Bailey and Scott's

DIAGNOSTIC MICROBIOLOGY

SYDNEY M. FINEGOLD,

WILLIAM J. MARTIN, Ph.D.;

SIXTH EDITION

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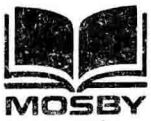

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PREFACE TO SIXTH EDITION

We were honored and delighted when Elvyn G. Scott invited us to become the new authors of *Bailey and Scott's Diagnostic Microbiology*, which we have long regarded as a classic in its field. We have strived to maintain the high standards of this important work so that it will continue to serve microbiologists and students of medical microbiology. We have made it our aim also to make the book useful to infectious disease clinicians and trainees, clinical pathologists, public health workers, and nurses and nursing students.

The text is changed significantly from the previous edition. There is some reorganization, a great deal of new material is added, and everything is updated. Only medically relevant material is included, and clinical correlations are presented. A new chapter on automation and rapid methods has been added. Almost all of the chapters have been completely rewritten. Among the new topics treated and areas given considerably more attention in this edition are new methods for detection of bacteriuria, the role of *Chlamydia* in disease, the new classification of Enterobacteriaceae, new descriptive material on certain nonfermentative gram-negative bacilli not previously discussed, newly described mycobacteria, new methods for susceptibility testing, an antibiotic removal device for blood cultures, and additional material on noncultural techniques for microbiologic diagnosis, laboratory safety, rapid processing for anaerobes, Legionnaires' disease, *Campylobacter*, *Yersinia*, antimicrobial-induced colitis, *Bacillus cereus*, sexually transmitted diseases, bite infections, bone infections, antibiotic tolerance, toxic shock syndrome, Kawasaki disease, monoclonal antibody, nutritionally variant streptococci, antibiotic-resistant pneumococci, *Vibrio vulnificus*, Actinomycetales, *Lactobacillus*, *Leptotrichia*, *Capnocytophaga*, *Prototheca*, hemotropic bacteria, DF-2, EF-4, HB-5, viral culture, fungi, and certain parasitic agents. It is beyond the scope of this book to discuss the role of the microbiology laboratory in investigations of nosocomial infection outbreaks. Interested readers are referred to an excellent recent article by Goldmann and Macone (Infect. Con. 1:391-400, 1980).

We acknowledge with gratitude the assistance

of numerous individuals. Particularly, we appreciate the help of George Berlin, Elaine Bixler-Forell, JoAnn Dizikes, Violet Fiacco, Carol Gagne, Janet Hindler, and Daniel Wong. We are particularly grateful to Elvyn G. Scott for his continued advice, assistance, and encouragement. We would like to thank Nobuko Kitamura for the preparation of a number of excellent new illustrations for Chapter 35. We express our deep appreciation to John G. Bartlett, Ann Bjornson, Diane M. Citron, V.R. Dowell, Jr., Paul Edelstein, Violet Fiacco, W. Lance George, Ellie J. C. Goldstein, Theo M. Hawkins, Hannele Jousimies, A. S. Klainer, Irving Krasnow, George Kubica, Martin McHenry, Marjorie Miller, Alan Morgenstein, Maury E. Mulligan, Charles V. Sanders, Elvyn G. Scott, Lakhbir Singh, Alex Sonnenwirth, Vera L. Sutter, Valerie Vargo, Trevor Willis, Analytab Products, Centers for Disease Control, General Diagnostics, The Mayo Clinic, Schering Corporation, and The Upjohn Company, for many of the color photos.

To our wives, Mary and Marcia, we express our thanks for their patience, indulgence, and assistance.

Sydney M. Finegold
William J. Martin

PREFACE TO FIRST EDITION

Diagnostic Microbiology is the first edition of a new series and not a revision of the former publication *Diagnostic Bacteriology*, the latest edition of which we revised (1958). This new title derives in part from the fact that the new volume includes microorganisms other than bacteria. The reader will note, for example, that the former Society of American Bacteriologists has now become the American Society for Microbiology.

Since this book is designed to be used as a reference text in medical bacteriology laboratories and as a textbook for courses in diagnostic bacteriology at the college level, the material has been consolidated and placed in separate parts and chapters. The selected sequence will be commensurate with the needs of both the diagnostician and the student.

For purposes of orientation in taxonomy and ready reference, an outline of bacterial classification has been included. For the student beginning diagnostic work, some pertinent background information is presented on the cultivation of microorganisms, the microscopic examination of microorganisms, and the proper methods for collecting and handling specimens.

A number of chapters include recommended procedures for the cultivation of both the common and the rare pathogens isolated from clinical material and should serve to familiarize the microbiologist with the wide variety of pathogens that may be encountered. An additional chapter has been devoted to the methods employed in the microbiological examination of surgical tissue and autopsy material.

To effect further consolidation of the book's content, one part has been devoted to a series of chapters which cover the various groups of bacteria of medical importance—their taxonomic position, general characteristics, and procedures for their identification. The chapter on the enteric bacteria introduces the new classification of the family Enterobacteriaceae, outlines the group biochemical characteristics, and discusses the serological aspects. The chapter on the mycobacteria includes a discussion of the increasingly important unclassified (anonymous) acid-fast bacilli, giving the methods for their identification, certain cytochemical tests, and

animal inoculation procedures.

The chapter on laboratory diagnosis of viral and rickettsial diseases includes a guide to the collection of specimens and offers recommendations for the appropriate time of collection. In the chapter on laboratory diagnosis of systemic mycotic infections, the biochemical approach in identifying the pathogenic fungi is brought to the reader's attention.

The remainder of the book includes prescribed tests for the susceptibility of bacteria to antibiotics, serological procedures on microorganisms and patients' sera, and a technical section on culture media, stains, reagents, and tests, each in alphabetical sequence.

We would like to express our gratitude to Mrs. Isabelle Schaub and to Sister Marie Judith for committing the continuation of the original publication, *Diagnostic Bacteriology*, to our care and responsibility. We also acknowledge the many kindnesses extended by a number of microbiologists and clinicians in permitting the use of published and unpublished materials.

W. Robert Bailey
Elvyn G. Scott

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PART I LABORATORY METHODS

1 GENERAL REQUIREMENTS FOR CULTIVATION OF MICROORGANISMS

Great advances relative to the nutritional requirements of bacteria and other microorganisms have been realized during the current century. Consequently, with the exception of a few fastidious forms, most pathogens can be cultivated in the laboratory on artificial media. Emphasis should be placed, therefore, on the proper preparation and selection of culture media in order to isolate and grow the various microbes. The need for quality control should be stressed. A good **culture medium** contains the essential nutrients in the proper concentration, an adequate amount of salt, and an adequate supply of water; is free of inhibitory substances for the organism to be cultured; is of the desired consistency; has the proper reaction (pH) for the metabolism of that organism; and is sterile. It is obvious that such a definition applies only to the preparation of inanimate media, because different requirements exist for obligate parasites.

Additional requirements must also be met, such as those generated by the temperature and oxygen relationships of the culture and by the lack of synthetic ability of certain fastidious pathogens.

Heterotrophic microorganisms, the group to which the pathogens belong, exhibit a wide variety of needs. Despite this, however, numerous dehydrated media that are stable and eminently suitable for use in a diagnostic laboratory are available commercially. This ready supply minimizes concern regarding deterioration of prepared media and reduces storage needs. It is strongly recommended that media makers follow the directions given on the container labels.

PREPARATION OF MEDIA

Clean, detergent-free glassware and equipment are essential to good media preparation. The screw cap, the metal closure, and the plastic plug have virtually replaced the cotton plug in tubes of media, but personnel are cautioned concerning the exclusive use of the push-on metal closure. When tubes of media are stored over a long period, air contamination is likely to occur. Tubes cooling after sterilization take in air—the most likely means of initial contamination.

If a culture medium is to be prepared from its ingredients, the latter should be weighed accurately in a suitable container. Approximately one half of the required amount of water is added first to dissolve the ingredients; then the remainder is added. If it is an agar-containing medium, heat will be necessary to dissolve the agar. Heating on an open flame is **not recommended,** although it is often practiced. The use of a boiling water bath or steam bath is preferred. Complete dissolution of the ingredients of the medium in the required amount of water before sterilization gives a consistent and homogeneous product. Flasks of agar-containing media should be mixed after sterilization to promote a uniform consistency for pouring plates.

For dissolving dehydrated agar media for autoclaving, the use of a microwave oven has been shown to result in a substantial reduction of heat generation and considerable savings in time.[5] The microwave oven has also been used for decontamination procedures in the laboratory.[6]

Media may be **liquid** (broth) or **solid** (containing agar). Sometimes a semisolid consistency is

desired, in which case a low concentration of agar is used. Culture media may be also **synthetic,** in which all ingredients are known, or **nonsynthetic,** in which the exact chemical composition is unknown.

The inclusion of small amounts of a carbohydrate, such as glucose, is often recommended for the enhancement of growth in routine plating or broth media. In some instances the presence of such a carbohydrate can lead to a product of fermentation that may alter the characteristic appearance of a reaction produced by a microorganism. Examples are cited later.

Dehydrated media or media prepared according to accepted formulas are now available for the cultivation of anaerobic bacteria. Thioglycollate broth, for example, is useful for biochemical tests on strains of anaerobes that have been previously isolated in pure culture. In the preparation of this medium (which contains peptone, an amino acid, and other ingredients), sodium thioglycollate is added as a reducing agent. This substance possesses sulfhydryl groups (SH—), which tie up molecular oxygen and prevent the formation and accumulation of hydrogen peroxide in the medium.

The cultivation of catalase-negative microorganisms, such as the clostridia, which are unable to break down this toxic substance, becomes possible under these conditions. The addition of thioglycollate or other reducing agents, for example, cysteine or glutathione, to culture media such as nutrient gelatin and milk renders them more suitable for anaerobic or microaerophilic cultivation.

STERILIZATION OF MEDIA

Sterilization may be effected by **heat, filtration,** or **chemical methods.** The method of choice will depend on the medium, its consistency, and its labile constituents. Moist heat rather than dry heat is employed in the sterilization of culture media, and the method of application will vary with the type of medium.

Moist heat

Steam under pressure

The usual application for most media is steam under pressure in an autoclave where temperatures in excess of 100 C are obtained. Although the principle of the autoclave need not be discussed here at length, one important rule about autoclave sterilization must be stressed. In the normal procedure, using 15 pounds of steam for 15 minutes, the autoclave chamber should be flushed **free of air** before the outlet valve is closed. A temperature of 105 C on the chamber thermometer is used as an index of complete live steam content. When this temperature is reached, the outlet valve may be closed, and the pressure allowed to build up to the required level of 15 pounds to attain generally a temperature of 121 C. Automation, however, takes care of such operational needs.

The time of exposure to this temperature and pressure may be allowed to exceed 15 minutes if large volumes of material are being sterilized. It is not recommended that an exposure of more than 30 minutes be used, because overheating can cause a breakdown of nutrient constituents.

At times a lower pressure may be necessary, such as in the heat sterilization of certain carbohydrate solutions. A pressure of 10 to 12 pounds for 10 to 15 minutes will reduce the possibility of hydrolysis.

The temperatures of the autoclave that correspond to the various live steam pressures (above atmospheric) are given in Table 1-1.

It is strongly recommended that the efficiency of the autoclave be checked at regular intervals. This may be conveniently done with the use of filter paper strips impregnated with spores of the thermophile *Bacillus stearothermophilus* and an appropriate amount of dehydrated culture medium with an indicator.

The paper strips,* contained in small enve-

*Kilit Sporestrips No. 1, Baltimore Biological Laboratory, Cockeysville, Md.

TABLE 1-1

Autoclave temperatures corresponding to steam pressures in the chamber

Pressure (pounds)	Temperature (° C)	Pressure (pounds)	Temperature (° C)	Pressure (pounds)	Temperature (° C)
1	102.3	10	115.6	15	121.3
3	105.7	11	116.8	16	122.4
5	108.8	12	118.0	17	123.3
7	111.7	13	119.1	18	124.3
9	114.3	14	120.2	20	126.2

lopes, are inserted in the center of a basket of tubes of media or other material to be tested. The basket or material is then placed near the bottom at the front of the autoclave chamber, and the usual sterilizing cycle is carried out. When the cycle is complete, the load is removed from the sterilizer, and the sporestrip envelope is sent to the laboratory.

In the microbiology laboratory the sporestrips are individually removed, using aseptic technique, and are placed directly into tubes containing 12 to 15 ml of sterile distilled water. After dissolution of the medium on the strips, the tubes are gently shaken and placed at 55 C and then observed daily for several days. An unexposed sporestrip is processed in like manner as a control with each sterilization check.

The control is examined after appropriate incubation. This should reveal a change in the color of the indicator, showing that acid (yellow if bromcresol purple is used) has been produced through fermentation of the glucose. If the remainder of the tubes have the same appearance, sterilization has not been effected.

Successful sterilization is indicated by the unchanged appearance of the heated tubes after 7 days' incubation at 55 C. The spores of *B. stearothermophilus* are destroyed when exposed to 121 C for 15 minutes.

A test kit more convenient for use in the microbiology laboratory consists of a sealed glass ampule containing a standardized spore suspension of *B. stearothermophilus*, a culture medium, and an indicator.* The ampule is exposed, incubated, and read as previously described for the sporestrip; an unheated ampule is also included as a positive control. These ampules are for professional use only and are used just once. Because they contain live cultures, they should be handled with care to prevent breakage. Each ampule is destroyed after use, preferably by incineration; unused ampules are stored in a refrigerator at 2 to 10 C.

A third but less reliable alternative is to use adhesive tape on which the word "sterile" is printed invisibly. The word becomes visible if the autoclaving is efficient. The tape may be placed on any suitable container to be autoclaved.

Flowing steam

The flowing steam procedure represents another application of moist heat and may be employed in the sterilization of materials that cannot withstand the elevated temperatures of an autoclave. **Fractional sterilization,** or **tyndallization,** introduced by John Tyndall in 1877, is a procedure involving the use of flowing steam in an Arnold sterilizer.

*Kilit ampule, Baltimore Biological Laboratory, Cockeysville, Md.

Material to be so sterilized is exposed for 30 minutes on 3 successive days. After the first and second days the material is placed at room temperature to permit any viable spores present to germinate. Vegetative bacteria are destroyed at flowing steam temperature, whereas their endospores are resistant. This procedure is used for media such as milk that contains an indicator and other media that may be precipitated or changed chemically by the normal autoclave treatment.

Inspissation

A third type of moist heat application is the process known as **inspissation,** or thickening through evaporation. This is used in the sterilization of high-protein-containing media that cannot withstand the high temperatures of the autoclave. The procedure causes coagulation of the material without greatly altering the substance or appearance. Materials such as the Lowenstein-Jensen egg medium, the Loeffler serum medium, and the Dorset egg medium are inspissated.

Modern autoclaves are equipped to allow inspissation procedures. If the Arnold sterilizer or a regular inspissator is used, the tubes containing the medium are placed in a slanted position and are exposed to a temperature of 75 to 80 C for 2 hours on 3 successive days. Precautions should be taken during such treatment to prevent excessive dehydration of the medium.

Manually operated autoclaves may also be used successfully. Tubes of medium are placed in the autoclave chamber in a slanted position in a rack with adequate spacing. The tubes may be closed with a screw cap, loosely fitted initially, when Lowenstein, Loeffler, and Dorset egg media are being prepared. For operation, the exhaust valve of the autoclave is closed, and the door is shut tightly. This traps air in the chamber. The steam is turned on, and the chamber contains an air-steam mixture. The pressure is then raised to 15 pounds and should be rigidly maintained for 10 minutes. The temperature ranges between 85 and 90 C. After this period, through manipulation of the steam valve and the exhaust valve, the air-steam mixture is replaced with live steam while the pressure is kept constant at 15 pounds. When the temperature reaches 105 C, the chamber contains only live steam. The outlet valve is then closed, and an additional 15 minutes at 15 pounds is allowed to effect complete sterilization. During the final phase the chamber temperature rises to 121 C. At the end of this period the pressure should be permitted to subside very slowly. This is achieved by closing the steam valve and keeping the outlet valve tightly closed. The chamber temperature should drop below 60 C before the door is opened. When the tubes of media are cool, their caps should be tightened.

Filtration

Certain materials cannot tolerate the high temperatures used in heat sterilization procedures without deterioration; thus, other methods must be devised. Materials such as urea, certain carbohydrate solutions, serum, plasma, and ascitic fluid must be filter sterilized. Filters made of sintered Pyrex, compressed asbestos, or membranes are employed. These are placed in sidearm flasks, and the entire assembly of filter and flask is sterilized in the autoclave. A test tube of appropriate length may be placed around and under the delivery tube of the filter and may be sterilized with the unit when only a small quantity of filtrate is required. Negative pressure (suction) is applied to draw or positive pressure is applied to force the material through the filter. Membrane filters are preferred to the Seitz asbestos filter for some materials because of the high adsorption capacity of the latter type. The Swinney filter,* consisting of a filter attachment affixed to a hypodermic syringe and needle, is fast and simple for small quantities of material. The nonsterile material is taken up in the syringe, the sterile filter attachment is

*Millipore Filter Corp., Bedford, Mass.

affixed, and the material is expressed by positive pressure (exerted by the plunger) into a sterile container. Disposable sterile plastic holders and membrane filters are now being widely used.

Chemical methods

In addition to or in lieu of some of the foregoing, chemical methods may be used. These normally comprise two main types: the use of chemical additives to solutions or treatment with gases. The latter applies primarily to the sterilization of thermolabile plasticware such as Petri dishes, pipets, and syringes, and ethylene oxide may be used. The efficiency of such sterilization may be determined by the use of commercially prepared sporestrips.* As this is not directly concerned with sterilization of media, it will not be discussed here. The use of chemical compounds, such as thymol, a crystalline phenol, as additives to concentrated thermolabile solutions is quite appropriate, however. For example, to sterilize a 20-times-normal concentration of urea or a 20% solution of carbohydrate, approximately 1 g of thymol per 100 ml of medium is added and allowed to stand at room temperature for 24 hours. The dilution serves to nullify any bactericidal effects of the thymol.

SELECTION OF PROPER MEDIA

The number of available media increases annually. Selection of the proper media for specific purposes requires judgment by an experienced person, but for the most part, commercially available media, used in nearly all laboratories today, carry a recommendation based on the broad experience of others in the field. A very important facet in medium selection is the purpose for which the medium is intended. Laboratory personnel are advised to keep their selections to a minimum to avoid duplication of purpose.

Culture media, by virtue of their ingredients, may be selected for general and for specific purposes. The reader will find various terms ascribed to media throughout the literature. For example, terms such as **enrichment, enriched, selective,** and **differential** are very common as applied to media and are found in various sections of this book. These terms are indicative of purpose. Usually the media are special, but for general use a good basic nutrient medium is needed in the preparation of broth and agar media. The choice of such a medium is governed partly by tradition and partly by the experience of the users. In the selection of a basic medium, however, it is generally understood that one that is glucose free tends to give more consistent and reliable results. The presence of small amounts of this carbohydrate in a medium tends to enhance growth; yet fermentation of glucose can result in a pH that is harmful to acid-sensitive organisms. For example, this has been recognized in the cultivation of pneumococci and beta-hemolytic streptococci. The presence of the carbohydrate in a blood agar base medium can lead to the appearance of a green zone around colonies of streptococci, making differentiation between alpha and beta hemolysis extremely difficult.

Trypticase or tryptic soy broth, a pancreatic digest of casein and soybean peptone, is a widely used general medium that will support the growth of many fastidious organisms without further enrichment. Because of its glucose content, however, one should be aware of its limitations in certain areas of application.

Thioglycollate medium, now available in a modified form containing 0.06% glucose, will support the growth of many facultatives, microaerophiles, and anaerobes. In the ensuing chapters of this book, which discuss the isolation and cultivation of various pathogenic microorganisms, recommendations relative to the selection and use of media are made. Chapter 42 gives the formulas and methods of preparation for many of the media in use at the present time.

Valuable information on the choice of media

*Attest Biological Indicators, 3M Co., St. Paul, Minn.