What Is the Relevance of Obtaining Multiple Blood Samples for Culture? A Comprehensive Model to Optimize the Strategy for Diagnosing Bacteremia

Brigitte Lamy,1,3 Pascal Roy,2,3 Gérard Carret,1,3 Jean-Pierre Flandrois,1,3 and Marie Laure Delignette-Muller4
1Laboratoire de Bactériologie and 2Service de Biostatistiques, Centre Hospitalier Lyon-Sud, Hospices Civils de Lyon, and 3Centre National de la Recherche Scientifique, Unité Mixte de Recherche 5558, Lyon, and 4Unité de Microbiologie Alimentaire et Prévisionnelle, Ecole Nationale Vétérinaire de Lyon, Marcy-L’Étoile, France

Through a heuristic and probabilistic approach, we evaluated blood culture operating characteristics (sensitivities, specificities, and predictive values) as a function of several pretest parameters, together with their variability. On the basis of a meta-analysis of quantitative data from the literature, a model was developed and an estimation of the operating characteristics through numerical simulations (Monte Carlo method) was performed. The model evaluates the influence of ordering and drawing parameters on the ability of blood culture to distinguish bacteremic from nonbacteremic patients, regardless of the causative species. By considering the total blood volume to be cultured (six 5–10-mL bottles), results were found to confirm the current guidelines. On the basis of this hypothesis, the results, together with an analysis of the literature, failed to show any benefit of a strategy that involves obtaining multiple samples. The best strategy when performing blood culture is to obtain blood for 6 bottles (for a total volume of 35–42 mL), preferably at the same time.

Detection of bacteremia by use of blood culture has been greatly improved since the beginning of the past century, but blood culture remains insufficiently reliable in terms of sensitivity (Se), specificity (Sp), and predictive value [1]. The practices of ordering and obtaining blood samples for culture have been proven to have a great impact on blood culture performance, because new blood culture systems have been found to be comparable in terms of Se, Sp, and predictive value [1]. Despite there being common guidelines, blood culture practices vary widely, either in the volume of blood sampled per culture or in the number of samples obtained for culture [2–5]. When the volume of blood cultured is small, some “negative” blood culture results may be falsely negative simply because the bacterial concentration in the blood is very low [6]. In addition, the skin preparation techniques used for obtaining the blood samples vary [7]. The lack of antisepsis may lead to the contamination of blood samples, and, considering that clinicians frequently and with ease order blood cultures and that the prevalence of bacteremia is low, a high rate of false-positive results is currently being reported. Up to one-half of all “positive” results are falsely positive [8–10].

The optimization of the blood culture strategy is particularly complex because it depends on several crucial parameters that directly and simultaneously impact blood culture performances. These parameters include the bacterial concentration in the blood, the volume of blood sampled per culture, the number of times that samples are obtained, and the risk of contamination.
Studies of blood culture usually focus on a sole factor without considering the other parameters. In addition, the variability of the parameters has hardly been considered, despite its potential impact. To our knowledge, there has not been a comprehensive study that includes the crucial parameters together with their variability. The aim of the present study is to determine whether the blood culture ordering strategy could be optimized by characterizing blood culture performances as a function of several pretest parameters together with their variability and whether the findings would differ from current recommendations. Technical factors, such as the culture media, the blood culture systems, and the duration of incubation, were deliberately not taken into account.

Empirical studies of blood culture ordering practices are tedious and difficult to perform, considering the low-level incidence of bacteremia, the presence of low-grade bacteremia, and the absence of a perfect “gold standard” test to identify patients with and without true bacteremia. Therefore, we chose to use a theoretical approach to define the optimal functioning of blood culture [1, 11]. We developed a model based on several parameters that influence blood culture performances to describe the distribution of Se, Sp, and predictive values that could occur. The parameters were the bacterial concentration in blood (\(\beta\)); the volume of blood sampled per culture, with its technical limitation (i.e., bottle size) (\(v\)); the number of blood samples (\(n_s\)); and the risk of contamination (\(\pi\)) [1, 12–14]. Numerical simulations were performed using quantitative published data (table 1) to deduce the model response (blood culture performance) from the parameters together with their variability.

### METHODS

#### The Model

\(\beta\) Defines the concentration of living bacteria. Culture characteristics are assumed to be perfect, so that any case of bacteremia or contamination is detected when \(\geq 1\) bacterium is present in a 5–10-mL culture bottle. Se was defined as a function of the bacterial concentration in blood (\(\beta\)), the volume of blood obtained (\(v\)), and the number of blood draws (\(n_s\)). The complete model is presented in the Appendix. Clinically significant bacteremia is characterized by a low concentration of

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description and comments</th>
<th>Probability distribution</th>
<th>References*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood bacterial concentration distribution (\beta), cfu/mL</td>
<td>Accurate distribution for adult patients only; (\beta) range, 0.01–1000; median = 1 cfu/mL ± SD was estimated from the data of Wain et al. [6].</td>
<td>Log normal (0, 1)(^{b,c})</td>
<td>[6, 14, 15–19, 20, 21]</td>
</tr>
<tr>
<td>Within-patient variability of (\beta) ((\delta))</td>
<td>Data on within-patient variability were rare and always from before the antibiotic era. One-log variation. Within-patient variability described as an added variability to the patient-to-patient variability ((\delta) sampled in the log-normal distribution).</td>
<td>BetaPERT (Log(\delta) – 0.5, log(\delta), log(\delta) + 0.5)(^d)</td>
<td>[15, 19]</td>
</tr>
<tr>
<td>No. of samples ((n_s))</td>
<td>Median proportion of patients with single blood culture is 26% [3, 4, 22]. Number of samples mostly varied from 1 to 4 and rarely exceeded 10 [3]. For (n_s) &gt; 4, the distribution was completed with data from Hospices Civils de Lyon.</td>
<td>Empirical distribution(^c)</td>
<td>[3, 4, 22]</td>
</tr>
<tr>
<td>Risk of contamination ((\pi)), %</td>
<td>No reference on accurate shape for (\pi) distribution; (\pi) range, 1%–8%; usually reported at 2%–3%, and frequently &lt;5%.</td>
<td>BetaPERT (0, 2.5, 8)(^c,d)</td>
<td>[2, 7–9, 23–25]</td>
</tr>
<tr>
<td>Blood volume per bottle ((v_s)), mL</td>
<td>More than 10% of bottles contained less than one-half of the requested blood volume [5, 26]. Technical limitations were taken into account to model blood volume per sample ((v_s)), with (v_s) defined with (v_s) and the number of bottles ((n_s)) filled; when using a vacuum drawing technique, the maximal blood volume to be injected in a recommended 10-mL bottle cannot be higher than 10 mL.</td>
<td>BetaPERT (3, 7, 10)(^c,d)</td>
<td>[5, 26]</td>
</tr>
</tbody>
</table>

*References in boldface are those with the most relevant quantitative data used to characterize the probability distributions.

\(^b\) Log-normal distribution is defined with respective mean and SD.

\(^c\) See figure 1.

\(^d\) BetaPERT distribution is defined with respective minimum, most likely, and maximum values [27, 28].
circulating bacteria, and the probability of finding $\geq 1$ bacterium is a rare event that is calculated according to a Poisson distribution of the number of bacteria in a sample unit. When a series of $n_s$ samples is obtained from a bacteremic patient, the probability of getting a positive result ($Se$) can be calculated according to a binomial distribution with this Poisson probability and $n_s$ as parameters. $Sp$ is defined according to a binomial distribution with $\pi$ and $n_s$ as parameters. The probability of a false-positive result depends on the risk of contamination $\pi$, which is related to a lack of antisepsis. When a series of $n_s$ samples is obtained, the probability of getting a false-positive result ($\geq 1$ positive culture result) also depends on $n_s$.

**Numerical Simulations**

The simulations were achieved in 2 steps.

**Step 1 analysis.** Step 1 analysis was performed to establish the impact of each parameter’s variability on blood culture performance and to describe the $Se$ and $Sp$ variability. Numerical simulations were performed by use of a Monte Carlo method, which enabled us to take into account several simultaneous variabilities [27]. This technique is classically used to perform quantitative risk analysis and requires the use of parameter probability distribution [29].

**Step 2 analysis.** The optimization of blood culture performance was studied using 25 scenarios. A scenario is a numerical simulation involving the control of several parameters, which is chosen according to the results of step 1 analysis.

**Parameter variability.** Quantitative data were obtained and compiled from the literature. Probability distributions were inferred to characterize the variability of each parameter (table 1) [2–9, 14–26]. The distributions fitted usually observed and possible rare values for each parameter. When there were too few data available on the shape of a distribution, the betaPERT distribution was used. This is a reparameterization of the beta distribution, which makes it convenient for modeling expert opinion formulated as the minimum, most likely, and maximum values [27]. This distribution is proposed in such commercial software as @RISK (http://www.palisade.com) and is recommended to replace the commonly used triangular distribution, the mean value of which is dependent on the specified minimum and maximum values and the shape of which is rarely justified [28]. The shapes of the distributions are presented in figure 1.

**The Monte Carlo method (step 1 analysis).** The parameters were sampled in a way that reproduces the distribution shape and were entered into the model. Two stages of parameter variability were taken into account: (1) patient-to-patient variability and (2) patient-to-patient variability together with sample-to-sample variability. The parameters $\beta$, $\pi$, $v_b$, and $n_s$ were sampled in the distribution presented in table 1. The number of bottles per sample ($n_b$) was set at 1. The variability of $Se$...
and Sp was characterized with 1000 iterations, after we checked to confirm that convergence of numerical simulations was adequate. The parameter variability influence on Se and Sp was evaluated by use of the Spearman’s rank correlation coefficients, according to equations (A1) and (A2), which are presented in the Appendix. The probability distributions of Se and Sp were determined by means of equations (A1) and (A2) (patient-to-patient variability). Additional simulations, performed by means of equations (A4) and (A5), were achieved with 3 levels of sampling, to take into account all of the sources of variability (patient-to-patient and within-patient variability), as shown in figure 2.

**Scenarios (step 2 analysis).** Scenarios were determined by means of equations (A4) and (A5) with parameters $n_b$ and $n_s$ successively fixed at 1, 2, and 3. Additional simulations were achieved with $n_b$ and $n_s$ at 4 and 6. The noncontrolled parameters were sampled in the probability distributions, as described above, in accordance with the sampling design presented in figure 2. The predictive values of blood culture, together with their variability, were also evaluated and were calculated with Bayes’s theorem from Se, Sp, and pretest probability $p$ successively set at 5%, 15%, and 30% [30]. Five percent is a reported frequency of bacteremia among a population of febrile hospitalized patients, 15% is a reported frequency among a population of febrile patients admitted in an emergency care unit, and 30% is a high prevalence of bacteremia [31–33]. The positive predictive value (PPV) describes the probability of a patient being bacteremic when a series is positive, regardless of the species yielded on culture.

The computation was performed on a Power Macintosh G3 computer (Apple) with Matlab software (Mathworks) and the NAG foundation toolbox (Numerical Algorithms Group; Mathworks).

**RESULTS**

**Evaluation of Se and Sp distributions.** The range of probability distribution for Sp was 0.65–1 (median, 0.94); the probability distribution for Se was highly dispersed (range, <0.1–1). Se was higher than 0.9 in ~75% of the trials, but the range for Se was <0.1–0.9 in ~25% of the trials. The probability distributions looked similar when the within-patient variability was also taken into account (data not shown), suggesting that the within-patient variability of the blood volume per bottle and of the bacterial concentration in blood had no impact on the Se global variability.

**The influence of parameter variability on blood culture performance.** The variability of Se was highly dependent on the variability of the bacterial concentration in blood (Spearman’s rank coefficient correlation $R_{S(Se/b)} = 0.94$). It was also slightly dependent on the number of samples ($R_{S(Se/n)} = 0.2$). These quantitative results showed the significant unreliability of Se in the case of very low-grade bacteremia, which is because the presence of any bacterial cell in the blood volume sampled is

---

**Figure 2.** Sampling design used for the characterization of the probability distribution of sensitivity and specificity. Patient-to-patient and within-patient variability were taken into account. Three levels of sampling were achieved: 1000 iterations of $b$ and $n_i$ were performed to produce patient-to-patient variability; for each of these iterations, $b'$ and $\pi$ were sampled $n_s$-fold to produce within-patient variability, and, for each of these iterations, $v_i$ was sampled $n_k$-fold to produce within-sample variability.
uncertain. The influence of the other parameters’ variability was not noteworthy ($R_\text{s} < 0.1$). With regard to Sp, $n_\text{b}$ variability was as influential as contamination rate variability ($R_{\text{s}[\text{Sp},b]} = -0.69$; $R_{\text{s}[\text{Sp},t]} = -0.7$).

**Scenarios (step 2 analysis): Se and Sp optimization.** The optimization of the blood culture performance was studied, with the controlled parameters being $n_\text{b}$ and $n_\text{t}$. The strategy of Se optimization consists of globally decreasing the number of cases submitted to unreliable-level Se (the case of very low–grade bacteremia) by increasing the blood volume cultured (i.e., $n_\text{b}$ and $n_\text{t}$). Sp optimization consists of decreasing the number of exposures to contamination risk (i.e., control $n_\text{t}$). Results are presented in tables 2 and 3. The total number of bottles cultured being equal ($N_{\text{c}} = (n_\text{b} \times n_\text{t})$), the probability distribution of Se was the same, whatever $n_\text{b}$ and $n_\text{t}$; it was consistent with step 1 analysis, which showed that within-patient variability had no impact on Se. The Se progressively improved until the total number of bottles cultured ($N_{\text{c}}$) reached 6. With a greater number of bottles, Se had no more substantial improvement.

With the total number of bottles filled set at 6, 89% of the trials were associated with an Se of ≥0.95; with up to 9 bottles filled, 90.5% of the trials were associated with an Se of ≥0.95. Nevertheless, the range of dispersion of Se could not be reduced (range, 0.1–0.9), regardless of the total volume of blood cultured. The overdispersion of Se was a direct consequence of the overdispersion of the bacterial concentration in blood (a 4-log range), and the lack of Se was a consequence of very low–grade bacteremia. With $N_{\text{c}}$ set at 6, this amount was limited to 10% of cases and could hardly be reduced with a higher blood volume cultured. Of course, Sp was maximized, with a sample number controlled at 1; >90% of the trials were associated with an Sp of >95% (median set at 97.5%).

**Scenarios and predictive values of blood culture results.** Results are presented in table 4. The PPV was all the more influenced by the impact of the number of venipunctures, and its variability was all the larger when the pretest probability was low. With 2 drawings of 3 bottles and with a pretest probability of 5%, the impact of the contamination probability ($p$) combined with the number of venipunctures ($n_\text{v}$) is so high that the PPV would be as low as 50%, which is equal to flipping a coin. The test would even generate more false-positive results than true-positive results with 3 drawings of 2 bottles (table 4). The negative predictive values were very high (data not shown) in all the scenarios, as expected, with very low pretest probabilities tested.

### DISCUSSION

The major difficulties of an empirical study led us to analyze this topic through a heuristic and probabilistic approach. There are 2 kinds of limitations to this study. First, the results of the simulations depend on the parameter distribution definition—that is, the shape of the bacterial distribution in blood ($\beta$) is of crucial importance, considering its impact on Se. Yet very few relevant data were available. The collection of such data requires the use of a quantitative blood culture technique, which is particularly labor-intensive; consequently, studies of bacterial concentration in blood are either old (from before 1920) or recent, the latter using the lysis-centrifugation technique (Isolator system; Oxoid) [34]. The old studies are usually inaccurate because of the methodology used (e.g., inappropriate culture medium, too short a range of variation for the parameter evaluated, or too few cases) or hardly comparable with each other because of unmatched class limits [14, 16–21]. Besides, we did not consider it relevant to use recent data obtained by means of the lysis-centrifugation technique (Isolator system) to simulate qualitative blood culture technique. These 2 techniques do not seem to measure the same parameters, because the culture of bacteria present in blood cells is uncertain with qualitative blood culture as opposed to the lysis-centrifugation technique [34].

We focused on the data of Warren and Herrick (1916) [20], Reynes (1947) [14], and Wain et al. (1998) [6] because of the high number of cases considered (134, 1026, and 349 cases, respectively). However, the data of Wain et al. [6] may have been biased by the bacterial species studied, the pathologic

### Table 2. Sensitivity of blood culture, according to the total number of bottles cultured.

<table>
<thead>
<tr>
<th>Total no. of bottles ($n_\text{b} \times n_\text{t}$)</th>
<th>Sensitivity of $&gt;$0.95, % of trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>81</td>
</tr>
<tr>
<td>6</td>
<td>89</td>
</tr>
<tr>
<td>8</td>
<td>90</td>
</tr>
<tr>
<td>12</td>
<td>92</td>
</tr>
</tbody>
</table>

**NOTE.** Results for sensitivity are presented according to the total number of bottles cultured ($N_{\text{c}} = (n_\text{b} \times n_\text{t})$), because, for a given $N_{\text{c}}$, the probability distribution of sensitivity was the same whatever $n_\text{b}$ and $n_\text{t}$.

### Table 3. Specificity of blood culture, according to the total number of draws.

<table>
<thead>
<tr>
<th>No. of draws</th>
<th>Median specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.98</td>
</tr>
<tr>
<td>2</td>
<td>0.94</td>
</tr>
<tr>
<td>3</td>
<td>0.91</td>
</tr>
<tr>
<td>4</td>
<td>0.87</td>
</tr>
</tbody>
</table>
Our results, combined with an analysis of the literature, failed to show any need for or benefit of repeated venipunctures during a short time period (<24 h). A rationalized bacteremia-diagnosing strategy should be defined by obtaining 6 bottles (i.e., 35–42 mL) of blood for culture during a single draw. Considering this total number of bottles and Se, this result takes into account technical limitations that are the result of bottle size, insufficiently filled bottles, and consequent variability of the total volume of blood cultured. This volume is recommended for any patient because patients with very low-grade bacteremia (20%–30%) are not identifiable; a higher blood volume would not substantially improve Se any more. This volume is consistent with previous experimental data and recommendations: it is often reported that >3 separate 10-mL samples for blood culture per septic episode are rarely necessary [1, 5, 24, 26, 36–41].

However, the effectiveness of a strategy that involves obtaining multiple blood samples (hereafter, “multisampling strategy”) is theoretical and has never been demonstrated. If one considers the high impact of repeated venipuncture, the multi- versus single-sampling strategy must be discussed. Our results imply that within-patient variability of the bacterial concentration in blood had no impact on Se. The increase in Se attained with separate blood cultures can simply be explained by the increase of the blood volume sampled, thus increasing the probability of drawing any bacterial cell, rather than by a true intermittent nature of the bacteremia, as is strongly suggested by the results of Li et al. [35]. The prevalence of true intermittent bacteremia is not substantial enough to justify a multisampling practice.

The need for a strategy to get the false-positive result rate under control is obvious, because false-positive results represent up to one-half of all “positive” results [10]. A contaminated blood culture may prompt the health care provider to administer inappropriate treatment, or it may prevent or delay the search for the cause of the signs or symptoms that prompted the decision to perform a culture of the patient’s blood. In addition, the economic consequences of contamination of blood cultures may be substantial and are reflected in the increased length of hospital stay, unnecessary antibiotic therapy, and additional testing and consultation [9]. Although strict compliance with the proper blood culture sample-collection technique or a better sterile technique obviously reduces the rate of false-positive results [7, 25], antisepsis cannot be absolute [42]. Because $\pi$ cannot be null, the impact of the multisampling practice on Sp becomes important.

This study shows that the variabilities of the number of blood draws and of the aseptic technique in obtaining samples equally affect the rate of false-positive results. Therefore, with Se being equal, it is obvious that a strategy devoted to improving the blood culture Sp should minimize $\pi$ and $n$, rather than the sole

### Table 4. Positive predictive values (PPVs), according to the number of samples ($n_i$) and the pretest probability ($p$) of testing of blood culture parameters.

<table>
<thead>
<tr>
<th>$p$, %</th>
<th>$n_i$</th>
<th>No. of bottles per sample ($n_b$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1</td>
<td>0.64 (0.55–0.73)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.46 (0.40–0.53)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.37 (0.32–0.42)</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>0.85 (0.81–0.90)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.74 (0.69–0.79)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.66 (0.62–0.71)</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
<td>0.93 (0.91–0.96)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.88 (0.85–0.90)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.83 (0.79–0.85)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are median (25th–75th percentile) of the distribution. The PPV describes the probability of a patient being bacteremic when $>1$ sample is positive, regardless of the species isolated. The total number of bottles filled was 6.
Physicians frequently and easily order blood cultures because contamination rate, as is often suggested [7, 25]. This obvious strategy contrasts with the current blood culture practice, which presents inherent paradoxes. The current practice is mostly based on a multisampling strategy, because, in addition to the theoretical detection of intermittent bacteremia, it is claimed that the current practice enables the differentiation between the true-positive blood culture result and results that reflect contamination, by means of guidelines. These guidelines are based on the number of positive culture results and on whether the culture yielded an organism regarded as a common contaminant (e.g., coagulase-negative staphylococci [CoNS]) [43].

In the case of a blood culture that yields CoNS, 2 positive blood culture results are usually required to consider the patient to be bacteremic. However, such a strategy is questionable. First, the principle of performing several blood draws is inconsistent with the need for early antibiotic treatment, which may lower the chance of finding microorganisms on culture. Second, a systematic use of these guidelines, which are aimed at identifying false-positive results, paradoxically generates false-positive results because the guidelines are based on a multisampling strategy. This kind of strategy also exposes to a neglected antisepsis that could merely, but falsely, be compensated by a result interpretation. Finally, the guidelines are based on a predictive approach and are not unfailing. The use of the guidelines may lead to the misclassification of 12%–30% of nonbacteremic patients (who are falsely considered to be bacteremic patients) and of 28%–35% of bacteremic patients (whose cultures are falsely considered to have been contaminated) [44, 45]. An unnecessary and systematic use of these guidelines may lead to an increased frequency of incorrect diagnosis and to the misuse of antimicrobial therapy [44].

Such misinterpretation may be substantial, especially if one considers the development of new medical practices and treatment modes and the prevalence of significant bacteremia involving CoNS, the rate of which is reported to be double the rate that was first estimated [44]. A multisampling strategy aimed at using these guidelines is irrelevant. On the one hand, common contaminants (e.g., CoNS) cannot be incriminates in bacteremia associated with a clinical setting (e.g., nephritis or pneumonia). The identification of a false-positive result is trivial in this case, and use of a multisampling strategy to interpret the result is not necessary. On the other hand, common contaminates can be incriminated in some cases of bacteremia (e.g., catheter-related bacteremia). In such cases, these guidelines may be ineffective in differentiating true-positive results from false-positive results.

Physicians frequently and easily order blood cultures because of the serious consequences that result from missing a diagnosis of bacteremia and because pretest probability is often low or very low [8, 9]. However, the impact of contaminated blood cultures should be considered when deciding whether to obtain blood samples from patients who have very low risk; in addition, the number of samples obtained should be minimized to 1. The PPV is a powerful parameter because it enables the evaluation of the global impact of \( p \), on Se and Sp for a given pretest probability (\( p \)). The relevance of a positive result is even more lowered by \( p \), when \( p \) is very low, because a multisampling strategy maintains a high probability of contamination. The quantitative approach evaluated the impact of this obvious phenomenon that is usually underestimated. An optimized strategy is to avoid—according to any guidelines—performing unwarranted blood cultures when the pretest probability of bacteremia is irrelevant [8, 31–33].

If we consider the total volume of blood cultured, our results were consistent with the existing recommendations, and we failed to find any argument based on culture performance to justify the nonobservance of the recommendations. Our approach, combined with a precise analysis of the literature and with the consideration of the limited definition of intermittent bacteremia, show the rationality of a 6-bottle, single-sampling strategy. A multisampling strategy is seldom necessary, and decisions about when to practice it should be made only after weighing the benefits against the disadvantages. A single-sampling strategy was successfully used by Arendrup et al. [24] with optimized resource use, workload management, and early treatment management, in addition to a consideration of the impact of diagnosing bacteremia. Obtaining blood samples for culture should be discouraged when the prevalence of bacteremia is irrelevant or excessively low, considering the level of PPV. However, when blood cultures are ordered, a single-sampling strategy is best.

Acknowledgments

We thank Pr. J. J. Lehot, Service de Réanimation, Hôpital L. Pradel, Hospices Civils de Lyon, France, for reading the article in manuscript and for his helpful comments.

APPENDIX

THE MODEL

Assuming there is no contamination, the probability \( P(S = |) \) that a blood sample obtained from a bacteremic patient contains no colony-forming units (cfu) of bacteria is a function of the mean number of cfu per sample unit [11]:

\[
P(S = |) = e^{-\beta v}
\]

This probability is calculated according to a Poisson distribution of the number of living bacteria in a sample unit [11].

\( \beta \) Denotes the bacterial blood concentration and \( v \) denotes the blood volume per sample unit. Because a sample comprises several bottles and because bottles may be filled with different
volumes of blood, the volume of a blood sample submitted for culture depends on the number of bottles filled \((n_b)\) and on the blood volume introduced in each bottle \((v_b)\). Hence, the probability that a blood sample obtained from a bacteremic patient is negative is \(P(S = 0/B) = e^{-\beta p} \), where \(v = \sum_{i=1}^{n} v_b\) and \(v_b\) is the volume of blood introduced in the \(i\)th bottle.

Now, let us take into consideration the contamination impact. A positive sample is positive as a result of bacteremia or contamination. Let us denote by \(\pi\) the probability that a sample is contaminated. The probability that the sample is not contaminated is \(P(S = -) = 1 - \pi\).

Given that the 2 causes of a negative result are independent, the probability of getting a negative sample obtained from a bacteremic patient is in fact \(P(S = 0/B) = e^{-\beta p}(1 - \pi)\).

When \(n\) samples are drawn from a bacteremic patient, a single positive sample is usually sufficient to consider the test result positive. Let us denote by \(X\) the number of positive samples. In the first approach, we simply consider that the values of the parameters vary from one patient to another but that, for a given patient, the parameters do not vary from one sample to another. Thus, the sensitivity \((Se)\) of the test is defined by the probability of getting \(\geq 1\) positive sample from a bacteremic patient \((B)\):

\[
Se = P(X > 0/B) = 1 - P(X = 0/B) = 1 - P(S = 0/B)^n . \tag{A1}
\]

The use of the binomial distribution supposes that \(\beta\) and the blood volume drawn are constant from one sample to another. Now, considering a nonbacteremic patient \((NB)\), the number of positive samples is \(X\). If \(\pi\) is constant from one sample to another, \(X\) is binomially distributed with parameters \(\pi\) and \(n\), and blood culture specificity \((Sp)\) can be written as

\[
Sp = P(X = 0/NB) = (1 - \pi)^n . \tag{A2}
\]

In a second stage, for a given patient, we consider that the values of the parameters also vary from one sample to another. Thus, (1) the bacterial concentration in blood may vary over time, (2) the probability of sample being contaminated may vary with the phlebotomist or with the skin aseptic procedure, and (3) the volume of blood obtained for culture may also vary from one sample to another. The \(j\)th sample is hence characterized by the bacterial concentration in blood \((\beta_j)\) at the time that the sample is obtained, by the probability \(\pi\), of the contamination of the \(j\)th sample, and by the volume of blood put in the \(i\)th bottle of the \(j\)th sample \((vi[j), j).\) Let us characterize blood culture performance in these conditions. When a blood sample is obtained from a bacteremic patient, a negative result \((S-)\) means that the sample contained neither contaminants nor significant bacteria—that is,

\[
P(S = 0/B) = [1 - \pi]e^{-\beta \sum_{i=1}^{n} v_b} .
\]

When \(n\) samples are drawn, the probability of getting all negative results is

\[
P(X = 0/B) = \prod_{j=1}^{n} [1 - \pi]e^{-\beta \sum_{i=1}^{n} v_b(j,i)} , \tag{A3}
\]

where \(\beta_j\) depends on a basal bacterial concentration in blood \(\beta\) defined for a given patient. Se is then defined by the probability that \(\geq 1\) sample is positive for a bacteremic patient:

\[
Se = 1 - P(X = 0/B) , \tag{A4}
\]

with \(P(X = 0/B)\) being defined by means of equation \((A3)\). Sp is defined by the probability of contaminating none of the samples obtained from a nonbacteremic patient:

\[
Sp = P(X = 0/NB) = \prod_{j=1}^{n} (1 - \pi) . \tag{A5}
\]

The sampling design used for the characterization of the probability distribution of Se and Sp is presented in figure 2.

References