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Preface

This book was written to provide microbiologists and researchers, involved in rapid microbial measurement methods, with a comprehensive and up-to-date account of basic principles and advanced techniques of impedance microbiology.

Initial efforts to study electrical impedance variations resulting from the metabolic activity of growing microorganisms may be traced to the late 1890's when G. N. Stewart described, before the British Medical Association, measurements of conductivity depression on samples of defibrinated dog blood allowed to putrefy. It took nearly a century to improve upon and implement the basic ideas in a commercial type instrument for rapid estimation of bacterial concentrations.

Impedance microbiology has become increasingly recognized in the field of rapid bacteriological methods. Interest is still growing rapidly in the fields of clinical and food microbiology. Each field emphasizes certain aspects such as correlation with standard plate counts, shelf-life, sensitivity to antibiotic susceptibilities, etc.

The bulk of the material dealing with impedance microbiology is widely scattered amongst technical journals and conference proceedings. Consequently, it is a rather difficult task, particularly for a newcomer, to learn the principles underlying the subject. This text attempts to put between the covers of one book information on the fundamental principles and up-to-date techniques

of impedance microbiology, and to organize it in a coherent and unified manner.

It is assumed that the reader has an adequate background in general microbiology. The "electro-bacteriological" model presented in chapter 3 and the statistical analysis presented in Appendix A require some background in mathematical analysis and statistics. Definitions of terms used throughout the text are provided in Appendix B. In presenting the material, emphasis is placed on the development of practical means to obtain reliable results in a variety of applications. Most of the theoretical and practical materials presented in this book are the results of research carried out over the last two years.

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Foreword

Few persons or events affected the course of a scientific discipline so profoundly as did Robert Koch and his publication in 1881 of an agar-based method for isolating bacteria. The track thus set for microbiology was cemented for the next century by Koch's assistant, Petri, with his description of '...a small modification to the master's apparatus.'.

Notwithstanding the development of numerous alternatives, most of the advances in analytical microbiology stem from a focussing on concepts of isolation, pure cultures, and colony forming units that the plating methods engendered. And microbiologists, in the main, have accepted prolonged incubation as an inescapable consequence of the plating procedure's sensitivity.

Today we cannot afford to wait so long to get results. Nor can we afford to centre attention so closely, merely on the properties of individual microorganisms or how many of them exist in the sample. Mixed populations are the rule rather than the exception and the interactions between microorganisms in a sample ensure that the whole does not behave like the sum of the parts. Usually it is how actively microbial contamination will do what it will do that matters, not how many of the individuals of a species are there.

The plating methods set a level of sensitivity that quite outperformed that of the early alternative methods. But limits of detection and analysis times of plate methods remained more or less

constant, while physical and chemical techniques advanced rapidly. Thus it is, that most microbiologists must now consider abandoning the old and trusted tool in favor of more efficient modern instrumentation. And of the available techniques, electrical impedance must - through its rapidity, versatility, compatibility with automation and computerized control, and pertinence to the total metabolic activity of the organisms - be considered a most desirable approach.

One can sense the quickening interest in impedance in almost every branch of microbiology. But the subject has long lacked the reference base needed to acquaint newcomers to the field with the theories and potentials that will allow them to adapt the technique to their individual problems. This obstacle has now been removed. With this book, Drs. Ruth and Gideon Eden have done a superb job of combining the history, concepts, mathematics, and procedural possibilities of the impedance method into one very readable volume. There is a great deal of information in this book. Approaching it from a background steeped in plate counts, you may often be startled at the conceptual jump needed to appreciate the beauty and potential of the impedance method. Even I, who have followed developments in the field for years, learned much from the manuscript. The Edens' influence may not ever rival Koch's, but will undoubtedly be felt for years as impedance microbiology permeates the analytical laboratory.

A.N. SHARPE

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CHAPTER 1
Introduction: Rapid Automated Methods

The age of automation has touched many areas of science and technology, and it seems as if microbiology is one of the last areas to be affected. This is not because there has been a lack of interest in the field of automation by microbiologists. The proceedings of international symposia (Johnston and Newsom 1976, Tilton 1982) demonstrated a great interest, at the research level at least, in a wide range of methods. The main interest has been in the medical area. However, industrial and food microbiologists seem to be similarly motivated to develop rapid automated methods. This increasing interest is doubtless caused by rising laboratory workloads without parallel rise in budget.

1.1 AUTOMATION OF ROUTINE PROCEDURES

The first area in microbiological laboratories to be affected by automation was that of routine and mundane procedures. In this category we include automated pipettors and dilutors, automated streaking and incubation, colony counters and staining machines. Two systems are worth mentioning in this category; 1. The spiral plater (Spiral System) which has a dispenser that distributes a continuously decreasing volume of liquid on the surface of a rotating petri dish. The dispenser moves from the center toward the edge, resulting in an attenuating Archimedes spiral of colonies. On these dishes a range of 10,000:1 can be read on a single dilution (Gilchrist et al. 1973). 2. The hydrophobic grid membrane filter

(ISO-GRID® HGMF, QA Laboratories) which is essentially a membrane base upon which a hydrophobic grid is applied to divide the membrane into a large number of individual growth compartments. The HGMF count is determined by a most-probable-number calculation (Brodsky et al. 1982). By providing an array of many (e.g. 1600) discrete growth compartments, it permits the organism to be enumerated over a range of nearly 4 log cycles on a single plate. This system retains many of the attractive features of membrane filtration (e.g. concentration of bacteria, removal of interfering substances, resuscitation of injured organisms). However, the need for filtration causes problems with certain samples which contain small particles, fat globules and other interfering substances. Some samples (e.g. dairy products) require an enzyme digestion step prior to filtration.

1.2 INSTRUMENTATION FOR IDENTIFICATION AND SUSCEPTIBILITY TESTS

Several instruments are available for the identification of microorganisms, or for testing their susceptibility to antibiotics. These instruments are mainly geared to the clinical area but may also be used in industrial and food microbiology. Most of these instruments analyze turbidity as a measure of the concentration or types of nutrients needed, or, conversely, the lack of growth in the absence of growth factor or in the presence of inhibitors. The Autobac (Pfizer Diagnostics), the MS-2 (Abbott Diagnostics Products) and AMS (Vitek System, subdivision of McDonnell Douglas Corp) are representative of the instruments available. The AMS System is the most automated instrument involving turbidity. It combines both detection and identification schemes which, in many cases, can bypass the initial requirement for a pure culture.

1.3 INSTRUMENTS FOR QUANTITATION OF MICROORGANISMS

The area where the penetration of instrumentation is the slowest is in estimation of microbial loads. The desire for direct enumeration has inhibited the development of appropriate

instrumentation and locked the microbiologist into the petri dish method that was introduced in the 1880's.

A. COUNTING INSTRUMENTS

Some of the instruments available in this area directly count numbers of organisms. An example of such instrument is the Coulter Counter (Coulter Electronics) which uses electronic particle sizing to detect and enumerate microorganisms. Direct microscopic counts can be done automatically using the Foss Bactoscan System (Dickey-John Canada Inc.). This system was mainly designed for the dairy industry. Similarly the direct epifluorescence filter technique (DEFT) can also be used. With the DEFT method the microorganisms are recovered from the sample on the surface of a membrane filter. The retained cells are stained with fluorescent stain, and counted by an epifluorescence microscope (Pettipher et al. 1980).

B. NON-COUNTING METHODS

The most promising rapid and automated methods for the estimation of microbial loads are those which do not involve counting. In this category we include very sensitive analytical instruments that can detect microbial components or metabolites. The impedance method belongs to this category. Since this book deals with the impedance method we will not discuss it here, but rather concentrate on other systems based on different principles.

i. Microcalorimetry

The minute amounts of heat produced by growing cultures can be detected by a sensitive calorimeter. Flow microcalorimetry was used to detect heat production by bacteria which commonly cause urinary infections. This method could detect the presence of bacteria at concentrations of 10^3 - 10^5 organisms/ml, depending upon the bacterial strain (Beezer et al. 1978). The profile of heat production versus time, or thermogram of microorganisms has been shown to be sensitive to growth

conditions. The change in response due to small changes in medium composition could be a serious limitation of microcalorimetry. Although this method was suggested over 10 years ago, this methodology has not been commercialized for quantitative tests of microorganisms.

ii. Radiometry

This method detects radioactive carbon dioxide produced by bacterial metabolism of a radio-labeled source of carbon incorporated into a culture medium. The time required to reliably detect radioactive CO_2, is inversely related to the initial number of organisms in the sample. The Bactec-460 (Johnston-Laboratories) tests the head space gases above inoculated, labeled media at a rate of 60 samples/hr. This instrument is widely used for clinical samples (mainly blood cultures); however, its use in other areas has not been widely reported.

iii. Bioluminescence

Microorganisms, like all living matters, contain ATP. When the luciferin-luciferase enzyme-substrate system comes into contact with ATP, light is emitted. Since the amount of light is proportional to the amount of ATP present in the system, the assay can be used to determine the number of organisms present. Non-microbial ATP is present in many systems. This must be destroyed before the microbial ATP assay begins. The sensitivity of the method depends on the quality of its releasing agent and the quality of the photometer. Lumac (Medical Product Division/3M) reported on a complete ATP System, including all needed reagents. The procedure, however, requires considerable sample preparation before the actual ATP measurement can take place. New instruments have been developed in which the reagents are added automatically. An example of such an instrument is the Picolite™ Luminometer (United Technologies Packard) in which 48

samples can be loaded into a sample changer, where the reagents are automatically injected.

1.4 COMPARISON BETWEEN QUANTITATIVE INSTRUMENTAL RESULTS AND STANDARD METHODS

Every new quantitative technique is eventually compared with existing standard methods. In most cases this entails regression analysis to show appropriate correlation and calculation of confidence limits for such comparisons. There are methods for which such comparison is straightforward. The comparison of direct microscopic count to plate counts is such an example, since both techniques estimate similar quantities, i.e. clumps of organisms which might be considered as colony forming units.

The comparison becomes more difficult with non-counting methods, since the different procedures are based on different assumptions. For example, in the comparison between ATP and plate count we could agree that ATP is a more basic unit than CFU since CFU can only be defined as an entity that gives rise to a colony (Wood and Gibbs 1982). However, since the ratio ATP/CFU is not necessarily constant, it is quite possible that new methods will bear a close relationship to the standard method only under very particular circumstances.

1.5 NEW PARAMETERS FOR QUANTITATIVE MICROBIOLOGY

In reviewing the new microbiological technologies one must raise the question of whether the current counting methods are the best, or even appropriate for estimating the microbial quality of products. It might very well be that a new method can better estimate shelf-life, safety, and acceptability of food products than the standard counting methods. Sharpe (1980) argues that microbiologists are obsessed with the counting of "clusters of bacteria which can grow on agar". This obsession has greatly retarded the development of more realistic methods and, particularly, instrumental methods for assessing the quality of products. Sharpe (1979) further indicates that the ability of food

to cause illness, or otherwise become unwholesome, depends on five factors; (i) number of organisms; (ii) their rate of multiplication in the food; (iii) the contribution of each microbial cell to the unwholesomeness of the food; (iv) inhibition or stimulation of (ii) and (iii) by other organisms; and (v) the consumer response thresholds to the levels of the various parameters. Counting microorganisms provides information on only the first item of the above list. Methods measuring levels of some physical, chemical, biochemical characteristics of the measured organism can provide information on factors (i) to (iv). Therefore, it is probable that these new methods, such as impedance, are a better direct measurement of the quality of foods than the standard plate count method.

CHAPTER 2
What is Impedance Microbiology?

2.1 HISTORY OF IMPEDANCE MEASUREMENTS IN MICROBIOLOGY

Although impedance monitoring, as performed by modern instruments, is a relatively new procedure, the observation of impedance variations due to microbial metabolism was reported to the British Medical Association as early as 1898 by Stewart (1899). He showed that the conductivity, measured daily, of defibrinated blood allowed to putrefy, increased tenfold over a course of 25 days. He postulated that ions were formed by bacterial decomposition of proteins and fats present in blood, and suggested that the rate of bacterial growth could be monitored by electrical means. This work is astonishing when one considers that Arrhenius had published his theory of ionic dissociation only eleven years earlier.

A few years later, Oker-Blom (1912) observed that conductivity measurements reflect collective changes taking place in media which can give a general picture of microbial metabolic processes, and lamented that this valuable technique had not been generally accepted among the major methods of measurement in bacteriology.

In 1926 Parsons and Sturges (1926 a,b) and later Parsons et al. (1929), working for a meat packing firm, reported upon the rather exact relationship between conductivity changes and ammonia production by cultures of *Clostridia* species grown in various media under anaerobic conditions. The conductivity bridge used had an accuracy of 0.5% and gave conductivity measurements which predicted ammonia production within 5% of the average of the