CUMITECH 1

Cumulative Techniques
and Procedures in
Clinical Microbiology

Blood Cultures

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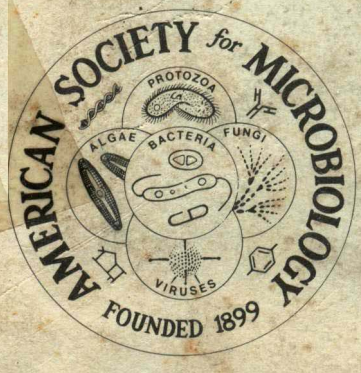

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The purpose of the Cumitech series is to provide consensus recommendations by the authors as to appropriate state-of-the-art operating procedures for clinical microbiology laboratories which may lack the facilities for fully evaluating routine or new methods.

The procedures given are not proposed as "standard" methods.

Blood Cultures

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GENERAL

Clinical indications. Blood cultures are collected whenever there is reason to suspect clinically significant bacteremia. Bacteremia may present in several ways.

(i) As a classical septicemia with invasion of the blood stream from a focus of infection accompanied by malaise, raised pulse and temperature, and chills followed by fever and prostration.

(ii) As "sepsis" in the newborn and immunologically compromised adult or child, sometimes with minimal clinical signs, such as failure to suck or thrive in the newborn, but with a rapid downhill course.

(iii) As part of a more chronic infection such as disseminated gonococcal disease or chronic meningococcal bacteremia.

(iv) As part of a "spillover" in certain severe infections such as meningitis, pneumonia, deep-seated abscesses (e.g., liver or kidney), or biliary infections. The presenting signs are those of the primary infection.

(v) As the result of a localized intravascular infection, such as on a heart valve (bacterial endocarditis) or in an infected and sometimes thrombosed blood vessel. In subacute bacterial endocarditis, there may be only low-grade intermittent fever and signs of peripheral emboli.

(vi) As part of the natural history of certain multisystem infections such as enteric fever, leptospirosis, or brucellosis. These often present as fever of unknown origin.

(vii) With sudden introduction of many bacteria into the blood stream by trauma to heavily infected areas (e.g., with urinary tract catheterization) or from contaminated external sources such as intravenous infusions. The patient may have symptoms and signs ranging from chills and fever to severe endotoxic shock.

(viii) As an asymptomatic, brief, and rapidly cleared introduction of bacteria into the blood stream from minor traumatic events, e.g., members of the normal microbial flora at the time of tooth extraction or endoscopic procedures.

In many situations, blood cultures are the only immediate source of the etiological agent of severe or life-threatening infections, and the diagnosis depends on them. In other cases, blood cultures indicate the severity and extent of dissemination of an infection. They are therefore among the most important tests that can be made in the clinical microbiology laboratory, and good technique, well-selected procedures, and avoidance of contamination are especially important for adequate patient care.

Time and number of cultures to be collected. Bacteremia is often continuous in the case of intravascular infections such as endocarditis, in severe uncontrolled infections, and in the acute phases of infections of the reticuloendothelial system. In these situations, it is apparently unimportant whether several blood cultures are taken within a brief period or whether they are spaced over a longer time. The determining factor is the clinical urgency of the situation and the need to collect samples before treatment begins. In other cases, bacteremia is intermittent and may precede episodes of fever and chills by about 1 h. In such situations, collection of blood cultures should be spaced at

intervals, with some being collected at the first sign of fever. In most adult infections, a maximum of only three to four 10-ml blood culture samples in the initial 24-h period need be collected (1, 17). Over 90% of positives will be obtained with this number, and decisions as to further blood cultures should depend on these results. In patients with severe infections already on chemotherapy, more blood cultures may be required, and some should be taken when the blood level of antimicrobial agent is lowest.

A general guide to blood culture collection in adults is as follows. (i) For severe life-threatening clinical septicemia: two 10-ml samples taken by separate venipuncture (preferably one from each arm) should be collected immediately before starting treatment. (ii) For suspected SBE or low-grade intravascular infection: three 10-ml samples should be taken within the first 24 h at intervals spaced no closer than 1 h. These should include two collected at the earliest sign of a febrile episode. (iii) For suspected bacteremia of unknown origin in patients already on therapy: if therapy cannot be suspended for a few days, four to six cultures should be drawn within the first 48 h. Cultures should be taken immediately before the next dose of antimicrobial agent if the patient is receiving intermittent parenteral therapy.

The volume of blood drawn from small children should be determined by the physician. In the case of infants, 1- to 2-ml samples of blood are usually collected. Two blood cultures usually suffice for diagnosing sepsis of the newborn.

RECOMMENDED BASIC PROCEDURE

Information required. The laboratory needs information on the suspected diagnosis and severity of the infection and on the nature, timing, and dose of any chemotherapeutic agent that is being given. If the laboratory personnel collect the blood cultures, the urgency and requested times of collection should be stated.

Skin preparation and collection of blood. When the optimal venipuncture site has been selected, careful attention to skin disinfection is essential to reduce the incidence of contaminated blood cultures. In the case of known hypersensitivity to iodine, the skin is prepared exclusively with 70% isopropyl or ethyl alcohol. Otherwise the skin is cleansed with 70% alcohol and then swabbed concentrically with 2% tincture of iodine. Alternatively, cleansing and decontamination may be accomplished by vigorous application of a povidone-iodine preparation. Potentially contaminated diaphragm tops of culture bottles or collection containers are simultaneously swabbed with alcohol or an iodine preparation, and surplus decontaminant is removed before injecting blood. In each case, the disinfecting agent should be allowed to act for approximately 1 min. Once disinfected, the venipuncture site should not be probed with a finger unless it has been similarly decontaminated or unless sterile surgical gloves are worn.

Blood may be drawn with a syringe and needle or, preferably, through a transfer set or double-ended needle directly into evacuated bottles of culture medium. An evacuated anticoagulant-containing collection tube may be used with subsequent distribution to culture media in the laboratory. Containers should be held below the level of the venipuncture needle to avoid the risk of reflux. The needle is inserted directly into the vein. If the vein is missed and a second venipuncture is required, a new needle or transfer set should be used. These procedures are designed to reduce the risk of contamination with skin flora. After collection, the contents of the bottles or tube are well mixed to avoid clotting. After the venipuncture, iodine should be removed from the skin with alcohol-soaked swabs. Adhesive or tight dressings should never be applied over skin treated with tincture of iodine because iodine burns may result.

Usually, 10 ml of blood in adults and 1 to 2 ml in infants suffices for culture. The blood is distributed between aerobic and anaerobic culture bottles at the time of collection or in the laboratory. The ratio of blood to medium should be 1:10 to 1:20.

Anticoagulant. Anticoagulant must be used for routine blood cultures to prevent clotting in the medium or the collection device. The best anticoagulant presently available for this purpose is sodium polyanetholsulfonate (SPS), which is also anticomplementary and inactivates leukocytes and certain aminoglycoside and polypeptide antibiotics (6, 11, 15). Blood culture yields with this agent are considerably greater than in its absence (13). Oxalate, citrate, and ethylenediaminetetraacetic acid are unsuitable anticoagulants because they inhibit some organisms. Fluoride is highly toxic to bactēria.

Culture media used for direct collection of blood should contain 0.025 to 0.05% SPS. Vacuum collecting tubes for transport to the laboratory should contain 1 ml of 0.25 to 0.5% SPS for 10 ml of blood. The volume of anticoagulant in the collecting tube should be reduced proportionately if less than 10 ml of blood is to be drawn.

Culture media and atmospheric conditions. Two evacuated screw-capped bottles containing 50 to 100 ml of medium and 0.1 atm (10%) CO_2 are recommended for the basic procedure when 10 ml of blood can be obtained. One is for aerobic and the other is for anaerobic culture. The bottles should have screw caps with underlying rubber or plastic diaphragm seals permitting access with a hypodermic needle without removal of the diaphragm from the bottle. With cultures from infants, 10- to 20-ml volumes of media are appropriate for 1 to 2 ml of blood, although 50-ml commercial bottles are acceptable. One or two sample bottles of each batch of culture medium should be incubated for 72 h to exclude contamination of the batch before it is released for routine use. The "aerobic" sample bottles should be vented as described below.

Aerobic culture. A bottle of soybean casein digest broth (e.g., Tryptic or Trypticase soy broth), brain heart infusion broth, Brucella, Columbia, or other good multipurpose broth is recommended for the aerobic culture. Preprepared media in evacuated bottles containing CO_2 are available from many supply houses. After 5 ml of blood is added, the bottle should be vented to replace the residual vacuum with air so that strict aerobes such as *Pseudomonas* spp. can grow. This is done by puncturing the diaphragm with a sterile cotton-plugged needle. Unless the bottle is placed in a CO_2 incubator, the needle should be removed when the pressure has equilibrated to avoid loss of CO_2.

Anaerobic culture. Prereduced media in vacuum bottles containing 0.1 atm (10%) CO_2 should be used. The media recommended for aerobic culture are appropriate but should include 0.05% cysteine to help maintain an Eh of −100 mV or less and permit growth of certain rare thiol-requiring organisms that have been described (3). The bottle should not be vented.

Additions to media to inactivate antimicrobial agents. Commercially obtained penicillinase may be added to the media in the amounts recommended by the manufacturers if the patient is receiving any of the penicillins. Since this carries some risk of contamination, penicillinase solutions should be sterility tested weekly and at the time of use by plating the same amount added to the blood culture bottles onto a blood agar plate. SPS itself antagonizes to some extent aminoglycosides and polymyxins. Other additions are not recommended for routine use; the dilution of blood with medium usually suffices to reduce the concentration of antimicrobial agent to below inhibitory levels.

Initial processing in the laboratory. If blood cultures are collected directly into culture medium, no additional processing is needed before incubation except venting the aerobic bottle and possibly adding penicillinase as described above.

Blood that has been drawn into evacuated collection tubes should be transferred to the culture media under closed conditions in the laboratory (5). This may be done with a double-ended needle, a syringe, or a transfer set. Before the transfer is made, the tops of the collection tube and culture bottles are disinfected with 70% alcohol. Transfers and subsequent subcultures are best performed in a quiet room free of air turbulence and with the door closed to reduce the risk of contamination.

Blood cultures should be incubated at 35 C. If infusion of contaminated blood or other products is suspected as a cause of bacteremia, room-temperature cultures (22 C) should also be set up to permit growth of psychrophiles. A direct Gram stain of the infused material may show bacteria in such cases, and direct quantitative plate cultures of the material should be set up at both temperatures.

Inspection, staining, and subculture routines. Blood culture bottles should be inspected daily for evidence of growth. Those received in the morning should be kept in the front of the incubator so that they may be inspected later on the same day. In cases of severe bacteremia due to staphylococci or enteric bacteria, growth may sometimes be detected in 6 h or less, and early inspection may yield valuable clinical data.

Bottles for inspection should be gently removed from the incubator to avoid disturbing sedimented blood and should be examined by transmitted and reflected light for evidence of turbidity, hemolysis, gas production, or bacterial colonies in or on the blood layer. With experience, even small colonies are readily distinguished from the buffy coat on the surface of the blood layer. If evidence of growth is seen, the contents of the bottle are mixed, the top of the rubber diaphragm is decontaminated with 70% alcohol, and about 0.25 ml of contents is aspirated with a needle and syringe. If only a few discrete colonies are seen, they may be removed aseptically by direct aspiration with a sterile capillary pipette to ensure enough bacteria for Gram staining and subculture.

The aspirated material is used to prepare a smear for Gram staining and to seed plates of (i) an enriched agar medium appropriate for isolation of fastidious facultative species, including *Neisseria gonorrhoeae* and *Haemophilus influenzae* (e.g., fortified chocolate agar); and (ii) freshly prepared or prereduced blood agar

medium suitable for growing anaerobic organisms. Other media may be added depending on the types of organisms observed in the smear. For example, MacConkey agar or eosin methylene blue should be inoculated when gram-negative rods are found. Differential media help to facilitate the detection of polymicrobial infection.

After inoculation, the aerobic plate is incubated in a candle jar or in the presence of 5 to 10% CO_2 and the anaerobic plate under anaerobic conditions. Subculture plates are incubated for 48 h before being discarded or for longer if organisms have been seen in the smear but have not been grown.

Certain organisms, particularly *H. influenzae*, pathogenic *Neisseria*, *Bacteroides*, *Fusobacterium*, *Pseudomonas* spp., and *Streptococcus pneumoniae*, may grow in the sedimented blood without producing visible turbidity (2, 8). "Blind" aerobic subcultures should therefore be made routinely on the day after the specimen is received, and anaerobic subcultures should be made after 48 h of incubation. Final aerobic and anaerobic subcultures are made after 5 to 7 days if the culture appears negative. For blind subcultures, it suffices to subculture both bottles to quadrants of two chocolate agar plates, one to be incubated aerobically in CO_2 and the other to be incubated anaerobically.

A total incubation period of 7 days is sufficient for the routine basic procedure.

Preliminary and final reports of negative and positive results of cultures. It is very important that the clinician know the progress of blood cultures in the laboratory. Therefore, preliminary reports are issued on both negative and positive cultures.

In the case of negative cultures, reports such as "Blood culture shows no growth after 3 days incubation: further report to follow" are appropriate when the first blind subcultures show no growth after overnight incubation and there is no macroscopic evidence of growth in the blood culture bottle.

When evidence of growth is found and this is confirmed by Gram staining, a telephoned preliminary report should be given; this should always be followed by a written report stating the general nature of the organisms observed, i.e., gram-negative rods, and that a further report will follow. It should be remembered that culture media sometimes contain small numbers of dead organisms derived from constituent reagents. These are probably species of *Bacillus* and may be seen as occasional irregularly stained gram-negative rods on the Gram stain. If there is uncertainty about the validity of the Gram stain, the culture should be reincubated for another hour or two and the test repeated before a report is given.

Final reports are normally made after the 5- to 7-day subcultures have been sterile after 48 h of incubation. They should be worded to the effect "Final report: no growth after 7 days of incubation." This describes the conditions used and is preferable to a report of "sterile."

Processing positive cultures. It is important to the clinician to have an indication of the probable identity of the infecting species as soon as possible. Specific procedures for presumptive early genus identification direct from blood cultures have been described (16), but it must be remembered that up to 15% of positive blood cultures are polymicrobial, and such procedures should be confirmed with single-colony isolates and standard procedures. Methods such as slide coagulase (clumping), oxidase, and other spot tests can be applied to colonies on initial subculture to aid in early identification.

Preliminary diffusion susceptibility tests may be set up directly from a positive blood culture bottle, although the inoculum will be unstandardized. Reports must indicate that results are preliminary, and the test must be confirmed under the usual standardized conditions. Dilution susceptibility tests should be used for susceptibility tests on clinically significant slower-growing organisms such as viridans streptococci. In cases such as bacterial endocarditis, it is often important to also determine bactericidal end points or the effects of combinations of antimicrobial agents. The procedures described in the second edition of the *Manual of Clinical Microbiology* (10) are recommended for these purposes.

Positive blood culture bottles should not be discarded for at least 7 days, and slants of any organisms isolated should be held for at least 3 weeks in case further studies are needed.

Significance of positive results. As discussed above, the detection of bacteremia and identification of the causal organism are often critically important laboratory procedures. Unfortunately, blood cultures are subject to contamination, particularly with skin bacteria, during collection and occasionally in processing. Even under optimal conditions, a contamination rate of 2 to 3% can be expected. However, contamination cannot be inferred simply because *Staphylococcus epidermidis*, diphtheroids, or propionibacteria have been isolated, because all of these organisms have been shown to cause subacute bacterial endocarditis

or infections on certain prosthetic implants. Furthermore, many species previously considered nonpathogenic have been seen in infections in the immunologically compromised host. Decisions as to the significance of such isolates will take account of clinical probabilities and are helped by knowing whether the organism was isolated from more than one of a series of blood cultures or more than one bottle on a single blood culture. If, for instance, *S. epidermidis* is isolated from two out of four blood cultures, one should test the two isolates simultaneously by a range of biochemical tests and determine their antibiograms. If the isolates are clearly different, their probable clinical significance is reduced.

The laboratory cannot state that a particular isolate is a contaminant and should never report an isolate in this way. The clinician may be advised as to probabilities, but the final decision on the significance of the isolate is his.

LIMITATIONS OF THE BASIC PROCEDURE

The procedure described above has the inherent limitations of basic cultural methods using liquid media. Cultures require at least several hours of incubation to allow growth of enough organisms to be detected by inspection and staining, and the procedures are nonquantitative. Organisms that cannot grow on the basic media used will obviously not be isolated; this applies to genera such as *Leptospira* and *Coxiella*, cell wall-deficient organisms, and probably *Francisella*, for which special media or procedures must be used. It is presently impractical for logistic and economic reasons to use basic routines covering every possible situation. Laboratories should have supplementary procedures available for these special situations.

Some laboratories routinely use pour-plate procedures, in which 1 ml of blood is incorporated in agar media in plates, in addition to liquid media. This permits quantitation and the ability to study early colony morphology and make some immediate spot biochemical tests. We do not feel that the additional yield of useful data is sufficient to recommend this as a basic procedure.

Recently, procedures based on early detection of microbial metabolic activity in blood cultures have been introduced or are being explored. One now available depends on the detection of $C^{14}O_2$ produced in the metabolism of labeled dextrose or amino acids (4, 12). These procedures supplement rather than replace those described here. Their value may be in the earlier detection of bacterial growth and initiation of procedures for identification. Gas chromatography is also under study for the early detection and identification of bacteria in blood or blood cultures. We cannot now assess the value and advantages of this approach.

RECOMMENDED SUPPLEMENTARY PROCEDURES FOR SPECIAL SITUATIONS

Supplementary media and procedures are always indicated when infection due to certain fastidious species is suspected. Modifications to the basic routine should also be considered on an individual basis whenever three or more negative routine blood cultures have been obtained from a patient with clinical evidence of bacteremia. Modifications for some specific situations are listed below.

Brucellosis. A Castaneda-type biphasic medium should be added, and all bottles should be incubated for 21 days with blind subcultures to appropriate media every 7 days.

Systemic fungal infections. Markedly improved yields of yeasts and fungi have been obtained by one of us (J.A.W.) with a biphasic (Castaneda-type) medium of brain heart infusion agar and broth incubated at 30 C for 4 weeks.

Tularemia. Liquid media containing added cystine and dextrose should be included.

Cell wall-deficient bacteria. The role of L-forms in infectious diseases presenting as bacteremia remains unclear. However, there is evidence that some organisms damaged by antimicrobial agents may be more easily isolated in broth of high osmotic pressure and that they will then grow on the usual media (14). The incorporation of 10% sucrose into one of the broth media recommended above is suitable for this purpose.

Leptospirosis. One to three drops of freshly drawn blood taken during the first week of the disease is inoculated into each of several tubes containing 5 ml of Fletcher medium. These are incubated in the dark at 30 C for 28 days. A portion of each culture is examined weekly by darkfield or fluorescence microscopy.

General. Whenever there is a strong suspicion of bacteremia but failure to isolate a causative agent, additional media such as those described above for *Brucella* and cell wall-deficient organisms should be added, the duration of incubation should be extended to 3 weeks, and all subculture plates should be incubated for 7 days. Omission of SPS from one bottle should also be considered because recent re-

ports indicate that certain strains of *Peptostreptococcus anaerobius* and of *Streptobacillus moniliformis* may be inhibited in vitro by this substance (7, 9). These procedures will occasionally permit isolation of an organism that would otherwise be missed. Occasional cases of endocarditis due to *Coxiella burneti* have been reported and should be kept in mind. The initial presumptive diagnosis is serological. Isolation of the etiological agent in animals or tissue cultures is an unsuitable procedure for most clinical laboratories and should be handled by reference laboratories.

UNSUITABLE PROCEDURES

Certain procedures or components of procedures that are sometimes used are considered unsuitable. They include:

(i) use of aqueous quaternary ammonium compounds (e.g., Zephiran) for skin decontamination;

(ii) use of anticoagulants such as citrate or oxalate rather than SPS;

(iii) use of more than 1 part of blood to 10 parts of medium;

(iv) *exclusive* use of thioglycolate-containing or prereduced anaerobically sterilized media;

(v) failure to use blind subcultures;

(vi) exclusive reliance at the present time on screening procedures dependent on $C^{14}O_2$ liberation;

(vii) procedures using blood culture bottles or flasks whose caps or plugs must be removed at the bedside. The methods described have a lower contamination rate.

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GENERAL REVIEW

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