Disinfection, Sterilization, and Preservation

2nd Edition

Edited by

Seymour S. Block

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Preface

The editors of the first edition were gratified by the wide acceptance of this book, which went through two printings. However, since its publication in 1968 the important field of microbial control has sustained rapid progress, which has led to the publication of this up-dated, revised, and enlarged edition. New subjects which have been introduced in this edition include disinfection and asepsis in dentistry, design of facilities for the control of microbial agents, the business and marketing of antimicrobial chemicals, the sanitary treatment of hospital wastes, and amphoteric surfactants used as disinfectants. It is hoped that these additions will give this already extensive book even broader scope and make it of value to a greater range of users.

This book should be useful to practitioners, research workers, teachers, and students; to those in industry, government, and universities; to public health specialists, hospital personnel, food processors, bacteriologists, plant pathologists, physicians, dentists, chemists, biologists, veterinarians, and engineers; and to professionals in many industries such as the production of pharmaceuticals, cosmetics, and paper. The broad range of subjects in this volume will enable the reader to draw on knowledge in related fields that may help him solve his own problems. In these times of increasing specialization it is hoped that this cross fertilization will aid in broadening and integrating the knowledge acquired by so many outstanding investigators.

We are fortunate in this second edition to have the contributions of many of the authors whose chapters led to the success of the first edition, as well as a number of new authors whose names and reputations are well known in this field of endeavor. We are deeply saddened, however, by the death, since the last edition, of several of our associates and friends who joined us in that work. Among them are Dr. L. S. Stuart, Dr. L. F. Ortenzio, and my fellow editor and esteemed colleague, Dr. Carl A. Lawrence. Dr. Lawrence and I planned the first edition together in his lovely hillside home in California and we worked harmoniously on that project for four years. He was a foremost scientist and teacher who will be missed by those who knew him.

I wish to express my appreciation to the authors for their cordial cooperation in the preparation of this edition and am also grateful to the staff of Lea & Febiger for their encouragement and ever-willing assistance during our long association.

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Part I
Methods of Testing

1

Introduction to Antimicrobial Testing Methods

WILLIAM G. ROESSLER, PH.D.

Many techniques have been employed in the laboratory by microbiologists to study the effects of physical agents and chemicals on fungi, bacteria and viruses since their role in the initiation of infection and disease was determined in the latter part of the nineteenth century by Semmelweiss, Pasteur, Koch and Lister. Progress toward improving techniques and developing new methods for testing disinfectants and antiseptics has been slow but steady since the publication of the first edition of this book. For the most part, the greatest interest in the field has been shown by investigators in industrial and government laboratories who are concerned with the requirements of federal statutes that regulate antimicrobial products, primarily the Federal Insecticide, Fungicide and Rodenticide Act (1947) and its amendment in 1972 (Public Law 92-516, the Federal Environmental Pesticide Control Act), and the Federal Food, Drug, and Cosmetic Act as amended in 1972.

Major contributions toward developing and improving testing methods have been made by investigators working through the programs of the Association of Official Analytical Chemists, the Chemical Specialties Manufacturers Association, The American Society for Testing and Materials, The American Association of Textile Chemists and Colorists, and the Society for Industrial Microbiology. The American Society for Testing and Materials established a new committee in 1973 to consider the testing of pesticides; a subcommittee was organized specifically to develop standard definitions, classifications, biological test methods and recommended practices related to efficacy of antibacterial and antiviral agents and devices. Other technical and professional societies, such as the American Society for Microbiology and the Society of Toxicology, also contribute to the development or improvement of testing methods through committee efforts and reports from individual investigators.

The Swiss Society of Microbiology Commission for Disinfection (Reber, 1974) cited the need for internationally accepted standard methods for evaluating disinfectants. Unfortunately, no set standardized procedure for the evaluation of antimicrobial products and devices is accepted internationally. This is partly because there exist between one country and another basic differences in concept, in definitions and in jurisdiction; it is also because no serious attempt has been made to bring about universal acceptance and standardization of methods. It is not inconceivable that this could be accomplished—or at least consid-

ered—through an international body such as the World Health Organization.

The Environmental Protection Agency administers the Federal Insecticide, Fungicide and Rodenticide Act and the Food and Drug Administration, Public Health Service of the Department of Health, Education and Welfare administers the Federal Food, Drug and Cosmetic Act. Certain definitions from these acts are pertinent to the testing of antimicrobial products and devices. The following definitions are taken from the former law:

Device. The term 'device' means any instrument or contrivance (other than a firearm) which is intended for trapping, destroying, repelling, or mitigating any pest or any other form of plant or animal life (other than man and other than bacteria, viruses, or other microorganisms on or in living man or other living animals), but not including equipment used for the application of pesticides when sold separately therefrom.

Fungus. The term 'fungus' means any nonchlorophyll-bearing thallophyte (that is, any non-chlorophyll-bearing plant of a lower order than mosses and liverworts), as for example, rust, smut, mildew, mold, yeast and bacteria, except those on or in living man or other animals and those on or in processed food, beverages, or pharmaceuticals.

Pest. The term 'pest' means (1) any insect, rodent, nematode, fungus, weed, or (2) any other form of terrestrial or aquatic plant or animal life or virus, bacteria, or other microorganism (except viruses, bacteria, or other microorganisms on or in living man or other living animals) which the Administrator declares to be a pest.

The following definitions are from the latter law:

Device. The term 'device means instruments, apparatus, and contrivances, including their components, parts, and accessories, intended (1) for use in the diagnosis, oure, mitigation, treatment, or prevention of disease in man or other animals, or (2) to affect the structure or any function of the body of man or other animals.

Drug. The term 'drug' means (A) articles recognized in the official United States Pharmacopeia, official Homeopathic Pharmacopeia of the United States, or official National Formulary, or any supplement to any of them, and (B) articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals, and (C) articles (other than food) intended to affect the structure or any function of the body of man or other animals, and (D) articles intended for use as a component of any articles specified in clause (A), (B), or (C), but does not include devices or their components, parts, or accessories. The representation of a drug, in its labeling, as an antiseptic shall be considered to be a representation that it is a germicide, except in the case of a drug purporting to be, or represented as, an antiseptic for inhibitory use as a wet dressing, ointment, dusting powder, or such other use as involves prolonged contact with the body.

The two federal agencies that administer these laws have published a memorandum of agreement which considers products that are both pesticides and drugs. The agreement (1973) states (in part): "Submissions for approval will be to the agency having primary jurisdiction in the format required by that agency which will be considered acceptable by the other agency in lieu of that normally required. Where specific requirements of the two agencies conflict in matters such as manufacturing, formulation, and labeling, the requirements of the agency of primary jurisdiction will apply." The memorandum lists the various uses of products and identifies which agency has primary jurisdiction.

A wide variety of the disinfectant type of product is on the market. A partial list as given in the *Guidelines for Registering Pesticides in the United States* (1975) includes those recommended for use in households and non-medical institutions, hospitals and related institutions, barber shops and beauty parlors, funeral homes, mortuaries and morgues, restaurants, taverns, etc., farms, laundries, chemical toilets, toilet bowls and urinals, dairy, food and beverage plants, and bird cages and animal litters; it also includes those used for antimicrobial fumigants, air sanitizers, treatments for drinking water, swimming pools,

industrial cooling water systems, pulp and paper mill systems, preservatives for raw feeds, material preservatives, bacteriostats and self-sanitizers, paper coating, wet-end additives and adhesives, textile additives, and additives for sugar mills.

The Food and Drug Administration published in the Federal Register a report from the Over-The-Counter (OTC) Antimicrobial I Drug Review Panel (U.S. Department of Health Education and Welfare, 1974). This panel was charged with the review and evaluation of safety and effectiveness data on antimicrobial ingredients and combinations in topically applied products sold over the counter—in other words, sold without prescription. Product categories included in this report were skin antiseptics, patient preoperative skin preparations, surgical hand scrubs, health care personnel handwashes, skin wound cleansers, skin wound protectants and antimicrobial soaps. In addition to a review of most of the active ingredients usually incorporated into these preparations, the report provided guidelines for safety and efficacy testing of these antimicrobial products that come under the purview of the Food and Drug Administration.

Many antimicrobial products can be evaluated for effectiveness and safety by methods provided in subsequent chapters of this book; however, because of the lack of a standard or established method, the unique use or claim of the product, or the difficulty in devising a suitable laboratory test, an "in-use" test or *in situ* test often is devised to demonstrate effectiveness. An "in-use" test or *in situ* test is a test that reflects the proposed use of the product on a particular inanimate surface under actual conditions encountered or usually prevalent in attempting to "prevent, destroy, repel or mitigate," as the law states, "the pest"—in this instance, the microorganism. The test devised must not only reflect the proposed use but also the claims made for the product. Wide variation in the magnitude of naturally occurring environmental contaminants, random distribution patterns and highly variable natural decay rates depending on the specific infectious agent or problem microorganism and conditions in the particular environment involved multiply the complexities of designing an "in-use" or *in situ* test. Nevertheless, it must be agreed that such tests are an essential part of any comprehensive antimicrobial evaluation program. In the absence of statistically significant data, it is not possible to make adequate interpretations of results obtained by various *in vitro* laboratory methods. The volume of testing required in terms of the number of samples and the multiple procedures for evaluating each sample to provide a statistically significant result usually precludes the use of such methods in routine regulatory evaluations. Thus, it usually is desirable to submit the protocol for the "in-use" test to the regulatory agency prior to actually conducting the test to assure that the efficacy data obtained are suitable to support registration or acceptance.

Clinical effectiveness studies, comparable to the *in situ* testing of antimicrobial products used on inanimate surfaces, are required for some topical antimicrobial products. The FDA report referred to above contains recommendations on specific protocols, testing procedures and analysis of data.

Of the many *in vitro* laboratory methods presently employed in comparing germicidal chemicals and providing, in a presumptive manner at least, an index to the concentration of products which can be employed in disinfecting inanimate surfaces where infectious organisms are suspected of being present, the Phenol Coefficient Method A.O.A.C. (1970) has received the most attention from the standpoint of evaluations as to both precision and accuracy. From the standpoint of precision or reproducibility of results, variations ranging from ± 12% to ± 23% have been reported (Stuart, Ortenzio and Friedl,

1958; Klimeck and Umbreit, 1948). These results clearly show that the precision of the procedure is not nearly so good as many investigators believed.

The errors inherent in converting phenol coefficient numbers to effective dilutions of products for practical disinfection are so great that the procedure must be considered as unsatisfactory from this standpoint; this is particularly true for nonphenolic types of compounds. This deficiency has been pointed out by numerous investigators. The various factors responsible for lack of accuracy in this respect have been summarized by Stuart, Ortenzio and Friedl (1955). In spite of its shortcomings, the Phenol Coefficient Method provides data for some antimicrobial products that are acceptable to regulatory agencies.

The Phenol Coefficient Method is basically a dilution-tube technique, and it can be assumed that the coefficient of variation in all other dilution-tube techniques employed up to this time is as great, if not greater, than found with this particular procedure. For standardization in this method as to test species and strain, culture media, test culture maintenance routines, test culture exposure manipulations, subculture routines, temperature for test organism exposure and subculture incubations have all been given meticulous attention. Even so, it can be anticipated that dilution-tube techniques will continue to be the primary methods of choice for most investigators desiring to compare chemical preparations for germicidal, fungicidal and virucidal activities. Such techniques are rapid, convenient and provide useful relative information. In the absence of confirmative data by other laboratory methods or extensive correlative *in situ* investigations, the result is unreliable and often misleading as an index to practical disinfecting value in any of the many applications for which different chemicals and chemical formulations are commonly recommended. It has been determined that the amount of testing in methods of this type and in other types of controlled laboratory procedures necessary to provide a result within a 95% confidence limit is much greater than is commonly recognized. It is essential to know what is required to provide this level of confidence with each test method accepted for regulatory or production control programs. Unfortunately, practical considerations are such that most routines of this nature fall considerably below this requirement.

While negative results (failures) in the relatively low volume testing programs commonly employed may constitute certain evidence that a product will fail in practical applications, positive results in such tests do not constitute certain evidence of effectiveness in any specified use. This type of evidence can be obtained only by increasing the number of samples tested and the number of tests made on individual samples. Needed improvements in test design to reduce the coefficient of variation have not been made, however.

The terms *disinfection* and *sterilization* have been so badly abused in the technical, semitechnical and popular literature as well as in the advertising media that many insist on considering them as relative in nature. They are, in fact, absolute terms. To sterilize is to destroy all forms of life that may be present. To disinfect is to destroy or eliminate entirely the infectious agent or agents present. The available laboratory methods for determining whether these absolute results have been achieved fall considerably short of providing absolute certainty, but this is no justification for redefining either term.

Early recognition of selective germicidal activities with chemical compounds resulted in the development of a specific connotation for the term disinfection. A disinfectant is commonly selected according to the specific infectious agent known or suspected to be present. This being the case, definition of the frequently encountered term *general disinfectant* becomes awkward. It seems reasonable to suppose that any preparation so represented should be

capable of destroying or eliminating a fairly wide spectrum of infectious agents under most of the environmental situations in which they are likely to be encountered. It is not reasonable to accept a wide spectrum activity short of the ability to kill or eliminate completely any individual infectious agent as fulfilling such a representation.

Since the objective of any disinfectant process is the elimination of the total population of the infectious agent against which it is directed, it is obvious that any laboratory test method designed to determine the concentration of a chemical or the mode of application of any device which will accomplish such a result must be a total end-point method. That is, the end-point in the method must be based on a total kill of the population exposed if a direct measure of the objective is to be obtained. If, as is frequently the case, a single determination in the best technique of this type available does not provide with certainty a result which can be relied upon to show that a total kill will be obtained, then it is the responsibility of the investigator to conduct the number of replicates in the method required to provide the necessary degree of surety.

Results obtained in methods using an arbitrary percentage reduction of the exposed population as an end-point are not acceptable as evidence of sterilizing, germicidal or disinfecting activities. Arguments in favor of such procedures based on inherent limitations in laboratory test procedures and lack of absolute knowledge on the dynamics of the germicidal process are specious in the face of the critical nature of the practical objectives.

The science of bacteriology is concerned with the smallest living creatures, the shortest generation times, the greatest population densities and the most rapid natural population decay rates in the test tube, the infected animal and the environment. Growth and death rates in microbial populations cannot be adequately described in terms of percentage. They are, for the most part, exponential in character. The bacteriologist who ignores this to insist that a 99, a 99.9 or a 99.999% reduction in a given population will in the face of current technical limitations suffice as an index to a germicidal or disinfecting result simply denies his fundamental training to mislead himself and the general public.

For determining general sanitizing values where there are no specific infectious agents known to be present as targets, as in the case with general disinfecting, there is no quarrel with this procedure provided application of the percentage reduction figure is restricted to a defined set of laboratory circumstances known through adequate *in situ* correlation studies to provide a meaningful result. There must be a vigorous challenge to all attempts to apply this percentage reduction figure as a blanket index to effective practical disinfection; it cannot be acknowledged that a chemical proposed for treating a polluted water supply containing 100,000 cells of *Salmonella typhosa* per ml which reduces the number of these organisms to 100 measurable viable cells per ml, easily recovered and identified by standard methods of water analysis, is an effective disinfectant for water or provides safe drinking water. Neither can it be agreed that a chemical laundry treatment which reduces the number of coagulase-positive *Staphylococcus aureus* cells by 99.999% is an effective disinfectant treatment for laundry sufficient to protect the subsequent user if the same coagulase-positive S. *aureus* can be readily recovered from any sample of the finished laundry.

While there is a substantial background of epidemiological evidence to support existing programs for the disinfection of public water supplies, very little epidemiological proof exists to support a great variety of sterilizing, disinfecting and sanitizing routines that have been established by medical authorities, public health officials and santitarians as necessary and representative