CLINICAL AND LABORATORY STANDARDS INSTITUTE®

2nd Edition

M47

Principles and Procedures for Blood Cultures

This guideline includes recommendations for collecting, transporting, and processing specimens for blood culture, as well as procedures for recovering pathogens from the blood of patients with suspected bacteremia or fungemia.

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

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Principles and Procedures for Blood Cultures

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Abstract

Clinical and Laboratory Standards Institute guideline M47—*Principles and Procedures for Blood Cultures* provides recommendations for laboratory detection of bacteremia and fungemia using blood cultures. This guideline also includes recommendations for collecting, transporting, and processing specimens for blood culture and for interpreting and reporting results. Critical factors in the recovery of pathogens from blood specimens and related topics are also covered.

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Contents

Contents (Continued)

Foreword

Because the morbidity and mortality rates attributable to sepsis are high, the prompt and accurate detection of bacteremia and fungemia is important to improving patient care. The laboratory test used to detect the presence of bacteria (bacteremia), mycobacteria (mycobacteremia), or fungi (fungemia) in the blood is the blood culture.[1] In the past 40 years, several studies[2-10] have been conducted to:

- Define the clinical significance of blood cultures.
- Identify the indications for blood cultures.
- Define the critical factors in the recovery of pathogens from blood.
- Identify the best medium formulations and establish best practices for facilities that perform blood cultures.
- Develop interpretive criteria to evaluate the significance of positive blood cultures.
- Evaluate and compare commercial blood culture systems.

The selection and use of optimal blood specimen collection, transport, and processing procedures is critical to ensuring accurate and timely results reporting. Since this guideline was first published in 2007, research and development in the preexamination, examination, and postexamination phases of blood cultures have advanced. In addition, changes in blood culture technology have resulted in new methods for recovering and identifying pathogens from blood cultures and for detecting antimicrobial resistance markers. Because the field continues to evolve, this guideline incorporates recommended practices for collecting, transporting, processing, testing, and storing blood culture specimens. This guideline is also intended to help control health care costs, because the costs attributable to the recovery and identification of pathogens from blood cultures are high.

Overview of Changes

This guideline replaces the previous edition of the approved guideline, M47-A, published in 2007. Several changes were made in this edition, including:

- Describing critical factors in recovering pathogens from blood specimens and providing guidance on selecting the best medium formulations, developing and applying breakpoints, and reporting results
- Describing existing technologies capable of identifying the etiology of bloodstream infections (BSIs) and the benefits of using these technologies (including culture-independent methods)
- Adding subchapters on special topics, including pediatric blood cultures, catheter-related BSIs, infective endocarditis, patients receiving antimicrobial therapy, and rare and fastidious pathogens
- Including example quality assurance indicators for preexamination, examination, and postexamination activities
- Removing descriptions of paper-based test ordering and results reporting, because the widespread use of electronic medical record systems has changed the way blood cultures are ordered and results are reported

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

Bacteremia
Bacteria
Blood culture
Bloodstream infection
Fungemia
Fungi
Mycobacteria
Sepsis

Chapter 1

Introduction

Principles and Procedures for Blood Cultures

1 Introduction

1.1 Scope

This guideline is intended to provide recommendations to clinical microbiologists, other laboratorians (eg, pathologists, supervisors and/or managers, phlebotomists), and health care providers (HCPs) for recovering pathogens from the blood of patients with suspected bacteremia, mycobacteremia, or fungemia. It is also intended for administrators who develop institutional best practices. Specific recommendations for collecting, transporting, and processing blood culture specimens are also included.

This guideline discusses the clinical significance of blood cultures, critical factors in recovering pathogens from blood specimens, selection of medium formulations and other laboratory practices, and development of interpretive criteria. It also discusses existing blood culture technologies and the relative benefits of these technologies. Special topics, including pediatric blood cultures; catheter-related bloodstream infections (CRBSIs); infective endocarditis; diagnostic testing for patients who are receiving antimicrobial therapy; rare and fastidious pathogens; and rapid diagnostic techniques, including culture-independent methods, are also covered.

This guideline does not include procedures for pathogen ID and antimicrobial susceptibility testing (AST). For guidance on bacterial AST, refer to CLSI documents M02,[11] M07,[12] M11,[13] M45,[14] and M100.[15] For guidance on fungal AST, refer to CLSI documents M27,[16] M38,[17] M44,[18] M51,[19] M60,[20] and M61.[21]

1.2 Background

The presence of living microorganisms in a patient's bloodstream has diagnostic and prognostic importance.[2] Therefore, when performed properly and in the appropriate clinical setting, blood cultures provide substantial value. Positive blood culture results either establish or confirm an infectious etiology of a patient's illness. Moreover, they also provide the etiological agent for AST, which, in turn, optimizes antimicrobial therapy.

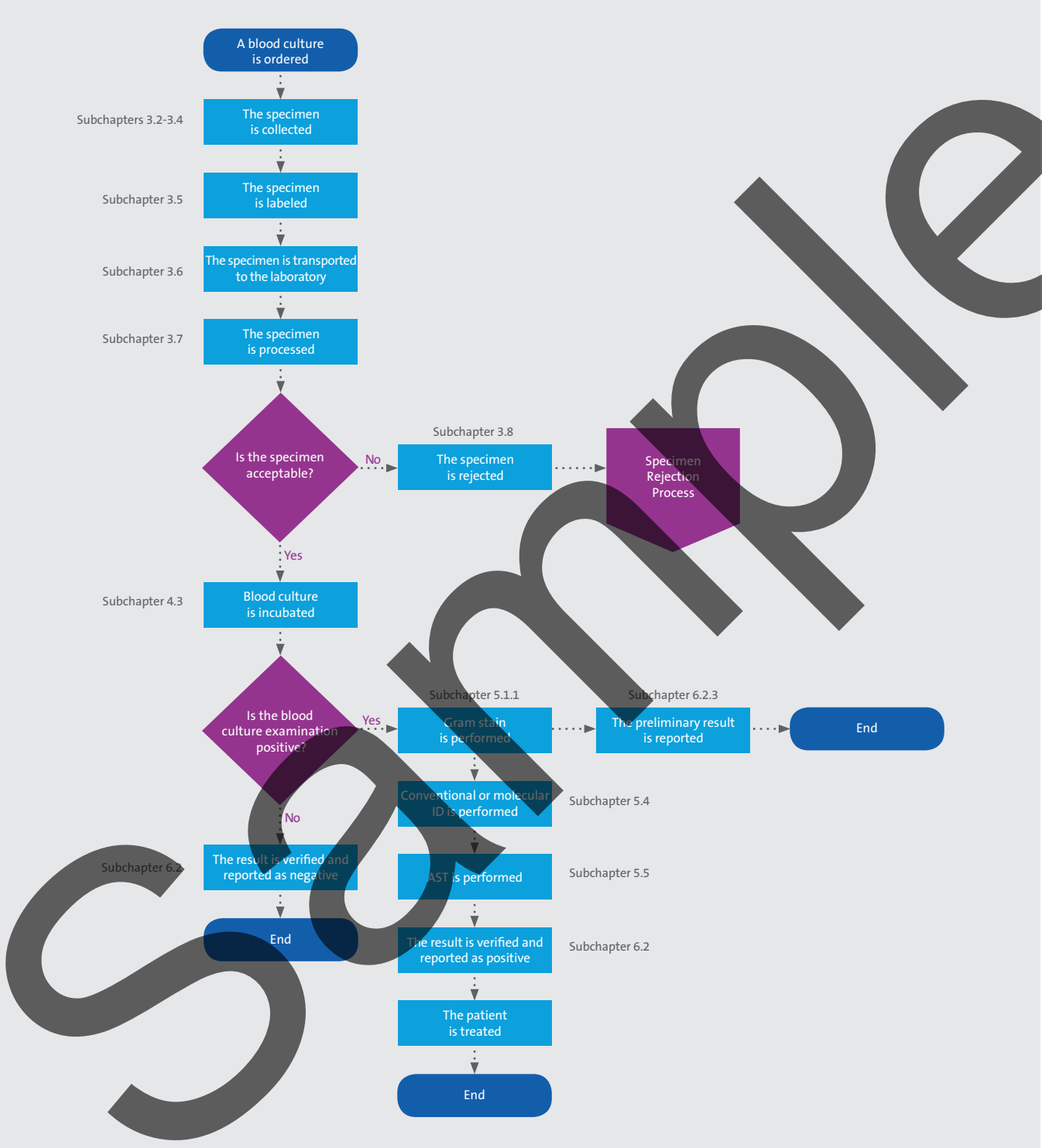
From a prognostic standpoint, a blood culture that grows a clinically important pathogen indicates failure of the patient's immune system to contain the infection at its primary location or failure of the patient's HCP to remove, drain, or otherwise eradicate the cause of an infectious disease. The type of pathogen recovered from the patient's blood also provides important prognostic information.[2]

1.3 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 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.[22] 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.[23]

2 Blood Culture Path of Workflow

Figure 1 outlines the blood culture process.



Abbreviations: AST, antimicrobial susceptibility testing; ID, identification.
[a] Five basic symbols are used in this process flow chart: oval (signifies the beginning or end of a process), arrow (connects process activities), box (designates process activities), diamond (includes a question with alternative "Yes" and "No" responses), pentagon (signifies another process).

Figure 1. Process for Blood Cultures[a]

7 Recovery of Rare and Fastidious Pathogens

Rare and fastidious microorganisms are not frequently recovered from blood. However, when encountered, they may represent severe infection. The recovery of a member of the HACEK group from blood is a major criterion for diagnosis of infective endocarditis.[214] Although recovery of these organisms used to require special procedures,[143,215] current automated technology enables fastidious isolates (eg, HACEK group) to be reliably detected within the same time frame as routine blood cultures.[216,217] Most blood cultures yielding nonfastidious pathogens become signal positive in the first 24 to 36 hours of incubation. In contrast, fastidious organisms may require up to five days of incubation before the blood culture becomes signal positive. Organisms such as *Legionella, Bartonella*, and *Mycoplasma* are optimally identified through immunodiagnostic or molecular techniques. The same is true of organisms such as *Coxiella, Chlamydia, Rickettsia*, and *Tropheryma*, which are uniformly uncultivable with usual bacterial culture systems.[218,219] Except for *Bartonella* spp., most rare or fastidious bacteria can still be cultivated by traditional bacterial culture systems and recovered through the blood culture protocols used by most medical laboratories.

Although numerous studies on blood culture methods for recovery and ID of rare and fastidious pathogens have been published,[220] the standard five-day incubation period recommended for CMBCS enables detection of most bacteria, even *Abiotrophia* and *Granulicatella* spp. and some strains of *Francisella* spp. Moreover, methods such as MALDI-TOF MS enable more rapid ID than did traditional phenotypic testing. However, the spectrum of pathogens identifiable through current molecular methods (especially DBTs) is limited.

Highly fastidious bacteria grown in blood cultures may produce a positive signal yet not be observed on Gram stain. Gram staining a positive blood culture may not reveal the bacteria for numerous reasons. Most commonly, atypically shaped or unusually small bacteria may not be visible on Gram stain. For example, *Abiotrophia* spp. may produce bizarre shapes or sizes, while *Francisella* spp. may be so small and indistinct that the organisms blend in with background on the slide. Some bacteria (eg, *Mycoplasma* spp.) may be difficult to visualize on Gram stain. In these cases, an acridine orange stain may reveal the bacteria.[219] For example, to visualize *Campylobacter, Helicobacter,* or *Brucella*, the laboratorian may need to perform either an acridine orange stain or Gram stain, using carbol fuchsin as the counterstain.

7.1 *Abiotrophia* and *Granulicatella*

Members of *Abiotrophia* and *Granulicatella* can cause clinically significant bacteremia and endocarditis, as well as other invasive infections. Recent studies suggest that 1% to 5% of endocarditis cases can be attributed to *Abiotrophia defectiva* or to *Granulicatella* spp.[221,222] Despite their fastidious nature, members of both genera can be reliably detected with CMBCS. Either media used with CMBCS already contain vitamin B6 and cysteine, or the human blood added to the culture medium provides them. The standard incubation time for other organisms is sufficient for growth. Extended incubation is not necessary.

Growth of *A. defectiva* or *Granulicatella* will not be observed when blood specimens are inoculated onto blood agar–based medium. Supplemented media, such as chocolate agar or some anaerobic agars, are needed to recover these organisms. Alternatively, suspected *A. defectiva* or *Granulicatella* may be cocultivated with staphylococci and the culture assessed for satellite colony formation.[223,224]

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