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Standard Guide for Determination of a Survival Curve for Antimicrobial Agents Against Selected Microorganisms and Calculation of a D-Value and Concentration Coefficient[1]

This standard is issued under the fixed designation E1891; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

INTRODUCTION

A variety of testing procedures have been devised almost from the beginning of disinfection and antisepsis as disciplines. From the first, there was recognition of the importance of time and rates of kill. After many decades and numerous test procedures involving carriers, the approach of establishing a death rate curve (often described as a survivor curve) is reclaiming its importance in establishing the basic kinetics of the killing process after exposure to antimicrobial chemicals.

D-values (historically, log death time or decimal reduction time), kill or survivor curves, processing calculations and rates of kill are discussed in many texts. There is extensive theoretical discussion but little applied material on how to perform testing to establish kill curves and D-values and associated calculations.

The guideline form has been selected to permit the inclusion of background information and a model procedure for determining D-values and their calculation. A related function, the concentration coefficient (η) can be calculated from a series of D-values calculated for different concentrations of the test antimicrobial and defines the loss of activity as the material is diluted. This information has value for application in disinfectants because many are sold to be diluted in use.

Specific procedural details are presented in descriptions of methods routinely used to establish a kill curve. The user should establish a protocol for the process that best fits their needs.

An experimental kill curve provides data for a calculated D-value derived from test data used to construct the kill curve.

BACKGROUND

Scientists concerned about antimicrobial testing have debated the value of suspension tests in contrast to tests using simulant carriers with dried microorganisms. U.S. regulation has been committed to carrier tests, while Europeans have emphasized suspension tests combined with practical applied tests using materials as carriers on which the disinfectant actually will be used.

The examination of the kinetics of kill for various disinfectants provides basic information on the activity of antimicrobials. The early history of microbiology reveals a strong momentum directed toward clarification of these reactions. From the earliest years of microbiology, the ideas of rate-of-kill and killing reactions as first order reactions (from chemical kinetics) have been involved in the estimation of antimicrobial activity.

Kronig and Paul (1897) were the early pioneers who developed the concept of bacterial destruction as a process. They used anthrax spores dried on garnet crystals and assessed the survivors by plating washings from the garments after treatment with disinfectants. Chick (1908) found that the number of survivors after disinfectant exposure, when plotted against time of treatment, produced a straight line that showed similarity to chemical, equimolecular reactions. Distortions in the expected straight-line reactions were noted by Chick as well as in subsequent investigations. Over the years, the most common type of deviation from the expected, straight-line survivor curve is a sigmodial one displaying a shoulder, a lag or delay in logarithmic kill, and ending in distinct tailing, sometimes indicating a resistant population.

There has been a variety of procedures advanced for accumulating data that can be used to calculate D-values and construct survivor curves.

Esty and Meyer (1922) introduced the terminology we currently use in relation to bacterial kill

whether for spores or vegetative bacterial cells in devising thermal processing to eliminate *Clostidium botulinium* in the canning industry. They also devised end-point analysis for interpretation of the results of heat exposure and for processing calculations. Their procedure involved sampling multiple tubes or other containers of product and analysis of the number remaining positive to determine the number of survivors by Most Probable Number (MPN) analysis using the pattern of positive and negative tubes. (**1**)[2] This analysis is done after an exposure period when there are fewer bacterial cells or spores in the container and positive and negative tubes can be expected on recovery.

Single-sample subculturing of aliquot samples from a reaction vessel containing the test organism and the test antimicrobial has been the basic means for establishing survival curves. Usually a suspension of target microorganisms is exposed to a disinfectant\sterilant and aliquots are withdrawn at specific time intervals and assessed for survivors, usually with plate counts. Because of tailing problems and difficulty in enumerating small numbers, when only a few survivors are left, MPN methods of enumeration are recommended and often used (**1, 2, 3**). A common method derived from thermal processing in the canning industry is the end-point method, described above, in which the number of positive and negative tubes from replicate sampling (such as tubes or cans) is used alone or in the combination with single sampling to construct a survivor curve and plotted to determine D-values. (**4**)

Many antimicrobial formulations available for test are diluted in use. When D-values are determined and calculated at more than one concentration (dilution) of an antimicrobial, the concentration coefficient, designated as the Greek letter eta or η, denotes the effect of dilution on the activity of a chemical or formulation.

[1] This guide is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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[2] The boldface numbers given in parentheses refer to a list of references at the end of the text.

1. Scope*

1.1 This guide covers the methods for determining the death rate kinetics expressed as D-values. These values can be derived from the construction of a kill curve (or survivor curve) or by using other procedures for determining the number of survivors after exposure to antimicrobial chemicals or formulations. Options for calculations will be presented as well as the method for calculation of a concentration coefficient.

1.1.1 The test methods are designed to evaluate antimicrobial agents in formulations to define a survivor curve and to subsequently calculate a D-value. The tests are designed to produce data and calculate values that provide basic information of the rate-of-kill of antimicrobial formulations tested against single, selected microorganisms. In addition, calculated D-values from survivor curves from exposure at different dilutions of antimicrobial can be used to show the effect of dilution by calculation of the concentration exponent, η (**2**). D-value determination assumes the ideal of first-order killing reactions that are reflected in a straight-line reduction in count where a count-versus-time plot is done. The goal here is not to determine the time at which no survivors are found, but to determine a standard value that can be used in processing and exposure determinations or used to estimate dilutions.

1.1.2 As an example of potential use of kill curve data, the published FDA, OTC Tentative Final Monograph for Health-Care Antiseptic Drug Products, Proposed Rule, June 17, 1994 has suggested the testing of topically applied antimicrobial products using survival curve (or kill curve) calculations. The methods described in this guide are applicable to these products, but adjustments such as the use of antifoaming agents when the reaction mixture is stirred may be necessary to counteract the presence of detergents in many formulations. Frequently the sampling for these tests is done after very short intervals of exposure to the formulation, such as 30 and 60 s. This methodology also has been applied to preservative testing of antimicrobial ingredients in more complex cosmetic formulations (**5**).

1.2 The test methods discussed should be performed only by those trained in microbiological techniques.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*