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Methods for Determining Bactericidal Activity of Antimicrobial Agents; Approved Guideline

This document provides procedures for determining the lethal activity of antimicrobial agents.

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Methods for Determining Bactericidal Activity of Antimicrobial Agents; Approved Guideline

Abstract

Established laboratory methods that can assess the bactericidal activity of an antimicrobial agent are needed, both because of the increase in the number of patients who do not have completely normal host immune defenses and because of the new classes of antimicrobial agents that have been introduced. Clinical cure depends largely upon host factors. Bactericidal tests can provide a rough prediction of bacterial eradication. It should be noted, however, that other factors (e.g., postantibiotic effect and the growth-inhibitory effects of sub-MIC concentrations of antibiotics) may also impact bacteriologic response of patients. The special susceptibility tests that assess lethal activity are not routinely applied to all microorganisms, but are applied in unusual situations; e.g., endocarditis. Uniform test procedures are thus needed to permit comparison of different datasets.

The methods for bactericidal testing are now evolving, but more work is needed with the methodological aspects and clinical correlations. The techniques described in this document are intended primarily for testing aerobic bacteria that grow after incubation in adjusted Mueller-Hinton broth or adjusted Mueller-Hinton broth supplemented with human serum or an ultrafiltrate thereof.

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Foreword

All of the susceptibility test methods commonly performed by clinical microbiology laboratories (e.g., disk diffusion, broth dilution, and agar dilution) measure the inhibitory activity (MIC) of an antimicrobial agent.[1,2,3] In most clinical situations, this is sufficient as the role of the antibiotic is to prevent the spread of bacteria from the focus of infection by preventing microbial replication at new sites; the active participation of the host's defense mechanisms finally achieves bacterial eradication and clinical cure.[4] Antimicrobial assays can provide additional valuable information on the pharmacokinetics of the agent(s) being used and, when combined with the MICs, can allow bacterial eradication to be predicted.

On occasion, it may be necessary to achieve bactericidal activity with an antimicrobial agent. This need has been well documented for endocarditis[5] and has been suggested by some for meningitis,[6] for osteomyelitis,[7] as well as for infections in immunocompromised patients.[8] The clinical occurrence of tolerance[9] may on rare occasion necessitate bactericidal testing.

When assessment of bactericidal activity is deemed appropriate, an *in vitro* test method such as the MBC determination or the use of time-kill kinetic methodology may be useful. Bactericidal activity against the patient's isolate by the antibiotic tested allows eradication to be predicted based upon the usual dosing of this antibiotic or based upon the results of an antimicrobial assay. When clinical experience is lacking and assay methods are not readily available, the serum bactericidal test which integrates both pharmacodynamic and pharmacokinetic properties may be more useful. Depending on certain modifications to the serum bactericidal test, the test can provide a quantitative assessment of bactericidal activity relative to the MBC (the serum bactericidal titer), a dynamic assessment of rapidity of killing over time (the serum bactericidal rate), or both the magnitude of serum bactericidal activity and its duration (the area-under-the-bactericidal-titer-curve). In addition, methods using serum from persons (e.g., volunteers) receiving antibiotics (*ex vivo*) can be used to assess antimicrobial bactericidal activity across drug classes or between members of a class against a wide variety of microorganisms.

Because of the complexity involved with the serum bactericidal test (including the particular method used, the proper collection of timed serum specimens, and the interpretation of results), and the lack of clinical data clearly documenting the usefulness of this test for most infections, it is recommended that consultation with the microbiology laboratory be obtained as a prerequisite for this test. The assistance of the laboratory's director is useful in (1) determining if such a test is needed; (2) selecting NCCLS recommended methodology for testing; and (3) interpreting the results. Techniques for the conduct of the serum bactericidal test may be found in the most current edition of NCCLS document M21—*Methodology for the Serum Bactericidal Test*.

This document describes the details of bactericidal testing and, in particular, the effects of variations in methodology. This information has been obtained largely from published data. Use of these guidelines should result in uniform methodology for bactericidal testing that is sufficiently practical for use in the clinical microbiology laboratory. The methodology described in this document does not imply *per se* that bactericidal testing is clinically relevant, but instead allows such testing to be used as a tool to assess clinical relevance. The techniques described in this document are intended primarily for testing aerobic bacteria that grow well after overnight incubation in either Mueller-Hinton broth or in Mueller-Hinton broth supplemented with human serum as described in Section 2.2.1. Modifications for more fastidious microorganisms such as anaerobes will be described in detail in the future.

Standard Precautions

Because it is often impossible to know what might be infectious, all human blood specimens are to be treated as infectious and handled according to "standard precautions." Standard precautions are new guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of any pathogen and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard precaution and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (Guideline for Isolation Precautions in Hospitals, Infection Control and Hospital Epidemiology, CDC, Vol 17;1:53-80.), [MMWR 1987;36(suppl 2S):2S-18S] and (MMWR 1988;37:377-382, 387-388). For specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure, refer to NCCLS document M29—*Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue*.

Key Words

Bactericidal activity, minimal bactericidal concentration (MBC), minimal lethal concentration, serum bactericidal concentration, time-kill determination

Methods for Determining Bactericidal Activity of Antimicrobial Agents; Approved Guideline

1 Introduction

1.1 Rationale

An increasing number of patients with infections do not have completely normal host immune defenses. In addition, classes of antibiotics that have been considered to be bactericidal can no longer be assumed to kill every clinical isolate due to the possibility of tolerance.[9] Finally, as new classes of antimicrobial agents are introduced, there is a need for established laboratory methods that can assess the bactericidal activity of these agents. Such methods for assessing lethal activity should be considered special susceptibility tests because they are not routinely applied to all microorganisms; rather, they are applied in unusual situations. Because of their specialized nature, complexity, and potential difficulty for interpretation, tests for bactericidal activity should be done in the context of consultation with appropriate persons (such as the microbiology laboratory director) who are aware of the potential problems involved in such testing.

1.2 Methods

The killing effect of an antimicrobial agent on a microorganism can be assessed in several ways:

(1) Lethal activity may be expressed as the rate of killing by a fixed concentration of drug under controlled conditions. This rate is determined by measuring the number of viable bacteria at various time intervals. The resulting graphic depiction is known as the time-kill curve. Bacterial killing rates are, in part, dependent on the class of antibiotic and the concentration of this agent. With certain classes of antibiotics (e.g., aminoglycosides and fluoroquino-lones), the rate of killing increases with increased drug concentrations up to a point of maximum effect.[10] This is termed *concentration-dependent bactericidal activity*. In contrast, the killing rates of β-lactam agents and vancomycin are relatively slow and continue only as long as the concentrations are in excess of the MIC.[10,11,12] This rate of killing is termed *time-dependent bactericidal activity.*

(2) The minimal concentration of drug needed to kill most (≥99.9%) of the viable organisms after incubation for a fixed length of time (generally 24 hours) under a given set of conditions is the most common estimation of bactericidal activity and is known as either the minimal bactericidal concentration (MBC) or the minimal lethal concentration (MLC). It will be referred to in this document as the MBC. Unfortunately, the definition of the MBC (99.9% killing of the final inoculum) is somewhat arbitrary and separates the bacteria into two populations – a segrega-tion which might not have biological relevance.[13] The determination of the MBC, moreover, is so subject to methodologic variables that the clinical relevance of MBCs is nearly impossible to assess, particularly for certain pathogen and drug combinations (e.g., staphylococci and beta-lactam agents).[14]

(3) The serum of a patient receiving an antibiotic may be tested against the infecting microorganism. This can be done using time-kill curve methodology (i.e., serum bactericidal rate) or using dilution methodology (i.e., serum bactericidal titer). The principles of these methods as well as the influence of biological and technical factors are similar.

1.3 Problems

Evaluating *in vitro* lethal effects of an antimicrobial agent is conceptually attractive and appears, at times, to be clinically necessary. However, many biological and technical factors are known to interfere with such *in vitro* measurement of killing.

The biological factors include:

- Persisters
- Paradoxical effect
- Tolerance