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Isolation of Enteric Fever Agents from the Blood*

JOEL ESCAMILLA, PhD**
U.S. NAVAL MEDICAL RESEARCH UNIT NO. 2
MANILA, REPUBLIC OF THE PHILIPPINES

ABSTRACT

A review of the literature on factors which influence the results of blood cultures for confirmation of typhoid and paratyphoid (enteric) fever is presented from two points of view. One view deals with aspects that may be peculiar to each patient: the stage and severity of the disease, prior use of antimicrobials, and the patient's body temperature at the time of obtaining the blood specimen. The second concerns technical aspects of the hemoculture procedure; this includes the volume of blood sampled, number of hemocultures performed, choice of bacteriological broth culture medium, and duration of incubation of cultures.

The literature reveals that, using proper culture media and techniques, hemocultures yield excellent results even beyond the third week of illness as well as among patients who have previously received partial treatment with antibiotics. Tentative conclusions of an ongoing study of various hemoculture media and techniques are discussed. Recommendations for hemoculture technique are presented.

INTRODUCTION

Salmonella typhi, the etiologic agent of typhoid fever, was first isolated from the blood of clinical cases of the disease by Vilchur in 1887 (24). Since then the value of blood cultures for confirmation of the disease has been well documented and authorities consider it as the routine diagnostic method of choice for such purposes (1, 12, 26, 30).

Because of the small number of organisms in the blood in typhoid fever and other infections (14, 26), little, if any, success in isolating the etiologic agent can be expected by simply inoculating a few drops of blood directly onto solidified bacteriological media. Hence, the broth culture technique has been the most commonly used method for blood culturing

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for many years. For this, blood is aseptically collected and inoculated into a sterile borth medium which provides the nutritional requirements for growth of bacteria. After a suitable incubation period the organisms will have greatly increased in number; samples of the broth culture are transferred onto the surface of agar-solidified media and these plated subcultures are then examined for growth after overnight incubation.

Although the broth culture technique for hemocultures appears simple, different degrees of success have been encountered with it. Sen and Saxena (21), in India, for example, reported that only 9.2 % of 5,735 suspected cases of typhoid were confirmed bacteriologically by hemoculture. Watson (29), however, reported that 93% of 99 cases were positive in Natal, South Africa. The variable success in confirming typhoid fever by hemoculture may reflect significant differences in the populations being studied; typhoid patients in India, for example, may present peculiar and more difficult challenges to the hemoculture procedure than do populations in South Africa. The possibility also exists, however, that the variable success of hemocultures may reflect some basic difference in laboratory methodology.

This report presents a literature review of clinical and laboratory aspects that are important to the success of blood cultures for isolation of enteric fever agents. Particular attention is given to features or aspects commonly regarded as adversely affecting the success of blood cultures. Techniques that have proved valuable in increasing the efficiency and expediency of the results are emphasized.

Clinical Aspects and Positivity of Hemocultures

A. *Duration of illness (or stage of the disease).* In general, it is commonly believed that a large degree of success is achievable in confirming enteric fever by hemoculture during the first few days of illness, that the returns of blood cultures rapidly diminish during the second and third week, and that there is little to be derived by culturing the blood of patients during or after the third week of illness (8, 13). Data compiled by Batty Shaw and Mackay (4), however, show that not all studies are in agreement. While the results reported by Coleman and Buxton (6), Mann et al. (17), and Stuart and Pullen (22) show the highest percentage of positive hemocultures among patients tested during the first week of illness, the data reported by Gay (9), and by Batty Shaw and Mackay (4) showed highest positivity among patients in the second week of illness. Chatterjee (5) on the other hand, reported the highest percentage among patients in the third week of illness. Perhaps the strongest conclusion that can be made from these data is that the percentage of positive blood cultures is definitely lower among cultients tested during or after the fourth week of illness; however, four out of the five studies mentioned above revealed that over one-third of the patients at this "later stage" of the disease still presented positive cultures. While the uncertainties inevitable in estimating the day of onset of the disease may be responsible for some of the differences observed in these studies, it must be concluded that much can be gained by culturing the blood of all patients suspected of having enteric fever regardless of the

duration of symptoms. Moreover, physicians should surely be "typhoid conscious", especially in typhoid-endemic areas of the world. In Jakarta, Indonesia, for example, a study conducted in 1976 revealed that enteric fever was the single most common, laboratory-confirmed diagnosis among a large group of patients admitted to hospital with fever (2); additionally, the single, most valuable laboratory examination for establishing a diagnosis proved to be the routine blood culture: 188 out of 741 (25.4%) of patients presented bacteremia with etiologic agents of enteric fever. Other tests, especially serological types, did not prove as useful largely due to mixed reactions and other difficulties in interpretation of the results (2).

B. *Severity of the disease.* The relationship between the severity of the disease and the results of blood cultures was studied at Acre, Palestine, during an epidemic which involved British troops and members of the Palestine Police, in 1948 (4). The report emphasized that the terms "severe, moderate, and mild" are certainly dependent on impressions of the clinician, and that the outbreak was probably a mild one with an overall mortality of 3.94%. Nevertheless, in the "moderate" and "mild" cases the percentage of positive blood cultures was highest in the second week, while the "severe" group showed a progressive rise in isolations from the first up to the third week of the disease. The percentage of positive hemocultures in the first two weeks was greater in the "moderately ill" than in the "severely ill" group. It was also noted that blood cultures remained positive in those cases that subsequently proved fatal (4). Even though differences were noted among the three study groups, it is noteworthy that the differences were rather small and that all groups presented positive blood cultures in over 73 of cases. The only exception was that of "mild" cases, which presented the lowest isolation frequency (60%) in the third week of illness.

C. *Effect of prior antibacterial therapy.* Guerra-Caceras et al. (11) reported that 13 of 23 (56.5%) of "previously untreated" cases of typhoid fever in Peru were positive for *S. typhi* by hemocultures; other cases were positive either by culture of bone marrow, stool, or urine. Nine of 22 (40.9%) of patients presenting evidence of previous treatment with a single antibiotic", and 4 of 15 (26.7%) of patients who had previously taken "combined drug therapy" presented positive blood cultures. Thus, these data showed only a 16% reduction in positivity of hemocultures among patients previously treated with one drug, and approximately a 30% reduction among patients previously treated with a combination of antibiotics. None of the differences were statistically significant. Schlack et al. (20), in Chile, obtained blood for cultures from children who had previously received various amounts of chloroamphenicol. Patients who had received the larger amounts (3.1 grams or more) presented the lowest culture positivity; however, positivity rates of the hemocultures in this group were only 10% lower than in the group of patients who had received either no antibiotic or only a total of 1.5 grams.

The ready availability and widespread use of antimicrobials in many countries of the world certainly makes isolation of etiologic agents more difficult and at times, impossible.

Enteric fever, however, is a disease that does not respond quickly and easily to antibiotics, even when aggressive treatment regimes are administered. Thus it is not surprising that a substantially large proportion of patients who undertake some form of "self medication" will nevertheless present positive blood cultures either during or soon after such treatment.

D. *Patients' body temperature.* It is generally recommended that, when possible, blood cultures should be drawn at the first sign of fever, or immediately before or after peaks of fever. These recommendations have undoubtedly garnered the false impression that there is little value to be gained in performing blood cultures when pyrexia is absent. Batty Shaw and Mackay studied the relationship between results of blood cultures and body temperature among 138 patients suspected of having enteric fever (4). The data showed that although the percentage of positive results was greatest at higher body temperatures (100% of 65 cultures from patients having a fever of 102 to 104°F. were positive), a significant number of positives was obtained from patients with lower body temperature. Of 53 cultures from patients presenting temperatures between 97 and 101°F., forty-five (84.9%) were positive, and three of four cultures taken from patients with subnormal temperatures also grew enteric fever agents (4).

Laboratory Aspects and Positivity of Hemocultures

A. *Amount of blood sampled.* As previously mentioned, cases of enteric fever generally present only a few organisms per milliliter of blood. Watson (26) found 0.5 to 22 bacteria per milliliter of blood in 15 patients with typhoid fever; blood from 11 of the 15 contained less than 10 microorganisms per milliliter. In another study, Kaye et al. (14) reported that 90% of blood specimens from 80 patients with *Salmonella* bacteremia contained less than 2 organisms per milliliter of blood. These results were obtained by plating 2 ml samples of blood directly into nutrient agar pourplates.

The relatively small numbers of organisms present in the circulating blood ultimately dictates that the blood sample which is withdrawn for culture should be large enough to insure that several bacteria are present in the specimen. Theoretically it is possible to obtain growth even with one organism in a broth culture system, however, there are several aspects, such as presence of inhibitory substances in the blood (to be discussed later), which make it difficult to achieve such results. Surprisingly, especially in view of the obvious importance of such information, no studies have been published regarding the minimum volume of blood to be cultured for optimal recovery of enteric fever agents. An examination of the literature, however, reveals that as little as three and as much as 20 ml of blood are commonly used for hemoculture of enteric fever agents. Most successful reports usually involve the culture of 8 to 10 ml. In a comparative study of various techniques for laboratory confirmation of typhoid fever, Gilman et al (10) used a 2 ml sample of blood for hemocultures; this amount of blood was grossly inadequate, according to Watson (28).

In general, a larger blood sample should lead to greater success than the use of a smaller one. In most cases a 10 ml sample from adults, and a 5

ml sample from children should be satisfactory. The blood-to-broth ratio however, is also critical: when a small volume of broth is used, the theoretical advantage of adding a large volume of blood to increase the chances of obtaining an infected sample may be outweighed by the disadvantage of increasing the concentration of bactericidal serum factors. Compatible ratios of blood-to-broth volume are influenced strongly by the specific type of broth culture medium used.

B. *Type of broth medium.* Bile has often been recommended as an excellent liquid medium for the isolation of *Salmonella* from the blood. Until recently, however, no data were available to show direct evidence that it is superior to other culture media frequently used for routine blood cultures. Kaye et al (14), in 1966, showed that a 10% solution of dehydrated beef bile (Oxgall, Difco Laboratories, Detroit, Michigan) is significantly better, both for isolation of *S. typhi* and *S. paratyphi A*, and perhaps also of *S. cholerasuis*, than trypticase soy broth (TSB). (According to the manufacturer's, label, 10% Oxgall is equivalent to undiluted beef bile.) The Oxgall medium yielded *Salmonella* in 88 of 100 cultures whereas TSB was positive only in 68. Moreover, none of the specimens cultured in the bile medium became contaminated during processing and subculturing, whereas 11% of the TSB cultures grew organisms commonly considered as laboratory contaminants. Bile cultures also became positive earlier than did TSB cultures. 78% of 41 positive bile cultures were positive at 24 hours of incubation, and all were positive after 72 hours. In contrast, none of 31 positive TSB cultures were positive after 24 hours, and only 55% were positive at 72 hours (14).

In a study using media artificially seeded with *S. typhi*, Kaye et al. (15) demonstrated that positivity and speed of results varied according to the type of broth medium and concentration of blood in the cultures. At most blood-to-broth dilutions, speed of growth and culture positivity was: bile $>$ 2.0% taurocholate $>$ 0.5% taurocholate $>$ TSB.

C. *Special culture techniques and culture additives.* Early investigators recognized that blood contained a bacteriocidal property and that it could be overcome by the use of high dilution ratios of blood to broth culture medium. Watson (25), for example, showed that a 30% increase in isolations of *Salmonella* could be achieved by simply increasing the broth volume from 35 to 50 ml while keeping the blood samples constant at 8 to 10 ml per specimen; the studies, however, were not conducted in parallel.

A dilution of one part blood in 9 parts broth medium has generally been considered as a *minimal* requirement for blood cultures, especially when nonselective, enriched media are used. However, the relatively large amount of blood required for successful hemocultures often necessitates large amounts of culture medium and thus large culture vessels. This particular problem has led to newer more practical approaches for overcoming the effect of bactericidal substances in the blood. One such approach has been the use of the "blood clot culture" technique. In this method, a sample of blood is allowed to clot in a sterile test tube. The serum is

then removed and mascerated and the clotted portion is added to a broth medium and incubated. Thomas et al (23), and Watson (29) reported that clot cultures were more often and more quickly positive when the clots were quickly dissolved by the addition of streptokinase. The enzymatic action of streptokinase was reported to be a purely mechanical one of dissolving the clot and thus allowing the entrapped organisms full access to the nutrient medium (23); streptokinase was later regarded as having complement-destroying properties (27). It was also reported that blood clots themselves exerted bactericidal action on *S. typhi*, particularly at 37°C. Therefore, it became imperative that the clots be maintained at refrigerator temperatures (about 4°C) until processing, and that the clots be dissolved rapidly upon incubation in broth medium at 37°C (23,29).

Mackie and Finkelstein (16) showed that the antibacterial effect of human blood was mediated by the combined action of naturally occurring antibodies and serum complement. The value of special additives for eliminating such bactericidal substances from blood cultures has been known for many years. In most well financed laboratories, sodium-polyanetholsulfonate (SPS) has become perhaps the single, most popular additive for such purpose, and its value in increasing the yields of many types of microorganisms from hemocultures has been well documented (3, 7, 18). Although several laboratories have utilized SPS as a culture additive to trypticase soy broth and to other enriched culture media for culturing enteric fever agents, there are, to date, no reports that have specifically documented a favorable advantage of such use. Nevertheless, SPS has been reported to be an excellent anticoagulant, and to be effective in inhibiting the bactericidal properties of complement and of the phagocytic action of the white blood cells (3, 18). Therefore, it is strongly suspected that use of this compound may be of great value for the isolation of enteric fever agents from the blood.

Other properties of SPS includes precipitation of B-lipoprotein, fibrinogen, and C_3, C_4, IgG, and inhibition of aminoglycoside and polymixin activity (3). One noteable disadvantage of SPS, however, is that it is inhibitory or toxic to some strains of meningococci and gonococci (3).

D. *Number of blood cultures performed per patient.* Sanborn and Dyer (19), in 1977, studied the value of obtaining multiple blood cultures from patients suspected of having enteric fever. Their data revealed that the first of a series of four cultures successfully identified approximately 85% of patients which were ultimately diagnosed as having *Salmonella* bacteremia. The second culture increased this percentage to 95%, while a third was sufficient for identifying 98% of bacteremic patients. The value of a second hemoculture on suspected cases of enteric fever is therefore, very clear; that of additional cultures, while adding little to overall positivity rates may be of value in special circumtances only.

E. *Duration of incubation of broth cultures.* While there is universal agreement that "positive" hemocultures should be reported immediately upon isolating an organism, there is no concensus on how much incubation time should elapse before reporting

a specimen as presenting no growth. Some workers recommend as little as 5 days, others as much as 30 days. In a study on this subject, Batty Shaw and Mackay (4) reported that, of 160 positive cultures, 34 (21.3%), 47 (29.4%), 31 (19.4%), 21 (13.1%), 19 (11.9%) and 8 (5.0%) were positive on days 1, 3, 5, 7, 9, and 11, respectively. The average period of incubation required to yield *Salmonella* was 4 to 5 days. Slightly more than 50% were positive by the third day, but 27 (17%) did not become positive until the ninth or eleventh day of incubation (4). One wonders if more cultures would have "turned positive" if they had been incubated for a longer period, say 21 or 30 days, before being reported as having "no growth".

In some laboratories, it is common practice to subculture only blood cultures showing evidence of turbidity. It should be pointed out, however, that it is often difficult, if not impossible, to determine the presence of turbidity when blood is cultured in SPS-containing media or in 10% Oxgall. Therefore, as a matter of routine practice, all specimens should be "blindly subcultured" after specific incubation periods, and most assuredly, before discarding and reporting as presenting no growth.

U.S. Naval Medical Research Unit No. 2 and San Lazaro Hospital Blood Culture Studies

During the past 9 months, the NAMRU-2 and San Lazaro Hospital bacteriology laboratories have collaborated in studies aimed at identifying the conditions necessary for optimal and expeditious recovery of etiologic agents from the blood of enteric fever suspects. When possible, a 12 ml sample of venous blood was withdrawn from each patient, and 3 ml aliquots were subjected to various media and/or culture techniques. All specimens were diluted 1:11 in the liquid media and incubated aerobically at 36° C.

The following conclusions have thus far been reached:

a) TSB (and probably nutrient broth, heart infusion, and most other non-selective broths) is inferior to ox bile (10% Oxgall, Difco Laboratories) for isolation of *Salmonella typhi* and *S. paratyphi-A*, the only salmonella thus far encountered.

b) The addition of 0.05% SPS to TSB improves the efficacy of the medium, up to, but not exceeding the levels observed in 10% Oxgall.

c) Blood clots mechanically disrupted by use of a microblender and samples of packed cells (ie., blood samples without serum or plasma) are useful material for culture of *Salmonella*. When these specimens are cultured in 10% Oxgall, however, they do not appear to offer an advantage over the culture of equivalent amounts of whole blood directly in either Oxgall or in TSB with SPS. The additional processing steps and increased potential for contamination thus makes the culture of blood clots or packed cells unwarranted for routine use.

d) A single, 3 ml volume of blood is insufficient sample for routine hemoculture of enteric fever agents. Data in support of this includes the observation that in over one-third of the cases positive for *Salmonella*, only one culture bottle presented growth out of a set of 3 or 4 bottles, each inoculated

with similar aliquots of the same specimen.

e) The addition of SPS to TSB not only improves the efficacy of the medium, but also speeds up the recovery of organisms. For example, 44% of TSB-positive samples presented growth upon the initial 24 h of incubation, whereas 66% of TSB-SPS-positive examples did so. In both systems, however, an additional 10-20% increase in positives was noted during each of three successive subcultures performed on a weekly basis.

Recommended Blood Culture Procedure

1. *Amount of blood sample.* A 10 ml sample from adults, or 5 ml from children, is probably adequate; one to 2 milliliters should suffice for infants. Specimens should be drawn before antibiotics are given, otherwise, the blood should be obtained immediately preceeding the next dose of antibiotics.

2. *Media.* Trypticase soy broth (TSB) containing 0.05% sodium polyanetholsulfonate (SPS) is preferred. This medium allows excellent and rapid growth of *Salmonella*, as well as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, and many other bacteria that may be encountered in blood cultures. Some starins of pathogenic *Neisseria*, however, may be inhibited. A 10% solution of Oxgall in water is excellent for *Salmonella typhi*, *S. paratyphi -A*, and other members of the enterobacteria; most other pathogens, however, will either not survive, or will be greatly inhibited.

3. *Blood to broth Ratio.* One part of blood should be added to a minimum of 9 parts of sterile broth medium. Although lesser dilution ratios of 10% Oxgall or SPS-containing broths may effectively inhibit complement mediated bacteriolysis, a 1:10 dilution will aid in diluting the presence of antibiotics to below their minimal bactericidal concentration. If plain TSB or another nonselective broth is used without special additives, a blood-to-broth ratio of 1:15 or 1:20 is recommended.

4. *Incubation and subculturing.* Broth cultures are incubated aerobically at 36° C. A loopful or a drop from each specimen should be subcultured onto either MacConkey, Eosin Methylene Blue, or other plated medium suitable for enteric bacilli, and also onto a chocolate agar plate for growth or more fastidious bacteria. The chocolate agar plate is incubated in a candle-extinction jar; others are incubated aerobically at 36° C. The inoculated plates are examined for growth after 24 and 48 hrs. of incubation. Hemocultures yielding no growth are again subcultured after 3, 7, and 14 days of incubation.

5. *Number of hemocultures per patient.* It is recommended that at least two hemocultures be performed, by separate venipuncture, from each patient. If possible both should be done before antibiotics are administered.

There are many factors that influence the outcome of blood cultures for isolation of enteric fever agents. Some are indeed beyond the control of both the physician and the laboratory staff. Nevertheless, it is clear that steps can be taken to increase the chances of isolating the etiologic agents and that success can be achieved in the majority of routine cases. It can not be emphasized too strongly that all patients suspected of having enteric

fever should have a hemoculture performed regardless of previous therapy and clinical condition. Moreover, serological tests should not displace hemocultures for routine diagnostic purposes.

In order to optimize the success of blood cultures each laboratory should establish a standard procedure for performing the test. Soon after the major deicisions are made regarding the amount of blood to be withdrawn from each patient, the choice of culture medium, the blood-to-broth dilution to be used, etc., the laboratory should initially incubate (and periodically subculture) all specimens for at least 21 days before regarding any as "negative". After evaluating the data of several months' blood culture results, the length of incubation of subsequent cultures can be reduced accordingly. If for any reason the methodology is later altered, the laboratory should again return to the 21-day incubation of cultures. In no case should presumed "negative" blood cultures be discarded before a final subculture at the end of two weeks of incubation.

Blood cultures, when properly performed, are a most valuable diagnostic tool. If cultures are persistently negative in large numbers of enteric fever suspects, physicians and laboratorians should meet to discuss possible sources of error. Ultimately, the services of a consultant may be necessary.

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A review of the literature on factors which influence the results of blood cultures for confirmation of typhoid and paratyphoid (enteric) fever is presented from two points of view. One view deals with aspects that may be peculiar to each patient: the stage and severity of the disease, prior use of antimicrobials, and the patient's body temperature at the time of obtaining the blood specimen. The second concerns technical aspects of the hemoculture procedure; this includes the

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volume of blood sampled, number of hemocultures performed, choice of bacteriological broth culture medium, and duration of incubation of cultures.

The literature reveals that, using proper culture media and techniques, hemocultures yield excellent results even beyond the third week of illness as well as among patients who have previously received partial treatment with antibiotics. Tentative conclusions of an ongoing study of various hemoculture media and techniques are discussed. Recommendations for hemoculture technique are presented.

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