ADMINISTRATIVE INFORMATION

This study was supported through funds provided by the Naval Medical Research and Development Command under Work Unit No. 3M161102 BS10.AF429.

Distribution of this document is unlimited.

W. H. SCHROEDER
CAPT MSC USN
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.
for many years. For this, blood is aseptically collected and inoculated into a sterile broth 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 (3, 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 the data is that the percentage of positive blood cultures is definitely lower among patients 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, phy-
sicians should surely be "typhoid conscious", especially in typhoid-en-
demic areas of the world. In Jakarta, Indonesia, for example, a study con-
ducted 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 dia-
gnosis 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 re-
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%. Never-
theless, in the "moderate" and "mild"
cases the percentage of positive blood
cultures was highest in the second
week, while the "severe" group show-
ed 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 note-
worthy that the differences were ra-
ther small and that all groups present-
ed positive blood cultures in over
73 of cases. The only exception
was that of "mild" cases, which pre-
vented the lowest isolation frequency
(60%) in the third week of illness.

C. Effect of prior antibacterial the-
rapy. Guerra-Caceras et al. (11) report-
ed 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 po-
sitive either by culture of bone mar-
row, stool, or urine. Nine of 22
(40.9%) of patients presenting evide-
ce of previous treatment with a
single antibiotic", and 4 of 15 (26.7%)
of patients who had previously
taken "combined drug therapy" pre-
sented positive blood cultures. Thus,
these data showed only a 16% reduct-
ion in positivity of hemocultures
among patients previously treated
with one drug, and approximately
a 30% reduction among patients pre-
viously 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 wides-
pread 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 lenteremia 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 (26).

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 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 macerated 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 enzy-
matic 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 prop-
ties (27). It was also reported that
blood clots themselves exerted bac-
tericidal action on S. typhi, particular-
ly at 37°C. Therefore, it became
imperative that the clots be main-
tained 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 anti-
bodies and serum complement. The
value of special additives for eliminat-
ing such bactericidal substances from
blood cultures has been known for
many years. In most well financed
laboratories, sodium-polyanethol sulfo-
nate (SPS) has become perhaps the
single, most popular additive for such
purpose, and its value in increasing
the yields of many types of micro-
organisms 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 cul-
ture media for culturing enteric fever
agents, there are, to date, no reports
that have specifically documented a
favorable advantage of such use. Never-
theless, 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, fibrin-
ogen, and C₃, C₄, IgG, and inhibition
of aminoglycoside and polymixin act-
ivity (3). One notable disadvantage
of SPS, however, is that it is inhibitory
or toxic to some strains of meningoc-
coci and gonococci (3).

D. Number of blood cultures per-
formed 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 ultimate-
ly diagnosed as having Salmonella
bacteremia. The second culture in-
creased 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
circumstances only.

E. Duration of incubation of broth
cultures. While there is universal ac-
ceptance 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 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 (i.e., 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 the 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 preceding the next dose of antibiotics.

2. Media. Trypticase soy broth (TSB) containing 0.05% sodium polyanethol sulfonate (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 strains 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 decisions 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.

REFERENCES


Isolation of enteric fever agents from the blood

J. Escamilla

U.S. Naval Medical Research Unit No. 2
APO San Francisco, CA 96528

Commanding Officer, Naval Medical Research and Development Command, National Naval Medical Center, Bethesda, MD 20814

1982

10

Unclassified

Distribution of this document is unlimited.


Enteric Fever Agents
Blood
Isolation

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 concern 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.