussed. Rapid and complex methods are compared for their ability to provide definitive identifications. Because the delivery of preliminary reports is vital to patient care when complex final reports are delayed, interpretations of direct smears and culture results are presented to help laboratorians confidently issue preliminary reports. Descriptions of anaerobes involved in human disease and a discussion of diagnostic methods for *Clostridium difficile* disease are presented. Guidelines for establishing competency testing to laboratories at their various levels of expertise and complexity are included. Because failures in good practices for preexamination, examination, and postexamination techniques can put patients at risk, a discussion of risk assessment during the design and implementation of anaerobe bacteriology protocols is included.

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Foreword

This document provides information on timely, standardized, and cost-effective methods that constitute best practices for anaerobe bacteriology and has been created to assist clinical laboratories in the selection and use of high-quality, efficient methods to improve patient care.

Anaerobes can be involved in infections of the head and neck, central nervous system, pleuropulmonary sites, intra-abdominal sites, female genital tract, blood, skin, soft tissues, and bones. These infections are known to contribute significantly to patient morbidity and mortality and adequate therapy plays an important role in the outcome. Previously, sites of anaerobe infection and the anaerobic microorganism's antimicrobial susceptibilities were thought to be predictable, but this is no longer true. Clinicians are seeing more anaerobe infections in immunocompromised patients and in those patients with complex diseases. Anaerobic isolates that are resistant to antimicrobial agents are encountered more frequently. This changing clinical picture requires an adequate laboratory response.

Anaerobe bacteriology can be challenging. The microorganisms are sensitive to oxygen exposure; thus requiring specialized supplies, equipment, and methods. They are part of the normal human flora often causing infections by penetration of the deeper tissues when physical damage to tissue or structure has occurred. Therefore, the diagnostic process must differentiate between the infecting and noninvolved microorganisms. The isolation and ID of those specific anaerobes involved in infections depend on appropriate methods beginning with specimen selection, collection, transportation, and, finally, ID determinations. New technical microbiology tools now allow IDs to be reported rapidly and to be more extensive and precise. Additional training and collaboration with clinicians will be required to interpret the extensive reports but this can improve patients' outcomes, which is the ultimate goal of testing, diagnosis, and treatment.

Key Words

Anaerobe identification, anaerobe taxonomy, anaerobes, anaerobic culture, antimicrobial susceptibility testing, *Clostridium difficile* detection

Principles and Procedures for Detection of Anaerobes in Clinical Specimens; Approved Guideline

1 Scope

This document provides guidance for preexamination, examination, and postexamination procedures associated with the culture of anaerobic bacteria. Because anaerobic bacteria are part of human normal flora and are sensitive to oxygen exposure, good preexamination methods are essential. These recommendations include methods for collecting proper specimens from appropriate clinical sites and for transport procedures that protect anaerobes from oxygen exposure so that all pathogens involved in infections can be detected. The optimal methods needed to provide accurate, timely, and sufficient information for appropriate medical decisions are included, along with a discussion of the use and value of partial and full isolate IDs. Also included in this guideline are recommendations for interpreting results, assistance in understanding the value of rapid preliminary results, and guidance on issues of QC, QA, and competency.

The intended audience includes medical technologists, infectious disease physicians, microbiology laboratory directors, pathologists, and researchers.

Because anaerobe antimicrobial susceptibility testing (AST) methods are presented in CLSI documents M11[1] and M100,[2] this document limits its discussion to the need and indications for AST.

2 Introduction

Institutions allocate varying resources to their laboratories for the development of the expertise and processes needed to work with anaerobic bacteria. The goal of this document is to present procedures that result in accurate and timely anaerobe bacteriology reports at different categories of expertise (refer to Table 1). Users of each laboratory should be aware of the level of expertise and extent of testing provided by that laboratory.

When an institution determines the extent of anaerobe bacteriology that will be provided, it is performing a risk assessment. An extreme risk for potential patient harm is introduced when a hospital decides not to provide resources for detection of anaerobes because of a perception that its patients do not have anaerobe infections or that those types of infections are not important. Other laboratories limit IDs of polymicrobial infections to less than four isolates. Some laboratories even recommend limited workup of specimens such as abdominal abscesses. This recommendation is based on the belief that empiric therapy does not require culture results; but it overlooks the fact that all organisms grown from an abscess were involved in its formation and that some may be antibiotic resistant. Because many anaerobic infections are polymicrobic and the bacterial constituents may act synergistically, ID of all organisms present may provide both prognostic and therapeutic guidance.

Newer techniques may allow the rapid ID of many microorganisms. If a decision is made to conserve resources by limiting the number of isolates identified, each case must be communicated to the clinician so that the risk of incomplete but potentially available information is known, and additional studies can be done if clinically indicated. Without this collaboration between the laboratory and clinician, the risks of arbitrary censure of the data may pose an unacceptable risk. The additional information provided by new technical tools such as 16s ribosomal RNA (rRNA) gene sequencing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) adds valuable clinical insight regarding the disease process and role of the pathogens. Although it is tempting to restrict the use of these new advances in laboratory medicine because of supposed users' unfamiliarity with new taxonomy

or financial costs, withholding or delaying reports from the clinician on the grounds that the report is not perceived by the laboratory to be clinically important can add to patient risk.

Risk for patients can also be introduced by a lack of rigor in specimen collection and transport or by a laboratory's failure to understand and use basic concepts of anaerobe bacteriology. Every delay and inaccuracy introduces potential harm. Every appropriate decision on specimen collection, transport, acceptance for processing, method of processing, and extent of testing can minimize risk and improve patient care.

Table 1. Categories of Anaerobe Bacteriology Expertise

Category	Extent of Expertise
1	Laboratory accepts specimens for anaerobic culture, but only detects the presence of anaerobes. Anaerobes are referred to another laboratory for ID and AST.
2	Laboratory screens for the major anaerobic groups. Definitive ID and AST are performed in a reference laboratory.
3	Laboratory can identify anaerobes to genus and species level using phenotypic and enzymatic tests. It may perform some AST.
4	Laboratory provides final definitive IDs using 16s rRNA gene sequencing or MALDI-TOF MS technology and may perform quantitative AST.

Abbreviations: AST, antimicrobial susceptibility testing; ID, identification; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; rRNA, ribosomal ribonucleic acid.

3 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 blood-borne pathogens. The Centers for Disease Control and Prevention (CDC) address this topic in published guidelines that address the daily operations of diagnostic medicine in human and animal medicine while encouraging a culture of safety in the laboratory.[3] 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.[4]

4 Terminology

4.1 A Note on Terminology

CLSI, as a global leader in standardization, is firmly committed to achieving global harmonization wherever possible. Harmonization is a process of recognizing, understanding, and explaining differences while taking steps to achieve worldwide uniformity. CLSI recognizes that medical conventions in the global metrological community have evolved differently in the United States, Europe, and elsewhere; that these differences are reflected in CLSI, International Organization for Standardization (ISO), and European Committee for Standardization (CEN) documents; and that legally required use of terms, regional usage, and different consensus timelines are all important considerations in the harmonization process. In light of this, CLSI's consensus process for development and revision of standards and guidelines focuses on harmonization of terms to facilitate the global application of standards and guidelines.

In order to align the usage of terminology in this document with that of ISO and CLSI document QMS01,[5] the terms *preexamination*, *examination*, and *postexamination* have replaced *preanalytical*,

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 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 as follows:

Organization	Personnel	Process Management	Nonconforming Event Management
Customer Focus	Purchasing and Inventory	Documents and Records	Assessments
Facilities and Safety	Equipment	Information Management	Continual Improvement

M56-A addresses the QSE 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.

Organization	Customer Focus	Facilities and Safety	Personnel	Purchasing and Inventory	Equipment	Process Management	Documents and Records	Information Management	Nonconforming Event Management	Assessments	Continual Improvement
		M29				X EP05 EP06 EP09 EP18 EP23 EP29 M02 M11 M35 M40 MM09 MM18			EP18	EP18	EP18
MM19 QMS01	MM19 QMS01	MM19 QMS01	MM19 QMS01 QMS03	MM19 QMS01	MM19 QMS01	MM19 QMS01	MM19 QMS01	MM19 QMS01	MM19 QMS01	MM19 QMS01	MM19 QMS01

Path of Workflow

A path of workflow is the description of the necessary processes to deliver the particular product or service that the organization or entity provides. A laboratory path of workflow consists of the sequential processes: preexamination, examination, and postexamination and their respective sequential subprocesses. All laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

M56-A 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	X	X	X	X	X	X	
				EP23	EP23	EP23		
				M02	M02	M02	M02	
				M11	M11	M11	M11	
				M35	M35	M35	M35	
		M40						
					M100	M100	M100	
	MM09	MM09	MM09	MM09	MM09	MM09	MM09	MM09
	MM19	MM19	MM19	MM19	MM19	MM19		
QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01	QMS01

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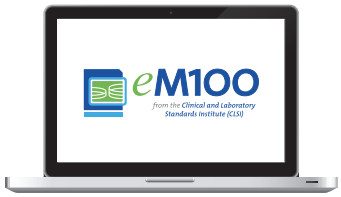

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