Can We Afford To Do Anaerobic Cultures and Identification? A Positive Point of View

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Because anaerobic bacteria cause significant human infections that require specific therapy and because anaerobes are resistant to certain antimicrobials, the isolation, identification, and determination of antimicrobial susceptibilities are as important for these bacteria as they are for other pathogenic bacteria. Anaerobic culturing can be made cost-efficient by strict adherence to several principles, including the selective culturing of only appropriate general specimens that are uncontaminated by normal flora (this can be achieved by educating physicians and nurses in recognizing likely sources of anaerobic infection); rapid transport of specimens and use of appropriate transport systems; use of a system of rejection and notification when inappropriate or when multiple specimens have been received; and use of a logical algorithm for determining the degree of isolation and identification to be performed, according to the numbers and types of organisms present. Testing of all significant gram-negative organisms for the production of \( \beta \)-lactamases can provide an early indication of antimicrobial susceptibility, and actual testing limited to screening of three or four drugs can be performed on selected isolates by using a rapid and simple method such as the Etest (AB BIODISK, Solna, Sweden). Although the number of anaerobic bacteremias has declined since the 1970s, this number has plateaued in recent years, and these infections are life-threatening. Routine culturing of blood for anaerobes is still indicated in many institutions because of the unpredictable clinical sources of some bacteremias and the improved yields of both anaerobes and some streptococci when anaerobic blood culture systems are used.

In an era of cost containment, there is a tendency for laboratories to cut back on the performance of those tests that are considered more “esoteric” than others, especially if they are costly to perform; anaerobic culturing and identification might be considered to be in this category by some laboratory personnel. However, the worth of a test should be measured by its diagnostic and therapeutic value to the physician and the patient. The cost of doing anaerobic cultures must be balanced by the potential costs of not doing them—i.e., misdiagnosis and mistreatment, leading to prolonged morbidity and hospitalization and even death.

Ample literature (including the proceedings of two prior symposia [1, 2] like this one) has accumulated over the past 20 years, and the following findings have been documented: (1) anaerobes are significant causes of human infections; (2) anaerobic infections are associated with significant morbidity and mortality; (3) improper diagnosis and treatment of anaerobic infections can have dire consequences; (4) broad-spectrum empirical antimicrobial therapy is expensive, may not be effective, and promotes the development of resistance to antimicrobials; and (5) resistance to antimicrobials is prevalent among anaerobes and is increasing; susceptibility patterns among certain anaerobes are much less predictable than they used to be. In spite of these facts, the real value of anaerobic culturing may be difficult to determine. One major reason for this difficulty is that anaerobic infections are often actually mixed infections involving a number of anaerobic, facultative, and aerobic bacteria. It may be difficult to determine which organisms are playing a primary role, and a response to antimicrobial therapy may be difficult to interpret because of the important role of surgery in many cases and the fact that multiple organisms may be susceptible to the antimicrobial(s) used. Therefore, it is only reasonable to allow the clinicians to determine how valuable anaerobic culturing will be to them in relation to the anticipated antimicrobial and surgical therapy and allow them to selectively order anaerobic cultures when they believe these cultures are needed (an exception is the routine performance of anaerobic blood cultures, which will be discussed later). It is incumbent upon laboratory personnel to find ways to make such culturing as efficient and economical as possible while still providing information that is clinically useful. Close and frequent communication with clinicians will help make this possible.
Laboratories can make anaerobic culturing more cost-efficient by adhering to the following principles: (1) perform selective rather than routine culturing on the basis of specific orders from physicians; (2) restrict culturing to those specimens collected so as to avoid contamination with the abundant normal anaerobic flora of the body; (3) use specimen transport methods that will preserve the anaerobic bacteria and prevent the proliferation of the less fastidious facultative organisms that may be present; (4) use ‘‘rejection criteria,’’ which will prevent the culturing of specimens that do not meet standards specified in items (2) and (3) above; (4) use a selective approach to the analysis of the culture that is based on the kind and number of isolates present and the specimen type; (5) identify isolates to the level useful to the physician by using rapid and inexpensive methods; and (6) selectively provide cost-efficient and useful antimicrobial susceptibility information, including the results of β-lactamase testing as well as actual susceptibility testing, as rapidly as possible.

**Specimen Selection and Collection**

The types of infections in which anaerobes often play a role, the types of specimens that are acceptable for culture, and suitable methods of collection have been extensively discussed and well described in the past [3, 4] and will not be discussed further herein. Several adequate anaerobic transporters are commercially available; those requiring the use of swabs should be avoided, since swab specimens are rarely, if ever, adequate. Stringent anaerobic culturing requires that abundant amounts of purulent fluid that is not contaminated with normal flora be obtained by needle aspiration. It is simply too easy to collect inadequate and/or contaminated specimens with a swab and/or to transport them suboptimally. Surgical tissue specimens are suitable and may simply be placed in rubber (removable)–stoppered tubes that have been gassed out with carbon dioxide. Anaerobes in these specimens are likely to be protected by the reduced environment within the infected tissue.

In the current era, laboratory personnel should develop criteria for rejecting specimens that are not acceptable for anaerobic culturing. This is best done by obtaining the input of clinicians who are knowledgeable about infectious diseases and by conveying an attitude of cooperation and helpfulness rather than confrontation. Clinicians should understand that a rejection policy will improve the quality of results reported to them and improve efficiency in the laboratory. Clinicians should be notified promptly and given a logical reason when a specimen is rejected, and they should be provided an opportunity to respond before the specimen is discarded. This communication should be informative and educational rather than punitive. ‘‘Precious’’ specimens (CSF, joint fluids, other normally sterile body fluids obtained by needle aspiration, and tissues and/or fluids obtained during surgery) should never be discarded without culturing before discussion with the clinician.

A recent review of 1 month’s experience at the Mayo Clinic (Rochester, MN) showed that ~15% of specimens submitted for anaerobic culturing were rejected by the laboratory. Most of these specimens (56%) were inappropriately collected and were swabs. The next most frequent problem (13% of specimens) was that specimens were collected from sites normally colonized by anaerobes (i.e., the vagina or upper respiratory tract). Seven percent of specimens were rejected because the source itself was inappropriate (i.e., stool or voided urine). Six percent of rejected specimens had been mistakenly ordered for culture. In the majority of these cases of rejected specimens, the clinician concluded that there was no need to have an anaerobic culture done after all, and there was no confrontation with the laboratory.

Laboratory personnel may also want to communicate with physicians who submit multiple specimens from the same site (these are usually surgical specimens, frequently from patients with orthopedic conditions) at the same time. It should be pointed out that cultures of such specimens almost always yield the same results and are very expensive to perform. A solution to the possible argument by a clinician that there may be different organisms in the different sampling areas of the same site would be to offer to pool the samples into one culture, thereby efficiently screening for all organisms present.

**Culture and Identification**

Cost-effective anaerobic bacteriology requires that specimens be processed as efficiently as possible. Use of selective and differential media is essential, and these media include bacteroides bile-esculin (BBE) agar, laked kanamycin-vanco-mycin (LKV) blood agar, and phenylethyl alcohol blood agar [4]. Use of these media allows rapid detection and separation of anaerobes from facultative organisms in the mixed flora that is often present. Anaerobic culturing can be adequately carried out by using anaerobic jars, but quality control measures must be applied to ensure that jar lids and catalysts are functioning properly. Although many anaerobes will grow within 24 hours of incubation, 48 hours are probably required to ensure that all organisms are recovered. Some more-fastidious organisms, such as species of Porphyromonas, may require 5–7 days of incubation. Pigment production may also require longer periods of incubation. While anaerobic glove boxes may be useful in certain circumstances, they are not essential for general anaerobic bacteriology, and the lack of these devices should not discourage laboratory personnel from performing these cultures.

The extent of efforts to isolate and identify anaerobes should be based on the growth on the primary culture plates. If five or more anaerobes appear to be present (and there are usually multiple facultative organisms in such cultures as well), there is usually no benefit to the clinician in identifying individual isolates, and a report of mixed flora may be sent. If one isolate type appears to predominate, laboratory personnel may choose
to identify only this isolate, especially if a “partial identification” or “rapid identification” scheme can be used and if a clinically significant and potentially resistant member of the “Bacteroides fragilis group” is suspected. If the culture contains more than two anaerobes (or more than a total of three organisms), partial identifications may be performed—i.e., the identification is limited to subculture for confirmation that the organisms are obligate anaerobes and to description by gram staining only (e.g., an anaerobic gram-positive bacillus). If cultural and microscopic morphologies suggest a genus, that notation may also be added (i.e., “. . . resembling Clostridium, Fusobacterium, Prevotella (Bacteroides) melaninogenica group [if pigmented], etc.”). A rapid β-lactamase test (Cefinase, BBL Microbiology Systems, Cockeysville, MD) should also be performed on all gram-negative bacilli, and the result included (i.e., “anaerobic gram-negative bacillus, β-lactamase positive”). Even this limited information may be useful to the clinician in mapping a strategy for treating a mixed infection due to multiple organisms.

More-complete identifications should be as rapid and abbreviated as possible. Clostridium perfringens can be identified on the basis of microscopic morphology, the presence of a double zone of hemolysis surrounding colonies on blood agar, and lecithinase production on egg-yolk agar. The “B. fragilis group” can be identified by typical growth on BBE and LKV agars, diagnostic disk antibiograms, and β-lactamase production. It may seldom be necessary for most clinical laboratories to identify anaerobic cocci to a level beyond the organisms’ cultural and gram-stain characteristics, and such isolates could legitimately be reported at the genus level (i.e., “Peptostreptococcus” or “Veillonella”).

Most complete identifications to the species level can be achieved by using simple and rapid kits that detect preformed enzymes and that are commercially available (Anldent [Analytab Products, Plainview, NY] or the RapID-ANA II System [Innovative Diagnostic Systems, Atlanta]). These kits are comparable in performance and cost (~$5–$6 per individual test strip). Many clinical laboratories will not pursue difficult identifications beyond this stage; an incomplete identification plus antimicrobial susceptibility results, when indicated, will suffice in most clinical situations.

Difficult identifications may be pursued in referral laboratories with use of additional rapid biochemical kits (e.g., Minitek; BBL) and gas-liquid chromatography (GLC). A recent review of our experience at the Mayo Clinic Anaerobe Laboratory (which I would classify as a referral laboratory) indicates that of the cultures that yielded anaerobes, 19% were reported only as mixed flora. For the other cultures from which isolates were identified, 52% of the isolates were reported as partial identifications, and 48% were reported as more complete identifications. Approximately 80% of the isolates in the latter group required only use of a rapid kit for identification, whereas 20% required additional biochemical and/or GLC testing. These data indicate that even in a referral laboratory setting, the evaluation of anaerobic cultures and identification procedures may be efficiently streamlined.

Cost of Anaerobic Cultures

How cost-efficient is culturing of anaerobes, even when policies that streamline the process are used? As previously mentioned, any conclusions drawn from analysis of these data must be tempered by consideration of the costs in terms of both money and the patient morbidity that may result if these cultures are not performed. Such costs are difficult to estimate but are potentially great, considering that even one additional day in an intensive care unit for treatment of an incorrectly diagnosed infection can cost many thousands of dollars. Rough calculations based on the estimated laboratory costs of Current Procedural Terminology (CPT)—coded procedures performed at the Mayo Clinic and on the usual Midwestern market fees (as determined by Mayo Medical Laboratory surveys) for these tests suggest that a small profit (~$3.00) can be made on cultures that do not yield anaerobes or from which only mixed flora are reported (i.e., no processing beyond culture, incubation, and inspection of the plates is done). A somewhat greater profit (~$10–$15) can be made from partial identification of anaerobic isolates. This is counterbalanced by losses of $10–$13 per identification of isolates with use of a rapid kit alone and losses of $4–$5 per isolate when additional tests, including GLC, are performed. The fact that Medicare reimbursement is considerably lower than market fees must also be taken into consideration.

Obviously, the balance figure for performing anaerobic cultures and identifications will vary considerably depending on the yield of the cultures, the degree to which the isolates are identified, and the amount of Medicare reimbursement received. Given all of these factors, it was possible to estimate that a laboratory that processed 750 anaerobic cultures per month, most of which required minimal processing and most of which yielded isolates that were only partially identified or identified with use of rapid kits alone, could realize a profit of $1,000–$2,000, providing Medicare reimbursement represented only a small portion of the fees collected.

These calculations are so general and speculative that they may be difficult to apply to any individual laboratory. However, they do suggest that anaerobic culturing need not be considered too costly for the average laboratory to perform or even necessarily a money-losing activity. Indeed, it is even likely that small profits can be made if proper principles of specimen selection and collection are adhered to and the streamlined processing described above is used. While the proper provision of clinically useful culture results should be the overriding principle of every anaerobe laboratory, avoidance of monetary losses or even generation of small profits will help keep cost-
conscious administrators from the door and ensure the continuation of this vital activity.

### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of clinical anaerobic isolates can also be made cost-efficient by streamlining the process. Because of the increasing unpredictability of susceptibility patterns and the desire on the part of clinicians to be aware of all susceptibility results for patients who are critically ill, timely testing should be available for anaerobes isolated from the following sites: blood and other normally sterile body fluids, bones and joints, surgical tissues, and needle aspirates from any closed abscess. Testing for β-lactamase production should be performed on all gram-negative bacilli and may be used in the testing algorithm outlined (in table 1).

The Etest (AB BIODISK, Solna, Sweden) is an efficient method for testing clinical isolates because of its great flexibility [5]. Even though the individual antimicrobial strips are costly (~$2 per strip), the ability to place up to four strips on a single agar plate and design a logical and efficient process (such as that outlined in table 1) makes the entire procedure relatively inexpensive (~$30 per organism). The Etest is also more practical than agar dilution, which requires a minimum of 15–20 organisms in a batch to be cost-effective, and more flexible than broth microdilution, in which numerous antimicrobials are included on each microplate. The cost of these plates will vary considerably, depending on whether they are prepared in-house or purchased commercially.

Other antimicrobials may be added if an organism is resistant to multiple agents or if a clinician wishes to use another agent. All antimicrobials tested should have had susceptibility breakpoints and quality control MIC ranges determined for a standard set of anaerobes, as established by the National Committee on Clinical Laboratory Standards (NCCLS; see NCCLS Document M11–A3). While some laboratorians may disagree with testing some anaerobes and antimicrobials for which the susceptibility patterns are considered predictable, increasing resistance and the desire of clinicians to have specific results for current isolates provide a rationale for this approach. In addition, knowledge of specific susceptibilities of an anaerobe can lead to substantial cost savings in treatment. For instance, typical costs per day for use of some inexpensive parenteral agents are as follows: penicillin, $3.50; clindamycin, $61; metronidazole, $3; gentamicin, $0.20. These figures should be compared with those for more expensive agents, which are often used for empirical treatment of anaerobic infections, including ampicillin/sulbactam ($41), imipenem ($82), and piperacillin/tazobactam ($61) (source: Mayo Medical Center Pharmacy).

### Anaerobic Blood Cultures

The need to perform routine anaerobic blood cultures has become a particularly contentious issue (except in the case of children, where there is a consensus that such cultures should not be performed routinely). During the 1970s, anaerobic bacteremias accounted for as much as 25% of all bacteremias at some medical centers. However, in recent years that number has dropped dramatically and is as low as ≤1% in some institutions [6], although at many centers it is more likely 3%–5% [7, 8]. At the Mayo Clinic, the average number of such bacteremias per year dropped from 117 in 1974–1978 to 58 in 1989–1993 and has remained steady at the latter figure since then. There has been a call to discontinue the routine performance of anaerobic blood cultures and depend on selective schemes, such as requiring a specific order by clinicians when they recognize a typical setting for anaerobic infection or culturing blood from patients most likely to develop anaerobic infection, such as those being treated by surgeons or obstetricians-gynecologists [6, 9].

The problem with this approach is that now a significant number of atypical anaerobic infections occur that would not have been predicted by many physicians. Recent studies have found that 16%–28% of anaerobic bacteremias would not have been predicted by ordering physicians because these bacteremias originated from unsuspected sources, and many of the patients had malignancies and/or were immunosuppressed [8, 10]. Our own estimate at the Mayo Clinic is that ~50% of the anaerobic bacteremias that occurred in 1995–1996 would not have been predicted. In addition, experience at several institutions where experiments with selective ordering by physicians or based on the type of hospital service were performed has shown that this approach failed to accurately target the appropriate patients. Furthermore, two studies conducted at Mayo institutions indicated that, with the blood culture systems studied, not only were anaerobes recovered at a higher rate in the anaerobic bottles, but the rate of recovery of some streptococci was also higher [11, 12]. In one of these studies, the optimum recovery of all organisms was achieved with a combination

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**Table 1.** Suggested scheme for susceptibility testing of anaerobes with use of the Etest.

<table>
<thead>
<tr>
<th>For anaerobic gram-negative bacilli</th>
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<tr>
<td>If β-lactamase positive, test ampicillin/sulbactam (piperacillin/tazobactam if resistant to ampicillin/sulbactam), imipenem, metronidazole, and clindamycin.</td>
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<tr>
<td>If β-lactamase negative, test penicillin, metronidazole, and clindamycin.</td>
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For nonsporing gram-positive bacilli, gram-positive cocci, and *Clostridium perfringens*, test penicillin, clindamycin, and metronidazole. For other *Clostridium* species, test penicillin, clindamycin, metronidazole, and imipenem.
of the Isolator (Wampole Laboratories, Cranbury, NJ) and an anaerobic bottle.

The decision to perform routine anaerobic blood culturing should be made at individual institutions on the basis of the experience at those institutions and on the specific blood culture system in use. The decision should not be made lightly, should not be based solely on economic factors, and should be made with the realizations that the predictability of anaerobic bacteremias is rather low, that the attributable mortality from anaerobic bacteremias is high (19%), and that inappropriate treatment of anaerobic bacteremias has dire consequences [13]. Clinicians should also be aware that several advocates of the discontinuation of routine anaerobic blood culturing have returned to routine culturing because of dissatisfactions with the selective approach.

References