BLOOD CULTURES FOR DIAGNOSIS OF BACTEREMIA

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THE diagnosis of bacteremia can be made with certainty only after a positive blood culture has been obtained. However, the clinical usefulness of a report on blood culture, whether positive or negative, depends upon the effectiveness of the technic employed in making the culture. In view of the importance of the blood culture procedure in clinical medicine it is surprising that none of the standard laboratory manuals, with the exception of that of Schaub, Foley, Scott, and Bailey,[1] offer more than a cursory description of the technic. Schaub and Foley and their newer collaborators have given an excellent account of their method of blood culture. While their method and our method are in some ways different their procedure also gives good results.

The procedure of blood culture used in our laboratory is a simple one and has been in use many years. Our results have been remarkably accurate, and although we do not use one of the recently devised "closed-system" procedures[2] to prevent contamination, nevertheless our contamination rate is minimal.

Blood Culture Procedure

Three principles. In preparing for a blood culture study, three important steps must be taken: (1) utilizing a technic that minimizes contamination; (2) providing the best conditions for bacterial growth, so that any microorganisms that are present in the blood may have an opportunity to grow; (3) rapidly reporting results so that the physician will be able to use the information while it still is clinically essential, and before it becomes merely a bit of data to be added to the patient's chart.

Preventing contamination. The blood culture method we use permits the growth of any bacteria that may be present, as evidenced by the many positive cultures that are obtained in tubes that have been inoculated with 0.5 ml. or less of blood. The bacteria that are responsible for the contamination of blood cultures come from the air of the room, and from the skin, and the flora of the nose and throat of the patient, of the technicians, and of the other persons in the vicinity. It is well for the technician and the patient to avoid speaking, sneezing, or coughing while the blood is being drawn and inoculated into the culture tubes. Oral spray may contaminate the syringe and, thus, transmit bacteria into the culture medium. In a similar fashion a cotton plug from a culture tube held too near to the operator's face may become contaminated.

Skin organisms can be controlled by scrupulous care on the part of the technician in preparing the site for puncture. The skin disinfectant we use is made up of one part of 2 per cent tincture of iodine, and nine parts of 95 per cent ethyl alcohol. A weak solution of iodine is used because it is light in color

and does not obscure the vein and, although bactericidal, it will not burn the skin. Indeed, the concentration of iodine is so low that it is not necessary to remove excess iodine after the specimen for culture has been taken. The iodine solution on a cotton ball is carefully applied to the center of the field and in the place where the needle actually will penetrate the skin. By beginning in the central area and working outward, the cotton swab is first used at the site of actual puncture, when the swab is clean and uncontaminated by bacteria that it is likely to pick up as it is drawn over the skin.

Sites for puncture. Ordinarily venous blood is cultured, but in some diseases, as in brucellosis, a definitive culture more often may be obtained from arterial blood or from bone marrow. In line with the present interest in microtechnics, especially for children, claims have been made of the usefulness of cultures of capillary blood obtained from a puncture in the heel. It is said that 0.5 ml. of blood obtained from the heel provides satisfactory cultures with minimal contamination;[3] we have had no personal experience with this technic. Several years ago it was suggested that it was feasible to culture petechiae of patients with meningococcal infections, in an effort to recover meningococci from the blood.[4] We have had no personal experience with this technic, but believe that it might be useful under appropriate conditions.

Culture of bacteria. To provide optimal conditions for bacterial growth, and thus obtain the highest incidence of valid positive cultures, it is necessary to draw the specimen of blood at the time that the maximal number of bacteria may be present in the peripheral circulation. This is generally thought to be the period during which the patient's fever is highest. Unfortunately, the laboratory technicians are not always summoned at the optimal time for drawing blood. Most clinicians order blood cultures at such times, but the order occasionally comes for a *stat.* culture to be made for a newly admitted patient whose temperature is normal, but who did have a high fever the night before. Furthermore, although in all probability the patient again will have an elevation of temperature in the afternoon, sometimes it is not feasible to wait that long for a specimen for culture because a course of antibiotics must be started immediately. Such cultures have very little chance of success. One circumstance that has contributed to the high incidence of successful blood cultures in our institution is not only that the laboratory is given the choice of selecting the time to draw the specimen, but also the clinicians whenever possible without danger to the patient make an effort to delay administration of antibiotic therapy until the specimen has been drawn and, if practical, until a definitive blood culture report is available.

Further attempts to provide optimal environmental conditions for pathogenic bacteria that may be present in a blood specimen must center around the culture media used. Many media have been advocated and continue in use. For many years our laboratory successfully used a dextrose infusion broth containing a few small pieces of calf brain.[5]

Since 1942 we have been using a commercially manufactured thioglycollate

medium* because it is simple to prepare and effective. This medium is available in the dehydrated form and may be made by dissolving the dried mixed ingredients in water. The formula is:

Yeast extract	5.0 gm.
Pancreatic digest of casein	15.0 gm.
Dextrose	5.0 gm.
Sodium chloride	2.5 gm.
L-cystine	0.75 gm.
Thioglycollic acid (or sodium thioglycollate)	0.3 ml.
Agar	0.75 gm.
Distilled water	1,000 ml.
Added just before the above ingredients are autoclaved—	
Sodium citrate	3.0 gm.
Para-aminobenzoic acid	0.05 gm.

Thioglycollate, dextrose, and l-cystine are reducing agents and serve to keep the medium in a highly reduced state. When dispensed in culture tubes of 200 mm. by 25 mm., less than 1 cm. of the upper layers of the medium is sufficiently oxidized to give color to methylene blue or other similar oxidation-reduction (Eh) indicators. The culture tubes are filled with the medium to a depth of about 125 mm. Because of the presence of such potent reducing agents as thioglycollate and l-cystine, it is not necessary to use rubber stoppers or oil seals, even for anaerobic cultures. In addition, there is a sufficient Eh gradient within the culture tube so that, regardless of their free-oxygen requirements, the various species of bacteria can find the location that is optimal for growth. The medium is available with or without an Eh indicator. The supply of medium is used rapidly in our laboratory and there is no need for an indicator to determine whether or not oxidation has taken place. Furthermore, we do not use the medium with indicator dye because of the possible bacteriostatic effects that such a chemical may produce.

The presence of the small amount of agar makes the medium sufficiently viscid so that the bacteria develop as colonies rather than as individual bacteria diffused throughout, as in broth medium. Thus, these colonies may be fished out of the culture medium to be stained and subcultured into other media for identification. Many organisms that form easily discernible colonies in the thioglycollate medium fail to grow on blood or on other agar plates unless they first are subcultured through another tube of thioglycollate medium. Agar also serves to prevent diffusion of oxygen from the air throughout the medium.

Para-aminobenzoic acid is added to the medium before it is autoclaved, as it acts as a growth factor and as a specific antagonist of sulfonamides in the blood, if the patient has been treated with any of this group of drugs. Sodium citrate is added at the same time, as an anticoagulant to prevent clotting of the blood after it has been added to the medium. Blood cultures made with clotted

*This medium is available from the Baltimore Biological Laboratory as Thioglycollate Medium Without Indicator, Brewer-modified (No. 01-135C); and from the Difco Laboratories as Bacto Thioglycollate Medium Without Indicator (B430).

blood often are objectionable because the clot binds the colonies of bacteria, which then become difficult to isolate. One or two marble chips dropped into each culture tube will neutralize excess acid that might result from bacterial growth.[6] Penicillinase must be added aseptically to the prepared and sterilized medium and is used only when the patient has a history of penicillin therapy. Penicillinase is not a really effective agent. It may inactivate the penicillin present in the blood but can do little to restore vitality to pathogenic organisms already damaged by penicillin.

Equipment and method. A conventional culture tube is preferable to a prescription bottle or a French square. The narrow neck of a bottle has certain theoretical advantages in regard to minimizing contamination from the air when the container is opened for any reason, but a uniformly wide container, with straight sides, offers a greater advantage when it is necessary to fish for a colony from the depths of the medium. Culture tubes have an added advantage over bottles, in that they occupy less space in the incubator and may be packed more easily in racks or wire baskets, and a number of them may be handled as a unit rather than as individual bottles.

In making blood cultures it is our practice to inoculate each of three thioglycollate-medium tubes with from 1 to 3 ml. of blood. After the blood has been drawn, the needle is removed from the syringe and the blood is expressed directly into the culture tubes. Care must be taken to avoid putting excessively large amounts of inoculum into the medium, because of the bacteriostatic action of fresh blood. Once the blood is in the culture tube, the cotton stopper is replaced without flaming the tube.

The question of flaming the mouth of the culture tube before and after inoculation has been the subject of some controversy.[7] Most technicians still flame the mouth of the tube immediately after removing the cotton or other stopper, and again after inoculating the tube and before replacing the stopper. This has certain theoretical advantages, but in practice it prolongs the time that the culture container is open, and thereby increases the risk of contamination. Frequently it is dangerous to use an open flame near to patients in oxygen tents or in the operating room. The alcohol lamp is a poor substitute for the Bunsen burner, and in order adequately to heat the tube, more care must be taken than usually is practicable. The alcohol lamp has been omitted from our technicians' trays since 1932, and contamination, as mentioned before, has been minimal.

Of great importance to the success of the blood culture is the amount of blood used as inoculum. Human blood when used in large amounts may actually inhibit the development of organisms in the blood culture. Many technics have been developed to overcome this action, since in the past an adequate blood culture was obtainable only when using as large an aliquot of the blood as possible. One technic, used for a number of years, required 50 ml. of blood in 500 ml. of culture medium. This was cumbersome and expensive and, in addition, was unsuccessful and resulted in a comparatively small number of positive cultures.

The cultures are incubated at 37°C. (98.6°F.) and are examined daily to detect possible growth. Care is taken not to agitate the culture tubes, because

many of the bacteria responsible for subacute bacterial endocarditis do not grow diffusely, but rather in the region of diminished oxygen supply. Only if diffuse growth is noted, are smears made from the upper regions of the medium. The zones in and above the erythrocyte layer should be carefully inspected for flaky or granular particles. A sterile pipet should then be lowered into this granular stratum and smears made from the material thus obtained. Negative cultures are examined daily for a minimum of two weeks. Positive cultures on patients who are free of antibiotics almost always show growth within 48 hours. The medium is not recommended for brucella. When brucella organisms are anticipated, a tryptose or trypticase soy bean medium* is used in Casteneda bottles.[8]

References

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These media are available from the Difco Laboratories as Tryptose Broth (B62) *and from the Baltimore Biological Laboratory as* Trypticase Soy Broth (No. 01-162).