WADSWORTH

ANAEROBIC BACTERIOLOGY MANUAL

THIRD EDITION

VERA L. SUTTER, Ph.D.

DIANE M. CITRON, B.S.

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with a chapter on gas-liquid chromatography by
KENNETH S. BRICKNELL

All from the Anaerobic Bacteriology Laboratory,
Veterans Administration Wadsworth Medical Center,
and Department of Medicine, UCLA School of Medicine,
Los Angeles, California

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Foreword

In reviewing the first edition of this manual, some eight years ago, I welcomed its appearance as a major event, since it presented much-needed, detailed, up-to-date, and authoritative information on modern anaerobic techniques, determination and evaluation of the properties of anaerobic bacteria, and their identification. Its emphasis on practical aspects and clinical laboratory procedures filled a real need, and strong demand for the manual resulted in the publication of a revised, second edition within 3 years.

In the present (third) edition Drs. Vera Sutter and Sydney Finegold, together with Diane Citron, have further enlarged and updated the wealth of material included in the earlier editions; as a result, it should be even more valuable as a guide to practical anaerobic methods.

The manual now includes data on the incidence of specific anaerobes in various infections (based on the Wadsworth Medical Center Anaerobic Group's unexcelled, extensive experience), new information on collection, transport, and cultivation techniques with discussion of new commercially available systems, and procedures emphasizing rapid presumptive identification based on initial observations of cultures and some additional tests (with comments about systems such as the L-D Presumpto Plate, API-20A, and Minitek). In addition, the broth-disc test and the National Committee for Clinical Laboratory Standards' (NCCLS) proposed reference agar-dilution procedure have been added to the susceptibility test techniques. New procedures for the study of vaginal and oral flora, together with detailed instructions for the study of the intestinal flora, provide a complete system for the study of the indigenous biota.

The manual is a welcome source of authoritative and comprehensive information for clinical microbiology laboratories, making available current thinking and well-proven practices that prevail today in anaerobic microbi-

ology; it should be highly useful for those with an interest in anaerobe bacteriology and the clinical significance of anaerobes, and for all clinical and public health microbiology laboratories.

Alex C. Sonnenwirth, Ph.D.

Director, Division of Microbiology Laboratories,
The Jewish Hospital of St. Louis,
St. Louis, Missouri

Preface

It has become clear in recent years that anaerobic bacteria are important causes of many different types of infection. Any type of bacterial infection in humans may involve anaerobes.

Anaerobic infections are beyond question the most frequently overlooked of all bacterial infections. The opportunity for overlooking anaerobes is greatest when they are present in mixed culture, as they commonly are. Isolation of an accompanying facultative organism, particularly if it has the same general characteristics on Gram stain (that is to say, both gram-negative bacilli), may lull the unaware bacteriologist into a false sense of security. When no aerobes or facultative organisms are present on culture and Gram stain has revealed bacteria, it is considerably easier to suspect and to isolate the anaerobes. The large number of recent reports of anaerobes (mostly gram-negative bacilli and especially the *Bacteroides fragilis* group) in bacteremia reflects the ease of recovery of these organisms in pure culture.

Failure to recognize the importance of anaerobic bacteria in infection in the past must be blamed on both clinicians and microbiologists. Fortunately, as one group becomes more aware of the role of these organisms, the other becomes equally so. However, there is still a need for education of both groups as to the importance of these organisms and optimum procedures for their recovery and identification. A monograph designed primarily for the clinician[34] provides a detailed review of the clinical aspects of anaerobic infection.

This manual is aimed chiefly at the laboratory worker. It is our intent to emphasize practical aspects of anaerobic bacteriology for clinical laboratories. Although certain fastidious anaerobes require very specialized anaerobic techniques (the roll-tube or prereduced medium procedures and the anaerobic chamber or glove-box procedure), such delicate organisms have rarely been involved in true infection.

While we emphasize the simpler, more rapid techniques suitable for processing clinical specimens in small laboratories, we also present descriptions of the two more rigorous techniques because they can be useful in clinical laboratories that process large numbers of specimens for anaerobic culture and because they are essential for normal flora studies.

This manual concerns only organisms encountered in humans. Animal strains may have different growth requirements and frequently have different antimicrobial susceptibility patterns.

We would like to express our appreciation to the many people who kindly offered suggestions on improvements since the first edition of our manual was published in 1972. We are especially indebted to Dr. Louis DS. Smith for his helpful suggestions for the section on *Clostridium* species.

We are indebted to Mrs. Kimi Ishii and Mrs. Lorrayne Adams for excellent suggestions for the arrangement of material and for typing the manuscript. We appreciate the help of Y-Y Kwok in providing excellent drawings.

Vera L. Sutter
Diane M. Citron
Sydney M. Finegold

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TABLES

1 General considerations

Since many anaerobes grow more slowly than facultative or aerobic bacteria and since clinical specimens yielding anaerobic bacteria not uncommonly contain several organisms, considerable time may elapse before the laboratory is able to provide a final report. Indeed, at times it may literally take weeks before certain specimens with a complex flora are worked up definitively. The question then naturally arises as to whether the bacteriologic data are really beneficial to the clinician. Aside from the time factor, how much data are useful to the clinician? Is the clinician interested in accurate species and subspecies identification or will general identification, with susceptibility data, suffice to permit effective treatment of the patient? Even when the clinician is interested in detailed, accurate bacteriologic data—if only for academic reasons—does cost-benefit analysis warrant providing this? Just how far should the laboratory go in processing anaerobic cultures? These are difficult problems, and the answers vary according to specific circumstances. Hard and fast rules cannot be set up.

Clearly, it is important that the laboratory provide as much information as possible as soon as it can after receipt of the specimen. This is particularly true, of course, in the case of very ill patients. It is the physician's responsibility to call such patients to the attention of the bacteriologist. A series of reports on each individual specimen would provide the clinician with information in optimal fashion. The initial report, at least in the case of very sick patients, should be an interpretation of the Gram stain and any other direct examination of the specimen. The bacteriologist must not hesitate to express judgment on likely possibilities, not only from direct examination, but (later) from observation of colonial characteristics. This presumes, of course, that the laboratory worker is well informed and applies rational judgment. After 18 to 24 hours, examination of aerobic cultures (and certain special anaerobic cultures) permits the microbiologist to provide a more reliable report. Simi-

larly, examination of routine anaerobic cultures after 48 hours provides even further information. A number of rapid diagnostic procedures may be employed to expedite presumptive or definitive identification.

Certain anaerobes should be identified, at least presumptively, as rapidly as possible. Two outstanding examples are the *Bacteroides fragilis* group and *Clostridium perfringens*, the former because it is the most commonly encountered and most resistant to antimicrobial agents and the latter because it may produce devastating disease. Fortunately, these organisms grow rapidly and with little difficulty. Means are available for readily recovering them from mixed cultures, and they can be identified quite simply and rapidly.

Any laboratory engaged in anaerobic cultivation should be able to recover in pure culture all anaerobes present in clinical specimens, to maintain them in a viable state, and to do at least preliminary characterization. This, with identification of certain key organisms such as *B. fragilis* and *C. perfringens*, provides the clinician with the data needed to successfully manage patients with anaerobic infections. The above two anaerobes, along with the *Bacteroides melaninogenicus-Bacteroides asaccharolyticus* group, *Fusobacterium nucleatum*, and the anaerobic cocci (these three groups are also readily identified) account for approximately two thirds of all clinically significant infections involving anaerobes. Broth disc susceptibility tests are easy to perform and should be available in most laboratories.

Although we recognize that a number of smaller laboratories may find it difficult to go beyond what has been outlined in the above two paragraphs and that cost may be a problem, we nonetheless urge full, definitive identification whenever possible. As an example, speciation within the *B. fragilis* group may provide useful information. *B. fragilis* and *B. thetaiotaomicron* are commonly found as pathogens; recovery of *B. ovatus* might mean contamination of a specimen with normal bowel flora. If an organism initially felt to be *B. fragilis* from a patient with bacteremia of unknown source was subsequently identified as *B. splanchnicus*, this would indicate the bowel as the likely source, whereas if it were *B. fragilis* the portal of entry might also have been the female genital tract or elsewhere. Identification of a *Clostridium* isolated from the blood as *C. septicum* provides the clinician with a valuable clue, as there is a strong association between bacteremia with this organism

and malignancy. Exact identification of an organism isolated from a patient with two or more episodes of infection helps distinguish between recurrence (which may imply tumor, foreign body, or an undrained abscess) and a new infection.

Short cuts to identification, if not applied intelligently, may lead to significant errors in the case of anaerobes, since these organisms are often pleomorphic (rods being mistaken for cocci and vice versa), do not always show spores readily, and gram-positive forms destain readily. We realize, of course, that the use of limited identification procedures is necessary in most clinical laboratories. However, careful application of knowledge of the significance of various organisms in specific situations and thoughtful use of limited approaches keep costs within reason and keep the laboratory's workload manageable without compromising patient care.

Quantitation of results is of particular importance in anaerobic bacteriology, since anaerobic infections are frequently mixed and the relative importance of various organisms in complex mixtures may often be deduced from this type of information. Formal quantitation is not usually necessary; designation as "heavy growth," "few colonies," and so on is adequate.

Accurate bacteriologic data permit one to define the role of various anaerobes in different infectious processes and the prognosis associated with these. Definitive identification is important in educating clinicians as to the role of various organisms in infectious processes and prevents deterioration of the skills and interest of the bacteriologist.

ANAEROBES AS NORMAL FLORA

A knowledge of the presence of specific anaerobes as normal flora at various sites in the body is important in several ways. Since most anaerobic infections arise in proximity to mucosal surfaces, where anaerobes predominate as normal flora, information on which organisms make up the indigenous flora at these sites enables one to suspect the presence of certain organisms in particular specimens and thus to assist the clinician in choosing the proper drugs for initiating therapy. This information also helps the microbiologist to choose selective and other media that might be particularly useful. Knowledge of the normal flora of various regions may also allow one to judge

more readily whether a given isolate is significant. For example, *Propionibacterium* in a single blood culture most often represents "contamination" from the patient's skin. Conversely, the presence of a particular organism in blood cultures may suggest the portal of entry for the bacteremia.

Table 1-1 indicates the incidence of certain anaerobes as normal flora at various sites in humans. Additional data on anaerobes as indigenous flora are found in Rosebury's classic book.[98]

CLINICAL BACKGROUND

Incidence of anaerobes in infection

Anaerobes may cause any type of infection in humans. There are a number of infections, however, in which anaerobic bacteria are the predominant pathogens or are commonly found; these are listed in Table 1-2. When information regarding incidence of anaerobes in these infections is available, it has been indicated. It must be emphasized, however, that the majority of published studies on anaerobic infections have been retrospective. The bacteriologic methods, particularly the anaerobic methods, were not uniform and in many instances not optimal. Therefore, some of these incidence figures are undoubtedly low. Several articles emphasizing various aspects of anaerobic infections are also indicated in the table and are recommended for those wishing to read further on this subject.

Other studies not included in Table 1-2 incorporate inaccuracies related to uncertainty about the clinical significance of isolates and to selection of certain types of specimens that would certainly contain elements of the normal flora. In this type of study, of course, the incidence of anaerobes may be falsely high.

Table 1-3 details our own experience with recovery of anaerobes from clinical specimens.

Clues to anaerobic infection

Certain hints suggesting to the bacteriologist that a given specimen is likely to contain anaerobic bacteria are listed below:

1. Foul odor to specimen
2. Location of infection in proximity to a muscosal surface

Table 1-1. Incidence of various anaerobes as normal flora in humans

		Non-spore-forming bacilli								
		Gram-positive					Gram-negative		Cocci	
	Clostridium	Actinomyces	Bifidobacterium	Eubacterium	Lactobacillus†	Propionibacterium	Bacteroides	Fusobacterium	Gram-positive	Gram-negative
Skin	0	0	0	±	0	2	0	0	1	0
Upper respiratory tract*	0	1	0	±	0	1	1	1	1	1
Mouth	±	1	1	1	1	±	2	2	2	2
Intestine	2	±	2	2	1	±	2	1	2	1
External genitalia	0	0	0	U	0	U	1	1	1	0
Urethra	±	0	0	U	±	0	1	1	±	U
Vagina	±	0	1	±	2	1	1	±	1	1

*Includes nasal passages, nasopharynx, oropharynx, and tonsils.

†Includes anaerobic, microaerophilic, and facultative strains.

U, unknown; 0, not found or rare; ±, irregular; 1, usually present; 2, usually present in large numbers.

Table 1-2. Infections commonly involving anaerobes

	Incidence (%)	Proportion of cultures positive for anaerobes yielding only anaerobes	Reference
Bacteremia	20	4/5	126
Central nervous system			
Brain abscess	89	1/2-2/3	55
Extradural or subdural empyema	10		121
Head and neck			
Chronic sinusitis	52	4/5*	42
Chronic otitis media	56	1/10	19
	33	0	65
Neck space infections	100	3/4	7
Wound infection following head and neck surgery	95	0	12
Dental, oral, facial			
Dental and oral			78
Orofacial, of dental origin	94	4/10	24
Bite wounds	47	1/34	45
Thoracic			
Aspiration pneumonia	93	1/2†	8
	62	1/3	79
	100	1/3	46
Lung abscess	93	1/2-2/3	9
	85	3/4	13
Bronchiectasis	76	1/3	10
Empyema (nonsurgical)	62	1/2	13
Abdominal			
Intra-abdominal infection (general)	86	1/10	41
	90	1/3	88
	81	1/3	122
	94	1/7	47
Appendicitis with peritonitis	96	1/100	2
Liver abscess	52	1/3	101
Other intra-abdominal infection (postsurgery)	93	1/6	51

*Twenty-three of 28 cultures (82% yielding heavy growth of one or more organisms had only anaerobes present).

†Aspiration pneumonia occurring in the community rather than in the hospital involves anaerobes to the exclusion of aerobic or facultative forms two thirds of the time.

	Incidence (%)	Proportion of cultures positive for anaerobes yielding only anaerobes	Reference
Wound infection following bowel surgery			34
Biliary tract	45	0	105
	41	2/117	28
Obstetric-gynecologic			
Miscellaneous types	100	1/3	124
	74	1/3	123
	72		74
Pelvic abscess	88	1/2	1
Vulvovaginal abscess	75	1/4	95
Vaginal cuff abscess	98	1/30	53
Septic abortion, sepsis	67		100
	63		106
Pelvic inflammatory disease	25	1/14	23
	48	1/7	30
Soft tissue and miscellaneous			
Nonclostridial crepitant cellulitis	75	1/12	81
Pilonidal sinus	73		96
Diabetic foot ulcers	95	1/20	80
Soft tissue abscesses	60	1/4	61
Cutaneous abscesses	62	1/5	83
Decubitus ulcers with bacteremia	63		22
Osteomyelitis	40	1/10	76
Gas gangrene (clostridial myonecrosis)			
Breast abscess			34
Perirectal abscess			34