# WADSWORTH

# ANAEROBIC BACTERIOLOGY MANUAL

# WADSWORTH

# ANAEROBIC BACTERIOLOGY MANUAL

Second Edition

Vera L. Sutter, Ph.D.
Valerie L. Vargo, M.S.
Sydney M. Finegold, M.D.

with a chapter on gas-liquid chromatography by
Kenneth S. Bricknell

From the Anaerobic Bacteriology Laboratory, Wadsworth Hospital Center, Veterans Administration, Wilshire and Sawtelle Blvds., Los Angeles, Ca 90073 and the Department of Medicine, UCLA School of Medicine, Los Angeles, Ca 90024

TABLE OF CONTENTS

# LIST OF TABLES

Table No.

Table No.

## *LIST OF FIGURES*

# INTRODUCTION

It has become increasingly clear in recent years that anaerobic bacteria are important causes of many different types of infection. These organisms may be responsible for all types of infections in man which may be caused by bacteria.

Anaerobic infections, beyond any question, are the most frequently overlooked of all bacterial infections. The opportunity for overlooking anaerobes is greatest when these organisms 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 (viz., both Gram-negative bacilli), may lull the unaware bacteriologist into a false sense of security. When no aerobes or facultatives 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 probably mostly Bacteroides fragilis) 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 one automatically becomes alerted to this. 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 (20) provides a brief review of the clinical aspects of anaerobic infection, while "Anaerobic Bacteria: Role in Disease" (8) covers major aspects of the bacteriology and clinical relevance of anaerobes. This manual is aimed chiefly at the laboratory worker. It is our intent to emphasize practical aspects of anaerobic bacteriology for clinical laboratories.

Certain fastidious anaerobes require very specialized anaerobic techniques (the "roll-tube" or pre-reduced medium procedures and the anaerobic chamber or glove-box procedure) but no one has established that such very delicate organisms have ever been involved in true infection. Certain reports purporting to show this are unsatisfactory in that one cannot be certain that the specimens cultured were not "contaminated" with normal flora; in other cases, clinical data is not adequate to establish the significance of the isolate in an infectious process.

TABLE 1. INCIDENCE OF VARIOUS ANAEROBES AS NORMAL FLORA IN HUMANS

| | Non-sporeforming bacilli | | | | | | | Cocci | |
|---|---|---|---|---|---|---|---|---|---|
| | | Gram-positive | | | | | Gram-negative | | |
| | Clostridium | Actinomyces | Bifidobacterium | Eubacterium | Lactobacillus † | Propionibacterium | Bacteroides | Fusobacterium | Gram-positive | Gram-negative |
| Skin | 0 | 0 | 0 | U | 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 | ± | ± | 1 | 1 | ± | ± |
| Vagina | ± | 0 | 2 | U | 2 | 0 | 1 | ± | 1 | 1 |

* = includes nasal passages, nasopharynx, oropharynx and tonsils

U = unknown

0 = not found or rare

± = irregular

1 = usually present

2 = usually present in large numbers

† = includes anaerobic, microaerophilic and facultative strains

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 processing large numbers of specimens for anaerobic culture and because they are essential for normal flora studies.

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

## 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 the anaerobes predominate as normal flora, information on which organisms make up the indigenous flora at these sites will enable 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 will also help the microbiologist to choose selective and other media which might be particularly useful. Knowledge of the normal flora of various regions may also allow one to judge more readily whether or not a given isolate is significant. For example, Propionibacterium in a blood culture most often represents "contamination" from the patient's skin. Conversely, the presence of a particular organism in blood cultures may also suggest the portal of entry for the bacteremia.

Table 1 indicates the incidence of certain anaerobes as normal flora at various sites in humans. Additional data on anaerobes as indigenous flora will be found in Rosebury's classic book (39).

## Clinical Background; Incidence of Anaerobes in Infection

As indicated in the introduction, anaerobes may cause any type of infection in man. There are a number of infections, however, in which anaerobic bacteria are the predominant pathogens or are commonly found; these are listed in Table 2. Where 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 bacteriological 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 2 incorporate inaccuracies related to uncertainty about the clinical significance of isolates and to selection of certain types of specimens which would certainly contain elements of the normal flora. In this type of study, of course, the incidence of anaerobes may be falsely high.

TABLE 2. INFECTIONS COMMONLY INVOLVING ANAEROBES

| | Incidence (%) | Proportion of Cultures Positive for Anaerobes Yielding Only Anaerobes | Reference |
|---|---|---|---|
| Bacteremia | 20 | 4/5 | 60 |
| Central Nervous System | | | |
| Brain abscess | 89 | 2/3 | 26 |
| Extradural or subdural empyema | 10 | | 56 |
| ENT-Dental | | | |
| Chronic otitis media<br>Chronic sinusitis<br>Dental and oral infections | 52 | 4/5* | 23<br>33 |
| Thoracic | | | |
| Aspiration pneumonia | 93 | 1/2** | 9 |
| Lung abscess | 93 | 2/3 | 10 |
| Bronchiectasis<br>Empyema (non-surgical) | 76 | 1/2 | 11 |
| Intra-abdominal | | | |
| Intra-abdominal infection (general) | 86 | 1/10 | 22 |
| Liver abscess (pyogenic) | 50-100 | 2/3 | 2,42 |
| Appendicitis with peritonitis | 96 | 1/100? | 1 |
| Other intra-abdominal infection (post-surgery) | 93 | 1/6 | 24 |
| Obstetrical-Gynecological | | | 41 |
| Vulvovaginal abscess | 74 | 1/2 | |
| Salpingitis and pelvic peritonitis | 56 | 1/5 | 16,17 |
| Tubo-ovarian and pelvic abscess | 92 | 1/2 | 57,58 |
| Septic abortion and endometritis | 73 | 1/5 | |
| Postoperative wound infection | 67 | 1/4 | |
| Total | 72 | 1/3 | |
| Soft Tissue and Miscellaneous | | | |
| Gas gangrene (anaerobic myonecrosis) | | | 3,13 |
| Gas-forming cellulitis | | | 34,36 |
| Peri-rectal abscess | | | |
| Breast abscess | | | |

* 23/28 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 2/3 of the time.

## Bacteriological Clues to Anaerobic Infection

Certain hints suggesting to the bacteriologist that a given specimen is likely to contain anaerobic bacteria are noted in Table 3.

TABLE 3. CLUES TO ANAEROBIC INFECTION

1. Foul odor to specimen
2. Location of infection in proximity to a mucosal surface
3. Infections secondary to human or animal bite
4. Gas in specimen
5. Previous therapy with aminoglycoside antibiotics (kanamycin, neomycin, gentamicin, in particular)
6. Black discoloration of blood-containing exudates; these exudates may fluoresce red under UV light (B. melaninogenicus infections)
7. Presence of "sulfur granules" in discharges (actinomycosis)
8. Unique morphology on Gram stain
9. Failure to grow, aerobically, organisms seen on Gram stain of original exudate
10. Growth in anaerobic zone of fluid media or of agar deeps
11. Growth anaerobically on media containing 100 μg/ml of kanamycin, neomycin, or paromomycin (or medium also containing 7.5 μg/ml of vancomycin, in the case of Gram-negative anaerobic bacilli)
12. Characteristic colonies on agar plates anaerobically (e.g., F. nucleatum and C. perfringens)
13. Young colonies of B. melaninogenicus may fluoresce red under ultraviolet light (blood agar plate)

## SPECIMEN COLLECTION

Since anaerobes may cause or contribute to infections of all types, it is clear that all specimens which are free of "contamination" with normal flora should be cultured anaerobically. The following specimens should not routinely be set up for anaerobic culture -- throat culture, gingival swab, gastric contents, small bowel contents, feces, coughed sputum (obtain specimen by transtracheal aspiration or direct lung puncture instead), voided urine (obtain specimen by percutaneous suprapubic bladder aspiration), and vaginal swabs. The above principle can be applied to other uncommon types of specimens which may be submitted. Exceptions will have to be made in certain instances. For example, in suspected "blind loop syndrome", quantitation of anaerobic and facultative or aerobic flora of the small bowel or afferent gastric loop contents may be very important diagnostically and in guiding therapy.

Proper collection, i.e., with care to avoid inclusion of normal flora, cannot be over-emphasized because indigenous anaerobes are often present in such large numbers that even minimal contamination of a specimen with normal flora can give very misleading results. Collection methods which avoid contamination with normal flora are listed in Table 4.

TABLE 4. RECOMMENDED SPECIMEN COLLECTION METHODS FOR ANAEROBIC CULTURE

| | |
|---|---|
| Pulmonary | Percutaneous transtracheal aspiration or direct lung puncture |
| Pleural | Thoracentesis |
| Urinary tract | Suprapubic percutaneous bladder aspiration |
| Abscesses | Needle and syringe aspiration of closed abscess. Use of swabs is much less desirable |
| Female genital tract | Use culdocentesis to obtain specimens, when possible, after decontaminating the vagina |
| Uterine | Use syringe and small plastic catheter to aspirate through a decontaminated cervical os (contamination from endocervical canal is unavoidable) |
| Sinus tracts or draining wounds | Aspiration by syringe and small plastic catheter introduced as deeply as possible through decontaminated skin orifice. Specimen obtained at surgery from depths of wound or underlying bone lesion always preferable |

Coughed sputum is unsuitable since it has become contaminated with normal flora anaerobes on its passage through the mouth and pharynx. For the same reason, bronchoscopic specimens or those obtained by naso-tracheal tube suctioning also should not be cultured anaerobically. The sampling tube may easily become contaminated on its downward path. Adequate pulmonary specimens for anaerobic culture can be obtained by transtracheal aspiration (TTA), thoracentesis, and direct percutaneous needle puncture and aspiration of lung infiltrates or abscesses. Tracheostomy specimens may be suitable.

Voided urine specimens are unsuitable for anaerobic culture because the distal portion of the urethra and the meatus are colonized with normal flora which will contaminate urine passing through these areas. If a suprapubic bladder catheter or cystostomy or nephrostomy tube is in place, reliable urine specimens may be collected from these sites.

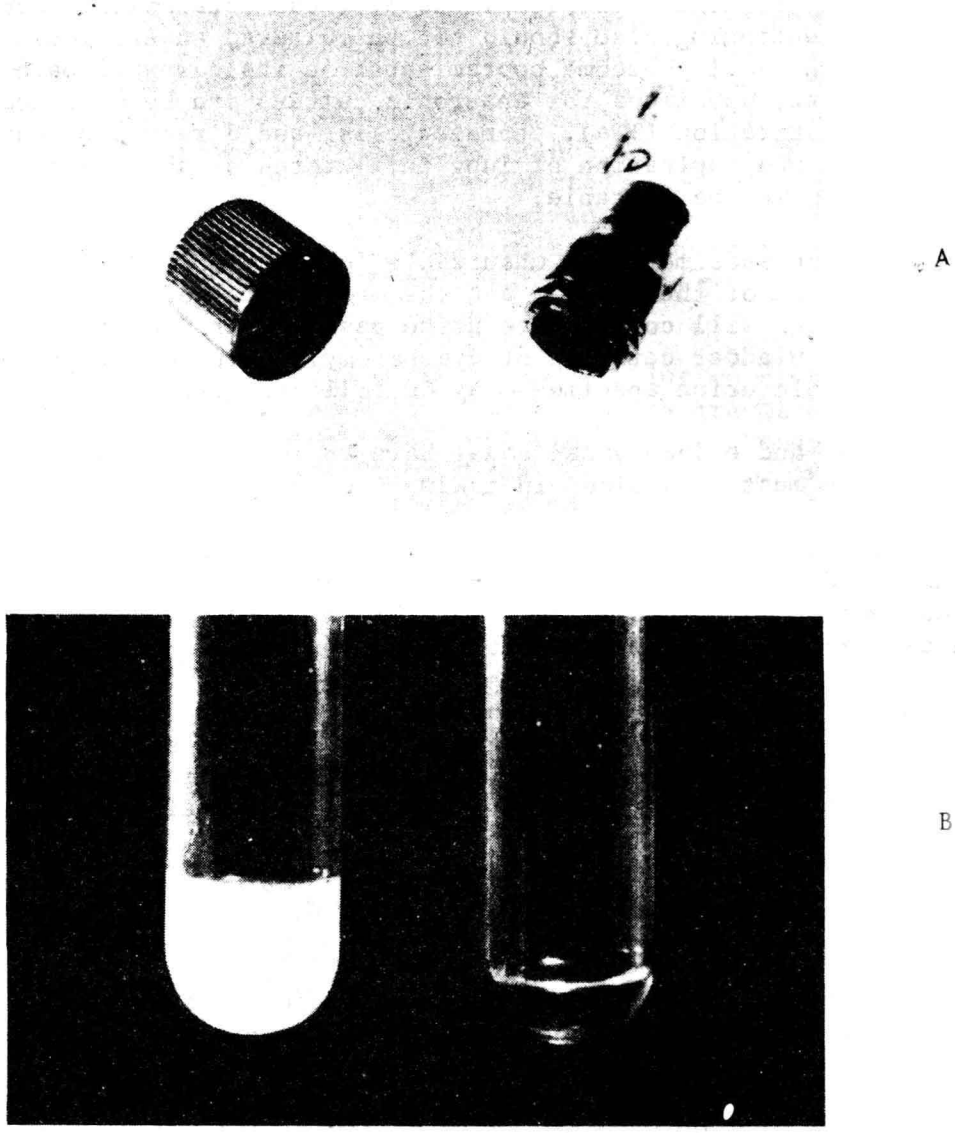
The vagina and endocervical canal have an indigenous population of anaerobes which must be avoided in taking uterine cultures.

Specimens which are normally sterile such as spinal fluid, blood and joint fluid may be collected in the usual fashion after thorough skin decontamination. Special anaerobic blood culture media are available and are discussed in a separate section.

In general, material for anaerobic culture is best obtained using a needle and syringe. All air must be expelled from the syringe and needle. Use of swabs is a poor alternative for a number of reasons, including excessive exposure of the specimen to the deleterious effects of oxygen and drying.

## SPECIMEN TRANSPORT

Another crucial factor affecting the ultimate success of anaerobic cultures is the transport of specimens. The bacteria must be protected from the lethal effects of oxygen during the period from time of collection of the specimen until it is set up anaerobically in the laboratory. Specimens should be placed, immediately following collection, into an anaerobic transporter, such as a modification of that described by Attebery and Finegold using a closed technique (6). (See Figure 1). This consists of a double-stoppered tube containing oxygen-free $CO_2$ or $N_2$ and a broth or agar indicator system. The specimen is injected through a recessed rubber stopper, avoiding introduction of air. The material can then remain in this anaerobic environment until just prior to media inoculation, when it is removed using a needle and syringe. Similar versions of this tube are now available commercially (Anaport vials, Scott Laboratories). An alternative open tube technique is illustrated and described in Figure 2. A syringe and



A

B

Figure 1. Transport tubes for anaerobic specimens. A) Liquid specimens can easily be injected through the recess in the butyl rubber stopper. B) The tubes contain no air, having been flushed out with oxygen-free gas before sterilization. Both tubes contain resazurin indicator. The tube on the left has an agar base. The other tube contains a reducing broth.

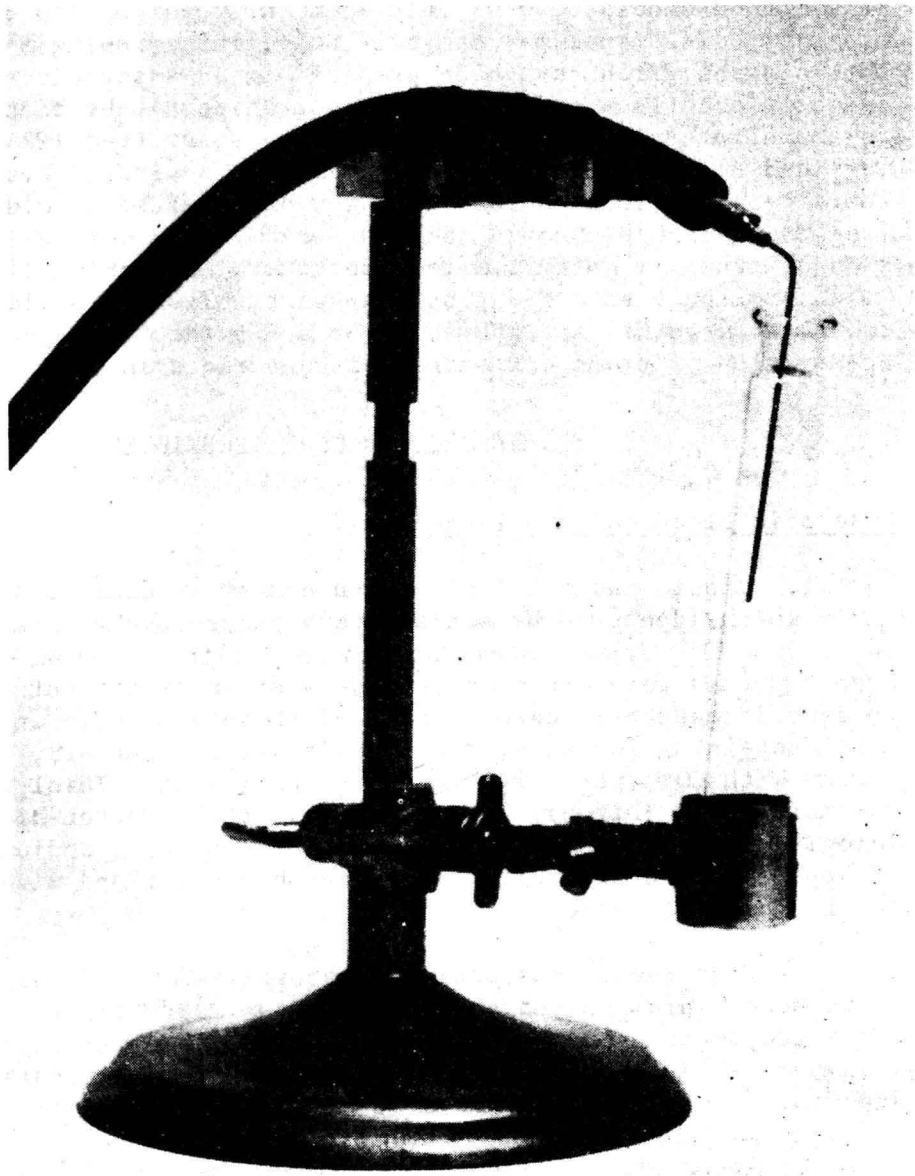


Figure 2. The "open" method of collection consists of removing the stopper from a sterile tube and placing a cannula in the tube so that a continuous flow of oxygen-free gas may be used to prevent ingress of air.

The tube remains anaerobic. Fluid specimens or solid specimens that can be placed within the tube can be transported to the laboratory in this fashion.