BLOOD CULTURES:

A Review of Contamination Rates and Antiseptic Methodologies with Preliminary Data on Using a New Pre-Venipuncture Antiseptic Kit

by

John E. Cronin, M.D., Ph.D.

Submitted as a critical review June 1990
I. Introduction

The rapid detection of bacteremia by routine blood cultures is an important task for both the clinician and the clinical microbiology laboratory. The literature is replete with articles concerning this topic. However, a number of reviews have been written in recent years that discuss the various aspects of the methodology of blood cultures, technical advances, interpretation of results and clinical significance of positive cultures (1, 2, 3).

The detection of microorganisms in a normally sterile fluid such as blood has great diagnostic and prognostic importance. Annually, some 200,000 cases of septicemia occur with a 40-50% mortality rate in the United States. The documented prevalence of septicemia is rising. In the United Kingdom, there was a 50% increase over a 10-year period from 10 to 15 cases per 1,000 admissions (4, 5). The most common anatomical sources of bacteremia are genitourinary, respiratory and biliary tracts as well as surgical wounds. A significant percentage of bacteremias are from unidentified sources (2). The clinical pattern of bacteremia can be transient, intermittent or continuous, and each of these patterns can be associated with different underlying etiologies (2). A positive blood culture in some cases may directly establish the diagnosis or, in certain cases, indirectly yield significant clinical information. However, not all positive blood cultures signify disease. The problem of the extent of false positive or contaminated cultures remains serious. In general, methodologies and techniques exist in order to minimize this difficulty (1, 2, 3). One aspect of this issue, antiseptic techniques during collection of cultures will be reviewed
A major problem in the interpretation of positive results is the contamination of blood cultures by resident skin flora (3,6,7). As the sensitivity of blood culture techniques increases, false positive or contaminated cultures prove to be a continuing confounding variable with valuable hospital resources consumed (5,7). Significant effort has been placed in defining characteristics to identify false positives (8,9,10). In an early study by MacGregor and Beaty (9) of 857 patients, 18.9% of blood cultures were positive. Of these, 47% were defined as false positives for an overall contamination rate of 8.9%. In a second, very comprehensive study by Weinstein et al. (10), over 500 episodes of septicemia were reviewed, and during this period, over 10,500 culture sets were submitted. Of these cultures, 8.1% were true positives representing true bacteremia, while there was a 2.3% false-positive rate.

In a series of recent reviews of this problem, rates of contaminated blood cultures were reported to range from 1.0 to 4.5% (4,8). A current review reported a range of circa 1% to greater than 8% in a survey of 183 laboratories in Australasia (7). Values as high as 14% have been reported (6). Contamination rates at UCSF have been reported to be 2.5-3.0% (York. pers. comm.) While a rate of zero percent false blood cultures may represent the ideal situation, less than 3% levels of contamination from all blood cultures drawn is one recommended practical goal (2,4).

Defining a false positive rate may not be simple, and is perhaps becoming more
complicated. The spectrum of pathogens and non-pathogens overlap and depend, to a large extent on the clinical picture. The recent increase in the number of immunocompromised patients due to naturally occurring disease (i.e., AIDS), or to treatment concomitant with organ transplantation, along with individuals who have had prosthetic devices implanted has led to a clear increase in commensals causing opportunistic disease.

Some guidelines have been suggested that are useful in identifying false positive blood cultures. For example, skin flora are usually considered to be contaminants. These usually include Corynebacteria (diptheroids), S. epidermidis, Bacillus sp. P. acnes and alpha Streptococcus. Secondly, contaminants are rarely isolated in subsequent cultures. Thirdly, multiple organisms isolated from one culture suggest contamination. In addition, delayed detection of growth in the culture is common for contaminants. And most importantly, noting the caveats above, clinical findings may not be consistent with sepsis. On the other hand, immunosuppression, intravenous drug abuse and prosthetic devices, as noted above, may predispose to infection with organisms previously considered non-pathogens. Sound clinical judgment along with laboratory data must be integrated to define the presence of true disease (8, 10).

A number of studies have attempted to characterize the nature of contamination found in blood cultures. In an early study, MacGregor and Beaty found that 61% of 150 false positive cultures were due to the organism S. epidermidis while 8% were found to be due to species within the group diptheroids. In an excellent comprehensive paper.
Weinstein et al (10) found that 55% of 276 false positives were due to \textit{S. epidermidis} while 36% were due to a diptheroid. Ninety-two percent of diptheroid isolates, ninety-four percent of Bacillus sp. isolated and ninety-four percent of \textit{S. epidermidis} isolated were judged to be contaminants after physician examination of the history of the cases. Conversely, essentially all isolates of \textit{S. pneumoniae}, 97% of streptococcus group A and B, 98% of \textit{Enterobacteriaceae} isolates, 91% of yeasts and essentially all isolates of \textit{H. influenzae}, \textit{N. gonorrhoeae} and anaerobic gram-negative bacilli were judged to be true positives. Yet, as can be clearly seen, some 1.0-9.0% of isolates of these groups were thought to be contaminants in spite of their usual pathogenicity (10).

It is well known that transient bacteremia may result from dental and GI procedures as well as from bowel movements or even from simple dental hygiene (8). Furthermore, even outpatients with no known infection have manifested a 2% (presumptively false) positive rate when blood cultures were drawn (8). Thus, the clinical picture is the \textit{sine qua non} of determinations of true disease from false positive results.

Because there is no "gold standard" to compare blood cultures with, sensitivity and specificity (with their concomitant true and false positive and true and false negative rates) can only be approximated. While sensitivity can be maximized by improvements in technology and by increasing the number of samples collected to the level with the requisite amounts of blood drawn, specificity can be maximized by adherence to antiseptic techniques (8). Specificity can be enhanced further by requiring that multiple cultures drawn at
different times be positive in a series for the results to be considered a true positive when pathogens are also common contaminants (8).

Rigorous antiseptic methodology should be employed in order to reduce the microbial level at the site of venipuncture when collecting blood cultures. As noted by Ackerman et al (7), the operational word is "reduce". None of the various antiseptic methodologies will do more. Updegraff (11) attempted to quantify bacterial counts by skin level from healthy subjects. There was wide variation in both the number of colonies, ranging from 0-300 per 4 cm sq. on the skin surface to deep within the stratum corneum. Yet, scrubbing the skin with 70% ethyl alcohol for one minute revealed significant numbers of residual colonies detected by culture throughout levels of the epidermis. Prior estimates, from studies cited in Updegraff's article, found bacterial counts of from 6 to 865,000 per cm sq. Anaerobes outnumbered aerobic species by 10 to 100 fold, depending on the area of the body sampled (11).

Numerous articles over the years have evaluated different antiseptic agents and techniques for preventing nosocomial infections by disinfecting skin before surgery or before an invasive procedure such as catheter placement or venipuncture (13-17). Kaul and Jewett, in an older review, detail the different antiseptics and disinfectants (16). Among all agents designated as antiseptics, four had been historically in general use or subject to continuing clinical trials. The first hexachlorophene, a chlorinated bisphenol, appears to be primarily effective against gram-positive bacteria. It has little activity against most gram-negative
bacteria or spores. The second group, Iodaphors, are complexes of iodine and organic compounds. One commonly used compound, Povidone-Iodine, is a combination of iodine plus polyvinyl pyrrolidone. This compound has a very broad spectrum of antimicrobial activity. The third agent is Benzalkonium chloride, an organic quaternary ammonium. It is little used now, but has activity against gram-positive and gram-negative bacteria, some fungi and certain protozoa. The fourth compound is chlorhexidine gluconate, a cationic bisbiquanide. This substance is most effective against gram-positive and gram-negative bacteria and fungi. It is usually used in combination with 70% isopropanol (16). The authors note "that the ideal antiseptic or disinfectant should significantly diminish or prevent transmission of disease. The agent and mode ideally should also be inexpensive, hypoallergenic, comfortable, innocuous to the user, persistent, timesaving and easy to use". They state that the ideal state has not been reached. Each agent has some limitations. For example, hexachlorophene has suspected teratogenicity, iodaphors fail to maintain adequate residual levels, benzalkonium chloride can be toxic, while chlorhexidine gluconate had been shown to be potentially ototoxic. Yet they recommended 0.5% chlorhexidine in isopropranol preparation as an antiseptic for use on a preoperative site. Curiously, they do not discuss alcohol in this review.

Laufman, in an excellent recent paper (17) reviews the development of both skin cleansers and antiseptics from the time of Maimonides in the 11th Century until today. He notes that by the end of World War II, there was general agreement that 2% iodine in a 70%
alcohol solution most often produced superior results as to residual bacteria levels (see References in 17). In addition to a review of the antiseptics discussed above, he reviewed the antibacterial activity of metallic salts, silver, diphenyl ether, aniline dyes, hydrogen peroxide and acridine dyes, as well as the alcohols (ethyl, n-propyl and isopropyl). The different alcohols have excellent activity against most gram-positive and gram-negative organisms as well as the tubercle bacillus. They also act against many fungi and viruses, but not spores. There are some toxic effects noted with alcohol. However, he reports that alcohol is the superior skin antiseptic. A one-minute immersion or scrub with alcohol is as effective as a 4-7 minute skin preparation with other antiseptics (see References in 17). The author states that the World Health Organization is preparing guidelines designating alcohol as the "gold standard" against which all other skin cleaning preparations would be measured. In summary, Laufman strongly recommends either alcohol alone or with the addition of chlorhexidine or an iodophor (such as povidone-iodine) as both a surgical hand scrub as well as a preprocedural skin preparative agent (17).

Specifically, with respect to the skin antisepsis prior to venipuncture for blood cultures. alcohol (isopropyl or 60%-95% ethanol) followed by 2% iodine or iodophor is the procedure in general practice (2.3.7.8.12). Lee (13) reports that a double application of 70% isopropyl alcohol was as efficacious as 2% iodine followed by alcohol application.

In addition, some authors recommend swabbing the tip of the blood culture bottle and changing needles on the venipuncture syringe prior to injections into the blood culture
bottle (7). However, in a recent study involving some 700 blood cultures, the rate of
contamination for the conventional technique of changing needles compared to that for no
change of needles was 1.28% versus 1.55%, a non-significant difference (Krumholtz et al.)(18).
Various authors also recommend pre-packaged swabs or scrub brushes rather than multi-use
disinfectected containers, as the latter have been prone to bacterial contamination and have
been reported to be responsible for false-positive results (7).

II. Pilot Study.

In an attempt to reduce the rate of false positives in blood cultures, specific
recommendations have been made by different authors in collection techniques and blood
culture procedures, as described above. Recently, as part of ongoing efforts to minimize
contamination rates, a new blood culture preparation kit was placed into use in our
Outpatient Clinics at the University of California Medical Center, San Francisco. An
ongoing preliminary evaluation has begun comparing results obtained with this kit against
standard techniques generally in use within the hospital as well as against prior rates of
contamination found at the Outpatient Clinics. The manufacturer of this kit is Mediflex.

The kit consists of a foam scrub brush with an ampule of 70% isopropyl alcohol and
10% acetone solution within the plastic handle and an ampule of 10% povidone iodine
solution. The manufacturer recommends application of the isopropyl alcohol and 10%
acetone solution by breaking the ampule within the scrub brush over the site of
venipuncture, cleaning the area thoroughhly, and then allowing the area to dry. The second step is to apply the povidone iodine to the center of the site and move in concentric circles to the periphery. The povidone iodine should be allowed to dry for 30-60 seconds before collection of the blood sample. Upon collection of the blood, the povidone iodine may be removed by application of alcohol.

Over a six-month period, from December 1989 to May 1990, blood cultures from 117 individuals were sent to the Microbiology Laboratory from the Outpatient Clinics. Each specimen had been collected using the Mediflex kit as described above. Over the course of the study, seventeen individuals had blood cultures that were positive for a rate of 0.137 (Table I). Ten episodes of clinical septicemia are represented by some 11 different usual pathogens. Two patients had double infections. Six patients had cultures positive for S. epidermidis. At least twice, S. epidermidis represented true infections. In the first case, sequential cultures drawn from the patient over a number of days were positive for the same organism. The second isolate of S. epidermidis represented an endocarditis in a patient with mitral valve prolapse. Four patients had only one of two culture bottles positive for this organism with no subsequent isolates. These represent probable contamination. Thus, four contaminants in approximately 120 different blood culture sets represents a rate of false positives of 0.034. While overall rates of contamination of blood cultures at UCSF range around 3%, blood cultures drawn in the outpatient clinics have had, historically, a higher contamination rate, around 5% (York. pers. comm.). September 1989 had 14 of 271
contaminated blood culture sets for a rate of 5.1%. Other months showed similar rates just prior to beginning the study. It is possible that contamination rates may have been lowered from 5 to 3-4% by the use of the Mediflex kit. However, our sample size is quite small. In order to rigorously evaluate the true statistical significance of these values, larger numbers of blood cultures should be examined comparing this technique to the standard technique currently in use. Ilstrup (19) in a review of statistical methods involving evaluation of changes in blood culture techniques (although specifically discussing comparisons of different media) recommends 200 positive cultures as the sample size to optimize analysis. Washington concurs with Ilstrup's calculations (4).

In general, careful adherence to antisepsis during collection of specimens for blood culture is key to minimizing contamination. Alcohol alone, alcohol with an iodophor or with hexochlorophene are acceptable agents to be employed for skin preparation. Acetone in combination with an alcohol followed by povidone-iodine deserves careful ongoing evaluation in order to determine its efficacy.
BIBLIOGRAPHY


10. Weinstein MP, LB Reller, JR Murphy and KA Lichenstein: The clinical significance

11. Updegraff DE: A cultural method of quantitatively studying the microorganisms in the skin. J Invest Dermatol 129-


Table I

Species Identification of Positive Blood Cultures

A. True Positives:

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) <em>S. pneumoniae</em></td>
<td>1</td>
</tr>
<tr>
<td>(2) Salmonella Group B</td>
<td>1</td>
</tr>
<tr>
<td>(3) Kingella sp.</td>
<td>1</td>
</tr>
<tr>
<td>(4) <em>S. aureus</em></td>
<td>1</td>
</tr>
<tr>
<td>(5) <em>C. neoformans</em></td>
<td>1</td>
</tr>
<tr>
<td>(6) <em>P. aeruginosa</em></td>
<td>1</td>
</tr>
<tr>
<td>(7) <em>E. coli</em></td>
<td>2</td>
</tr>
<tr>
<td>(8) <em>Streptococcus sp.</em> and <em>Neisseria sp.</em></td>
<td>1</td>
</tr>
<tr>
<td>(9) <em>E. coli</em> and <em>K. pneumoniae</em></td>
<td>1</td>
</tr>
<tr>
<td>(10) <em>S. epidermidis</em></td>
<td>2</td>
</tr>
</tbody>
</table>

B. False Positives:

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) <em>S. epidermidis</em></td>
<td>4</td>
</tr>
</tbody>
</table>
BLOOD CULTURES