APPLIED MICROBIOLOGY, Dec. 1968, p. 1892-1894
Vol. 16, No. 12

System for Inoculation of Blood in the Laboratory

PAUL D. ELLNER

Diagnostic Service, Department of Microbiology, Columbia University College of Physicians and Surgeons New York, New York 10032

Received for publication 20 September 1968

A system is described that provides for the collection of blood in Vacutainer tubes containing Liquoid (sodium polyanethol sulfonate) and for the transport of such blood to the laboratory for inoculation. The procedure is compared to one utilizing open flasks of media, and the advantages of the closed system are discussed.

The high rate of contamination of blood cultures associated with the practice of inoculating open flasks of broth at the bedside led us to investigate the possibility of transporting the blood to the laboratory for inoculation. The continued use of the bedside inoculation method has been justified by the adverse effects of coagulation upon recovery of bacteria (13), and by the toxicity of citrate and several other anticoagulants for certain species of bacteria (7, 13). However, the demonstration that bacteria survive up to 24 hr in blood containing Liquoid (sodium polyanethol sulfonate; 4-6, 9, 11) opened the way for the development of a system for culturing blood by collecting it in closed tubes and transporting it to the laboratory for inoculation.

MATERIALS AND METHODS

Equipment. The following materials are used for the processing of blood specimens.

(i) Sterile 10-ml vacuum tubes containing approximately 1.7 ml of 0.35% Liquoid in saline (Vacutainer S3200XF177; Becton, Dickinson and Co., Rutherford, N.J.).

(ii) Sterile disposable holders for the above tubes with a needle attached (3200HN; Becton, Dickinson and Co.).

(iii) Four-ounce (0.118 liter) bottles fitted with a rubber diaphragm kept in place by an aluminum screw cap with a small central hole (McCartney Culture Bottle, M-102; Albimi Laboratories, Flushing, N.Y.). These bottles are filled with approximately 50 ml of Trypticase Soy Broth (BBL) and are capped loosely to permit the escape of air. The metal screw caps are covered with an aluminum-paper cap, and the bottles are autoclaved for 15 min at 121 C. The caps are tightened immediately after removal from the autoclave; this results in a negative pressure within the bottle upon cooling.

(iv) One-ounce (0.029 liter) bottles (McCartney Culture Bottle, M-101) prepared as above but filled with 25 ml of Trypticase Soy Broth.

(v) Sterile, 20-gauge, 1.5-inch (3.8 cm) disposable Vacutainer needles (no. 5746; Becton, Dickinson and Co.).

(vi) A tank of 10% CO_2 in air, fitted with a needle valve. The needle valve is attached to an anesthesia bag (5-053-100; Foregger Co. Inc., Roslyn Heights, N.Y.), and a tube long enough to reach the work bench leads from the other end of the anesthesia bag to a one-way stopcock (MS-01; Becton, Dickinson and Co.). The stopcok is connected to an autoclavable, plastic membrane-filter holder (no. 4320; Gelman Instrument Co., Ann Arbor, Mich.). A sterile, 20-gauge, 1.5-inch (3.8 cm) disposable needle (no. 5179; Becton, Dickinson and Co.) is attached to the discharge end of the filter holder.

(vii) Penicillinase (Neutrapen; Riker Laboratories, Northridge, Calif.).

Collection of specimens. The skin is prepared with 2% tincture of iodine. The concave rubber cap of the Vacutainer tube is also wiped with the iodine solution prior to insertion into the Vacutainer holder. Venipuncture is performed in the usual manner, and the entire tube is filled with blood. The Vacutainer tube is removed, inverted several times to mix the blood with the Liquoid solution, and sent to the laboratory together with a requisition. Tubes should not be refrigerated at any time.

Inoculation. Upon arrival in the laboratory, the Vacutainer tube is inverted several times to mix the specimen. The concave top is swabbed with 2% tincture of iodine and the short end of the Vacutainer needle is pushed through the rubber top of the tube; the aluminum-paper cap is removed from one 1-oz (0.029 liter) culture bottle and from one 4-oz (0.118 liter) culture bottle, and the plastic guard is removed from the long end of the Vacutainer needle. The needle is first inserted through the diaphragm of the 1-oz bottle, and approximately 1 ml is drawn into the bottle in about 2 sec. This serves as the anaerobic culture bottle. The needle is withdrawn from the 1-oz bottle and, in a similar manner, inserted through the diaphragm of the 4-oz bottle. The remaining blood in the tube is permitted to flow into this bottle. Ten per cent CO_2 in air is added to the 4-oz bottle only, by filling the anesthesia bag with the gas mixture, flaming the needle attached to the filter holder, and thrusting it through the diaphragm of the bottle. The vacuum remaining in the bottle draws in the gas mixture in 3 to 4 sec and the needle is withdrawn. The needles are always flamed between bottles and are changed fre-

quently throughout the day. The filter holders are replaced daily.

When the requisition indicates that the patient received one of the penicillins, 0.1 ml of penicillinase (40,000 units) was added to both bottles with a syringe and needle.

Both bottles are incubated at 35 C and are examined daily for signs of growth as evidenced by turbidity changes in the appearance of the blood layer. Gram stains are prepared from bottles showing growth, and subcultures are made by unscrewing the cap and removing the broth with a loop or a capillary pipette. Two blood-agar plates are inoculated; one of these plates is incubated anaerobically. When the Gram stain indicates the presence of gram-negative rods, an E M B agar plate is also inoculated. Bottles that fail to show signs of growth are routinely subcultured, both aerobically and anaerobically, on blood-agar plates on the seventh day after inoculation. When the subcultures remain free from growth after 48 hr, the culture is considered to be sterile.

RESULTS

To evaluate the new system, the number of bottles showing growth during two similar 9 month periods were compared (Table 1). The adoption of the closed system resulted in a marked decrease in contaminated cultures. Thus, the probability that a positive culture represents an etiologic agent rather than a contaminant was almost doubled and was about 80%. In the 15 months following January 1967, an additional 8,382 blood cultures were examined. In this latter series, with the same criteria as previously, the contamination rate was 1.4%.

The species isolated in each of the 9-month periods is shown in Table 2. Alpha-hemolytic streptococci, nonhemolytic streptococci, enterococci, *Escherichia coli*, *Klebsiella*, and yeasts were isolated with greater frequency with the closed system, but the significance of this is not completely understood.

TABLE 1. *Comparison of two systems for blood cultures*

Specimens	Open flasks (May 1965–January 1966)	Closed tube (May 1966–January 1967)
Total specimens cultured	3,977	4,426
Positive	244 (6.1%)	357 (8.1%)
Contaminated[a]	327 (8.2%)	107 (2.4%)
Total showing growth	571 (14.3%)	464 (10.5%)

[a] Cultures were considered to be contaminated when single specimens grew out *S. epidermidis* or *Micrococcus* spp., or when one or more cultures grew out nonmotile diphtheroids or *Bacillus* spp.

TABLE 2. *Species isolated by both open and closed method*

Species	Open flasks	Closed tube
Streptococcus, viridans group	37	65
Enterococcus	22	45
Pneumococcus	12	12
Staphylococcus aureus	46	33
E. coli	31	54
Klebsiella pneumoniae	17	36
Hemolytic streptococci	8	6
Nonhemolytic streptococci	0	14
Anaerobic streptococci	3	2
S. epidermidis	0	0
Neisseria meningitidis	1	2
N. gonorrhoeae	0	1
Listeria monocytogenes	0	1
Clostridium perfringens	1	4
Diphtheroids	3	0
Mimeae-Herellea group	13	17
Pseudomonas	24	11
Alcaligenes	1	1
Serratia	2	1
Escherichia sp.	4	5
Proteus sp.	6	8
Salmonella sp.	7	11
Bacteroides sp.	0	3
Brucella sp.	1	0
Yeasts	5	25
Total	244	357

DISCUSSION

The study of Conner and Mallery (2) suggested that contamination of blood cultures is primarily due to manipulation of syringe and flasks. They demonstrated the advantages of a closed system in which blood is drawn directly into an evacuated bottle containing the medium. The difficulty of maintaining sterility at the bedside is considerable and is increased by each additional manipulation. Evacuated bottles containing broth are commercially available, but the cost of these units precluded their adoption by this institution. Some of these units are now available with broth containing Liquoid, and since Rosner's study (8) demonstrated the advantage of Liquoid-containing media, the use of such units would be an acceptable alternative, where cost is not a primary factor. The technique of drawing a sample into a single tube, as described here, greatly minimizes the risk of contamination. An additional benefit of having the sample transported to the laboratory is that a variety of media can be inoculated at the discretion of the bacteriologist.

In the early part of this study, Thioglycollate Medium without Indicator (BBL), Brewer-modified, was used to fill the 1-oz (0.029 liter) culture

bottles. However, when a number of cultures grew out *Clostridium perfringens* or *Bacteroides* spp. in the 4-oz (0.118 liter) bottle of Trypticase Soy Broth and failed to grow in the Thioglycollate Medium, it was suspected that the Thioglycollate Medium was slightly inhibitory. Trypticase Soy Broth was then substituted for the Thioglycollate Medium and a number of anaerobic species were recovered. It is obvious that the favorable surface-volume ratio and the unrelieved vacuum after inoculation provide adequately reduced conditions for the recovery of anaerobes.

The addition of an excess of *Bacillus cereus* penicillinase to cultures obtained from patients receiving penicillin therapy proved to be a worthwhile procedure. Carleton and Hamburger (1) showed that the antimicrobial activity of a number of the penicillinase-resistant semisynthetic penicillins can be reversed with this preparation as well as that of penicillin G. It is preferable to add the penicillinase at the time of inoculation. On several occasions, however, this was deferred for 5 days, with the result that cultures became positive within 24 hr after the addition of the penicillinase.

Before the initiation of this study, blood cultures were routinely held for 14 days or for 21 days when designated "possible endocarditis." The study of Effersoe (3), which suggested that 7 days of incubation was adequate, led us to examine our records of the past 10 years. A review of approximately 40,000 blood cultures failed to reveal a single instance in which a culture became positive after the fifth day. Although a recent study (12) claimed that occasionally cultures demonstrated growth after 1 or 2 weeks of incubation, we adopted the practice of discarding cultures that fail to show growth after 7 days, regardless of the clinical diagnosis.

The successful use of Liquoid as an anticoagulant when culturing for *Leptospira* has been demonstrated (10). In our laboratory, L forms of *Staphylococcus aureus*, enterococci, *Pseudomonas*, *E. coli*, *Klebsiella*, and *Erysipelothrix insidiosa* were not affected by 4 hr of exposure to Liquoid in the concentration routinely employed (600 μg/ml). However, a strain of *Mycoplasma hominis* type 1 was inhibited by 1 hr of exposure to 100 μg of Liquoid per ml (*unpublished data*).

It was also shown in our laboratory that Liquoid is able to reverse the antimicrobial effect of streptomycin (*unpublished data*).

LITERATURE CITED

1. Carleton, J., and M. Hamburger, 1963. Unmasking of false-negative blood cultures in patients receiving new penicillins. J. Am. Med. Assoc. **186**:157–159.
2. Conner, V., and O. T. Mallery. 1951. Blood culture: a clinical laboratory study of two methods. Am. J. Clin. Pathol. **21**:785–788.
3. Effersoe, P. 1965. The importance of the duration of incubation in the investigation of blood cultures. Acta Pathol. Microbiol. Scand. **65**:129–133.
4. Elliot, S. D. 1938. The use of saponin in blood culture media, with special reference to blood cultures in subacute bacterial endocarditis. J. Pathol. Bacteriol. **46**:121–131.
5. Ellner, P. D., and C. J. Stossel. 1966. The role of temperature and anticoagulant on the in vitro survival of bacteria in blood. J. Infect. Diseases **116**:238–242.
6. Hoare, E. D. 1939. The suitability of "Liquoid" for use in blood culture media, with particular reference to anaerobic streptococci. J. Pathol. Bacteriol. **48**:573–577.
7. Rammell, C. G. 1962. Inhibition by citrate of the growth of coagulase-positive staphylococci. J. Bacteriol. **84**:1123–1124.
8. Rosner, R. 1968. Effects of various anticoagulants and no anticoagulant on ability to isolate bacteria directly from parallel clinical blood specimens. Am. J. Clin. Pathol. **49**:216–219.
9. Stuart, R. D. 1948. The value of liquoid for blood culture. J. Clin. Pathol. **1**:311–314.
10. Van Reil, J., L. Szpajshendler, and M. Van Riel. 1956. Etude clinique bacteriologique et epidemiologique d'un nouveau foyer de leptospirose au Congo. Bull. Soc. Pathol. Exotique **49**:118–143.
11. Von Haebler, T., and A. A. Miles. 1938. The action of sodium polyanethol sulfonate (Liquoid) on blood cultures. J. Pathol. Bacteriol. **46**:245–252.
12. Werner, A. S., C. G. Cobbs, D. Kaye, and E. W. Hook. 1967. Studies on the bacteremia of bacterial endocarditis. J. Am. Med. Assoc. **202**: 199–203.
13. Wright, H. D. 1925. The bacteriology of subacute infective endocarditis. J. Pathol. Bacteriol. **28**: 541–578.