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Comparison between two blood collection techniques for the diagnosis of bacteremia in patients with sepsis: unique blood culture vs. the standard method of multiple blood cultures

Juan Camilo Motta-Valencia[1,*], Camilo Rodríguez-García[2], Álvaro Ignacio Arango[3], Magda Sánchez-López[4], Ana María Barragán[5]

Abstract

Introduction: In clinical practice, bloodstream infections, which often occur alongside sepsis, remain among the most serious and life-threatening conditions, with mortality rates as high as 50%. This study aimed to compare the diagnostic performance of a single venipuncture blood culture strategy (30 mL) with the standard method of multiple blood cultures in patients with sepsis.

Materials and methods: A prospective paired within-patient diagnostic comparison study was conducted at the Fundación Cardioinfantil (Bogotá, Colombia) between April 2019 and May 2020. Adult patients with sepsis (SOFA >2) for whom blood cultures were ordered by the treating physician were included in the study. Each patient underwent three venipunctures according to the institutional protocol (10 mL each), and an additional 30 mL sample was collected through a single venipuncture for blood culture. A total of 113 patients were included in the analysis.

Results: The single blood culture technique showed a relative sensitivity of 97.6% (95% CI: 87.1–99.9) and relative specificity of 95.8% (95% CI: 88.3–99.1), with high agreement between both strategies (AUC 0.967; 95% CI: 0.934–1). Compared to the standard technique, the single blood culture demonstrated a comparable diagnostic yield while reducing collection time and patient discomfort.

Discussion: The single blood culture technique (30 mL) may represent a feasible and less invasive alternative, showing comparable diagnostic yield and agreement with the standard method. These findings should be interpreted as a comparison between sampling strategies, given the absence of a truly independent gold standard. Multicenter studies with larger blood volumes are needed to confirm these findings, and their impact on clinical outcomes should be evaluated before supporting their incorporation into clinical practice guidelines.

Keywords: Blood Culture; Bacteremia; Sepsis; Diagnosis; Clinical Laboratory Techniques.

Comparación entre dos técnicas de toma de muestras de sangre para el diagnóstico de bacteriemia en pacientes con sepsis: hemocultivo único vs. el método estándar de múltiples hemocultivos

Resumen

Introducción: En la práctica clínica, las infecciones del torrente sanguíneo, que a menudo ocurren junto con la sepsis, siguen estando entre las afecciones más graves que ponen en peligro la vida, con tasas de mortalidad que se han reportado hasta en un 50%. El objetivo de este estudio fue **c**omparar el desempeño diagnóstico de una estrategia de hemocultivo mediante punción única (30 mL) con el método estándar de múltiples hemocultivos en pacientes con sepsis.

Materiales y métodos: Se realizó un estudio prospectivo de comparación diagnóstica pareada en el mismo paciente, en la Fundación Cardioinfantil (Bogotá, Colombia) entre abril de 2019 y mayo de 2020. Se incluyeron pacientes adultos con sepsis (SOFA >2) en quienes el médico tratante solicitó hemocultivos. A cada paciente se le realizaron tres venopunciones según el protocolo institucional (10 mL cada una), además de una muestra adicional de 30 mL obtenida mediante una única venopunción para procesamiento microbiológico. Se analizaron un total de 113 pacientes.

Resultados: La técnica de hemocultivo por punción única mostró una sensibilidad relativa de 97,6% (IC 95%: 87,1–99,9) y una especificidad relativa de 95,8% (IC 95%: 88,3–99,1), con alta concordancia entre ambas estrategias (AUC 0,967; IC 95%: 0,934–1). En comparación con la técnica estándar, el hemocultivo único demostró un rendimiento diagnóstico comparable en términos de detección, con reducción en el tiempo de toma y menor incomodidad para el paciente.

Discusión: La técnica de hemocultivo por punción única (30 mL) puede representar una alternativa factible y menos invasiva, mostrando un rendimiento diagnóstico comparable y alta concordancia con el método estándar. Estos hallazgos deben interpretarse como una comparación entre estrategias de muestreo, dada la ausencia de un estándar de referencia independiente. Se requieren estudios multicéntricos con mayores volúmenes de sangre para confirmar estos resultados, y su impacto en desenlaces clínicos debe evaluarse antes de considerar su incorporación en guías de práctica clínica.

Palabras clave: Hemocultivo; Bacteriemia; Sepsis; Diagnóstico; Técnicas de laboratorio clínico.

1 Internal Medicine service, Fundación Cardioinfantil- Instituto de Cardiología, Bogotá, Colombia. Infectious Diseases Service, Universidad Nacional de Colombia, Bogotá, Colombia. https://orcid.org/0000-0002-0847-7343
2 Internal Medicine service, Fundación Cardioinfantil- Instituto de Cardiología, Bogotá, Colombia. https://orcid.org/0000-0003-4684-050X
3 Infectiology Department, Fundación Cardioinfantil- Instituto de Cardiología, Bogotá, Colombia. https://orcid.org/0000-0003-2346-3860
4 Microbiology Department, Fundación Cardioinfantil- Instituto de Cardiología, Bogotá, Colombia. https://orcid.org/0000-0003-3735-389X
5 School of Medicine and Health Sciences, Public Health Research Group, Universidad del Rosario, Bogotá, Colombia. https://orcid.org/0000-0002-4646-4324

* Autor para correspondencia:
Correo electrónico: jcmotta@lacardio.org

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Introduction

In clinical practice, bloodstream infections, often occurring alongside sepsis, remain among the most serious life-threatening conditions, with mortality rates reaching up to 50%[1,2]. Prior to initiating therapy, obtaining blood cultures is recommended to support the diagnosis of these infections, provided that this procedure does not delay the timely administration of antibiotics[1,3,4]. Sepsis is widely recognized as a time-dependent condition in which earlier initiation of treatment is associated with improved survival. Evidence suggests that survival may reach up to 80% when antibiotics are administered within the first hour following diagnosis; however, delays beyond four hours have been associated with mortality rates approaching 50% [2,3,5].

In Colombia, the diagnosis of bacteremia relies on blood cultures obtained through three separate venipunctures, each collecting 10 mL of blood (30 mL in total), typically drawn from different anatomical sites at intervals of 20–30 minutes[6–8]. This 30 mL sampling approach is lower than that recommended by long-established international guidelines, which advise a total blood volume of 40–60 mL; nonetheless, this practice remains in use in some institutions. Considering that blood volume, rather than the anatomical site of collection or the number of venipunctures, is the key determinant of diagnostic performance[9–11], a single blood culture collection has been proposed as a valid alternative for the diagnosis of bacteremia, given its favorable performance in other clinical settings[10,12].

However, in the Americas, current guideline recommendations for the diagnosis of sepsis and bloodstream infections indicate that blood cultures for bacteremia are still commonly obtained through multiple venipunctures. This approach is historically grounded in the concept of intermittent bacteremia, whereby sampling from different anatomical sites was thought to increase the likelihood of detecting colony-forming units[1,7,13,14]. However, this assumption may not be entirely accurate, as multiple studies have shown that the volume of blood collected is the primary determinant of blood culture diagnostic performance in this clinical setting[10–12,15–17].

Accordingly, this study aimed to compare the diagnostic performance of a single-venipuncture blood culture strategy (30 mL) with that of the standard multiple-venipuncture approach in patients with suspected bacteremia. The objective was to evaluate its diagnostic yield, agreement with the standard method, and operational characteristics, including the potential to reduce the number of venipunctures, shorten collection time, and decrease the risk of sample contamination, rather than to establish equivalence or definitive clinical superiority between both strategies.

Materials and methods

This study was designed as a prospective, within-patient comparative diagnostic study. The study population included adult patients (≥18 years) treated in the emergency department, general wards, and intensive care unit of the Fundación Cardioinfantil–Instituto de Cardiología, Bogotá, Colombia. Patients with sepsis, defined by a Sequential Organ Failure Assessment (SOFA) score >2, were enrolled between April 2019 and May 2020. Blood cultures were obtained as part of routine clinical management at the discretion of the treating physicians. The sample size was estimated based on prior evidence and calculated using the method described by[10]. was replaced in the formula suggested by[18], resulting in a required sample size of 117 patients to estimate a sensitivity greater than 90% with adequate precision.

Patients with confirmed or suspected catheter-associated infections, as well as those in whom a single 30 mL venipuncture was not feasible or in whom the required blood volume could not be obtained, were excluded. The sample size was determined using consecutive sampling. In each patient, three venipunctures were performed at different anatomical sites, yielding a total of six blood culture bottles: three processed using the standard technique and three processed using the single-collection method. Blood volumes were allocated as follows: 10 mL was collected from two venipunctures according to the institutional protocol, and 40 mL was obtained from a third venipuncture to ensure that both methods received 30 mL each.

According to the available literature, the first two milliliters of blood collected through venipuncture contains skin appendages, thus increasing the probability of contamination of the sample. To avoid favoring any technique and minimize systematic bias between both sampling strategies, the amount of blood needed for the unique blood culture (30 ml) was randomly collected in a balanced way in any of the three venipunctures performed using the standard technique. Upon collecting 40 ml of blood in this venipuncture, a randomization table was used to determine how this amount should be distributed. Whether the standard or the single-venipuncture allocation would be performed first. Once all samples were collected, they were sent to the laboratory, where they were processed. This sampling strategy was designed to ensure comparability between both techniques within the same patient; however, it does not fully reproduce two completely independent sampling approaches as performed in routine clinical practice, since both strategies share the same venipuncture events and blood volume allocation.

Each 30 mL blood sample was distributed into three blood culture bottles according to standard processing procedures. All samples were analyzed by the clinical laboratory following established protocols, and the results were interpreted by the treating physician. Relevant data were subsequently extracted from the patients' medical records.

Blood culture positivity was defined using similar parameters in both techniques, that is, in at least one blood culture obtained from any of the three venipunctures in the standard technique and the unique blood draw, a pathogen consistent with

the clinical signs of the patients was detected, provided that the isolate was monomicrobial (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella spp*., *Enterococcus spp*., and *Candida spp*.). When the isolate was polymicrobial, each germ was analyzed separately to classify the isolates. The classification of isolates as true pathogens or contaminants, findings were based on predefined microbiological and clinical criteria, including the type of microorganism, number of positive bottles, time to positivity, and consistency with the patient's clinical presentation. Classification was initially performed by the treating physician and subsequently reviewed by the research team. Importantly, this adjudication process was conducted independently of the comparative results of both sampling strategies in order to minimize classification bias.

Sample contamination was defined using the same criteria for both blood collection techniques: the growth of non-pathogenic microorganisms, such as coagulase-negative *staphylococcus*, *Bacillus spp*., *Corynebacterium spp*., *Propionibacterium* spp., and *Micrococcus spp*., positivity time greater than 20 h [19], and positivity in only one of the three blood culture tubes, in case any of these microorganisms were isolated. However, when positivity to the same isolate was reported in 2 or more tubes, pathogenicity was determined using the algorithm proposed by[20]

The Ethics and Research Committee of the Fundación Cardioinfantil approved the study protocol. Informed consent was obtained from all the participants.

Statistical analysis

Statistical analyses were performed to compare the diagnostic performances of both sampling strategies. Descriptive statistics were calculated using frequencies and percentages for qualitative variables and measures of central tendency and dispersion for quantitative variables.

A 2×2 contingency table comparing the single venipuncture strategy with the standard method was constructed. Sensitivity, specificity, predictive values, and likelihood ratios were calculated; however, these measures were interpreted as relative estimates, given the absence of an independent gold standard and non-independence of both strategies.

The agreement between the two sampling strategies was assessed, and 95% confidence intervals were calculated for all estimates. Statistical analyses were performed using STATA version 15, and post-test probabilities were estimated using Epidat software

Results

A total of 118 patients were included in the study. The mean age was 59.1 years (SD 18.2), and 60.2% (n=71) were men. Most patients were managed in the emergency department (49.2%), followed by general wards (34.7%). The mean body mass index was 25.3 (SD 5.3).

The diagnostic performance of the single blood culture technique compared with that of the standard approach, as well as the patient inclusion flowchart, are presented in Figure 1.

Diabetes mellitus was the most frequent comorbidity (22%), followed by liver cirrhosis (18.6%), hypertension (17.8%), and hematologic malignancy (16.9%). Corticosteroid use and other immunosuppressive therapies were reported in 17 (14.4%) and 15 (12.7%) patients, respectively. In addition, 32 patients (27.1%) were receiving antibiotics at the time of blood culture collection or had received them within the preceding week. The overall mortality rate was 15.3% (n=18), with septic shock being the leading cause of death (61.1%).
As a result of the randomization process, 40 mL of blood was collected during the first, second, and third venipunctures in 43 (36.4%), 38 (32.2%), and 37 (31.4%) cases, respectively. Regarding allocation, the 30 mL sample corresponding to the single blood culture technique was obtained first in 52 (44.1 %) cases. The most frequently used site for collecting 40 mL of blood was the right basilic vein (26.3%), followed by the right internal jugular vein (20.3%).

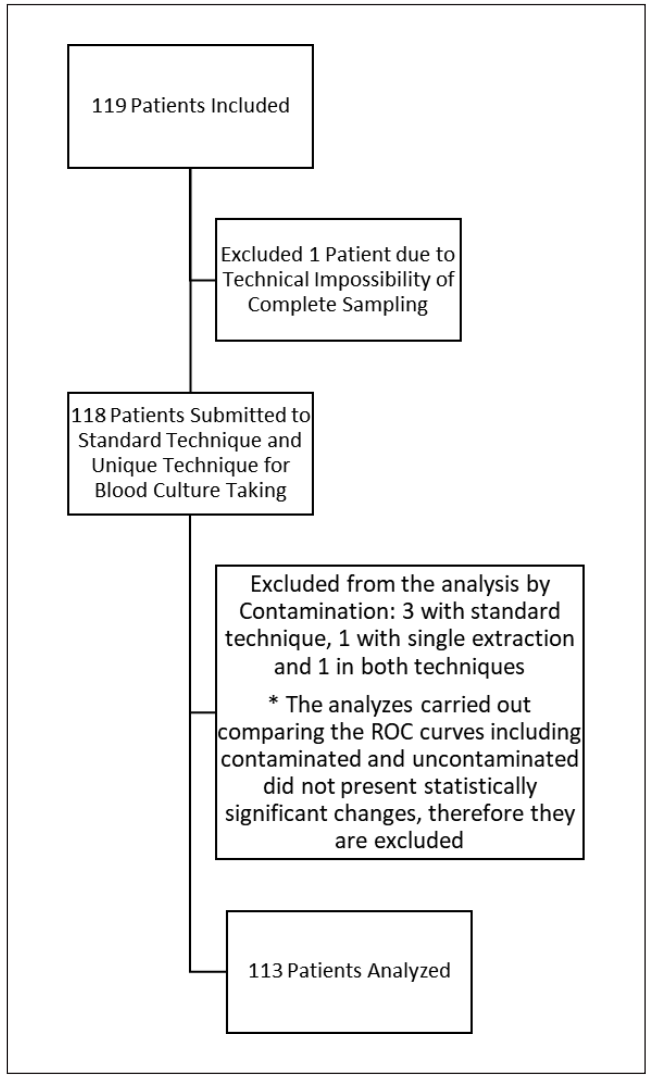

Figure 1. The patient inclusion flowchart

Table 1. Characteristics of the blood cultures collection process.

Characteristic	n	%
Venipuncture in which 40 ml blood were collected		
First venipuncture	43	36.4
Second venipuncture	38	32.2
Third venipuncture	37	31.4
Initial distribution of 40ml of blood		
10ml for the standard blood culture collection technique	66	55.9
30ml for the unique blood culture collection technique	52	44.1
Vein used for performing the unique venipuncture		
Right Basilic	31	26.3
Right jugular	24	20.3
Left Basilic	15	12.7
Left cephalic	15	12.7
Right cephalic	12	10.2
Median antebrachial Right	6	5.1
Right Arterial Line	6	5.1
Right Brachial	2	1.7
Left Radial	2	1.7
Left Brachial	1	0.85
Left Cubital	1	0.85
Right Dorsal Vein	1	0.85
Right Dorsal Metacarpal	1	0.85
Left Dorsal Metacarpal	1	0.85
Vital signs at the time the blood sample was collected		
	Mean	**Standard deviation (SD)**
SBP (mmHg)	107,4	24,1
DBP (mmHg)	61,9	16
MAP (mmHg)	76,2	17,4
HR (heart beats per minute)	98,1	23,1
RR (breaths per minute)	20,7	4,3
Glasgow Coma Scale	13,4	3,6
Degrees (in C°)	37,5	1,3

SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, TAM: Mean Arterial Pressure, HR: Heart Rate, RR: Respiratory Rate

The detailed characteristics of the blood culture collection process and patients' vital signs at the time of sampling are provided in Table 1. The SOFA scores and laboratory parameters on the day of blood culture collection are presented in Table 2.

Regarding blood culture results, the single-collection technique yielded a positivity rate of 37.3% (n=44), with contamination observed in 1.7% (n=2) of the samples. In contrast, the standard technique (three venipunctures) showed a positivity rate of 34.8% (n=41) and a contamination rate of 3.4% (n=4). These differences in contamination rates were descriptively explored as part of the overall performance of both strategies.

Escherichia coli was the most frequently isolated microorganism (28.3%; n=13), followed by *Staphylococcus epidermidis* (13.0%; n=6), and *Staphylococcus aureus* (8.7%; n=4). Blood cultures were most commonly obtained in the context of urinary tract infections (26.3%; n=31), which represented the leading source of bacteremia (Table 3). The mean time from hospital admission to blood culture collection was five days The mean time to blood culture positivity was 21.6 h (SD 17.6) with the standard technique and 22.2 h (SD 17.5) with the single-collection method. In contrast, the time required for sample collection differed substantially: the standard technique required a mean of 28.4 min (SD 13.3), whereas the single-collection method required only 1–2 min.

A contingency table was constructed to assess the diagnostic validity of the single blood culture collection technique, using it as the index test and the standard method as the reference standard (Table 4). The single-collection approach demonstrated high sensitivity for the diagnosis of bacteremia (97.6%), corresponding to the detection of approximately 98 cases per 100 affected patients (Table 5).

The overall agreement between the two sampling strategies was high, with concordant results observed in 109 of 113 cases (96.5%). Discordant results were identified in four cases, including three cases in which the single blood culture was positive and the standard method was negative and one case with the opposite pattern.

The unique blood culture technique had a positive predictive value of 93% based on a bacteremia prevalence of 36%. The clinical utility was explored descriptively using likelihood ratios, although these measures should be interpreted cautiously given the absence of an independent gold standard. The clinical utility of the results was descriptively explored using likelihood ratios and Fagan's nomogram. However, these measures should be interpreted with caution, as they are derived from sensitivity and specificity estimates based on imperfect and non-independent reference standards. Therefore, in the context of this study, likelihood ratios were presented as complementary descriptive metrics rather than definitive indicators of clinical decision-making or post-test probability (Figure 2).

Discussion

This study evaluated two blood culture collection strategies in patients with sepsis using a within-patient comparative design. Rather than establishing diagnostic accuracy against an external reference standard, the findings should be interpreted as a comparison of the diagnostic agreement and relative yield between the two approaches that capture the

same underlying biological process.

The results showed comparable diagnostic yields and elevated levels of agreement between both strategies, suggesting that a single-venipuncture approach using an adequate blood volume (30 mL) may achieve a detection capacity similar to that of the standard multiple-venipuncture method. These findings should not be construed as evidence of equivalent diagnostic accuracy in absolute terms but rather as estimates of relative performance, given that both strategies are based on the same microbiological principles and are not independent.

The analysis of discordant cases provides additional clinical insight. Discordant results were uncommon and were observed in cases where the single venipuncture strategy identified pathogens that were not detected by the standard method. Clinical correlation indicated that several of these cases were consistent with true infections, suggesting that the observed differences between strategies may reflect sampling variability rather than true diagnostic failure.

To our knowledge, this is the first diagnostic study conducted in Latin America to evaluate the performance of a single blood culture collection approach for diagnosing bacteremia. To date, only three studies have assessed the performance of this strategy in the diagnosis of bacteremia or bloodstream infections[10,21,22]; however, in those studies, a total blood volume of 40 mL was collected, in contrast to the 30 mL used in our study. Additionally, our study population consisted of patients with sepsis and suspected primary bacteremia. Patients with catheter-related infections were excluded, as their management protocols do not permit the application of this sampling approach

According to the institutional blood culture collection protocol, the standard technique requires a total of 30 mL of blood obtained through three venipunctures at different anatomical sites, with 20–30-minute intervals between each draw[6]. However, in real-world settings, complete adherence to this protocol is not always achieved[6]. This limitation highlights that the standard technique itself may be imperfect and should not be considered a definitive gold standard, even though it represents the routine clinical practice in our institution. This situation may explain the apparent classification of some cases as false positives in the single blood culture strategy, which, upon clinical correlation, were consistent with true infection.

A key strength of this study is the inclusion of patients across multiple clinical settings, including the intensive care unit, general wards, and the emergency department. In addition, both sampling strategies were performed for each patient by the same nurse, minimizing operator-related variability. Furthermore, the venipuncture used to obtain the 40 mL sample and the allocation of blood between techniques were randomly assigned, thereby strengthening internal validity and reducing potential sampling bias.

Regarding the interpretation of isolates detected using the single blood culture collection approach, evidence remains limited, and to date, only one study has addressed this aspect, namely that conducted by[22]. Based on the isolated microorganisms and the number of positive culture bottles, the positive predictive values for growth in more than one bottle were reported as 88% for Escherichia coli, Staphylococcus aureus, and Candida spp. and 100% for Pseudomonas aeruginosa. However, in that study, a total of 60 mL of blood was collected across six culture bottles—twice the volume obtained in our study. In light of this difference, we defined a clinically significant isolate as a microorganism identified as a pathogen and consistent with the patient's clinical presentation[9,21 22].

Regarding the definition of contamination in the single blood culture collection approach, the proportion of positive bottles obtained from different anatomical sites cannot be used as a criterion. Instead, contamination was considered when the isolates corresponded to microorganisms commonly associated with the skin microbiota[23]. However, in recent years, infections caused by coagulase-negative Staphylococcus (CoNS), both nosocomial and community-acquired, have increasingly been recognized as causes of bacteremia, particularly in association with catheters, urinary tract infections, surgical site infections, and prosthetic devices[24,25]. *Staphylococcus epidermidis* is the most frequently isolated bacteria

Table 2. SOFA score and paraclinical test results of participants on the day the blood cultures were collected.

Paraclinical test	Mean	(DE)
Blood Gases		
Fraction of inspired oxygen (%)	30,9	18,7
Partial pressure of oxygen (PaO2) (mmHg)	67,5	20,2
Partial pressure of carbon dioxide (PaCO2) mmHg)	30,3	7,6
Bicarbonate (meq/L)	19,8	4,6
PAFI or Kirby Index (PaO2/FiO2)	251	79
Lactate (mmol/L)	2,9	2,25
Complete blood count and blood chemistry tests		
Hemoglobin (mg/dl)	11,5	2,8
Hematocrit (%)	35	8,1
Leukocytes (absolute count)	14086	10652
Platelets (absolute count)	196411	146011
Total bilirubin (mg/dl)	3,5	6,9
Direct bilirubin (mg/dl)	2,2	4,4
Creatinine (mg/dl)	2,1	2,5
Ureic Nitrogen (mg/dl)	33	27,6
C-reactive protein (mg/l)	13,4	10,1
Albumin (mg/dl)	2,9	0,78
SOFA score	6,5	4

SD: Standard Deviation; SOFA: Sequential Organ Failure Assessment

Table 3. Characteristics of isolates detected in the blood cultures.

Characteristic	n	%
Result of the blood culture collected using the unique blood culture technique		
Negative	72	**61**
Escherichia coli	13	**11**
*Staphylococcus Epidermidis***	6	**5.1**
Staphylococcus Aureus	4	**3.4**
Salmonella spp	3	**2.5**
Candida parapsilosis	2	**1.7**
Klebsiella Pneumoniae	2	**1.7**
Candida Glabrata	1	**0.85**
Enterobacter Aerogenes	1	**0.85**
Enterococci Faecalis	1	**0.85**
Klebsiella Oxytoca	1	**0.85**
Morganella Morganii	1	**0.85**
*Propionibacterium acnes**	1	**0.85**
Proteus mirabillis	1	**0.85**
Pseudomonas aeruginosa	1	**0.85**
Pseudomonas aeruginosa y Escherichia Coli	1	**0.85**
Serratia Marcescens	1	**0.85**
*Staphylococcus Haemoliticus***	1	**0.85**
Streptococcus pyogenes	1	**0.85**
Streptococcus Agalactie	1	**0.85**
Streptococcus Anginosus	1	**0.85**
Streptococcus Pneumoniae	1	**0.85**
Streptococcus mitis	1	**0.85**
Source of infection		
Urinary tract infections	31	**26.3**
Gastrointestinal infections	19	**16.1**
Pulmonary infections	19	**16.1**
Biliary tract infections	15	**12.7**
Skin and soft tissues infections	8	**6.8**
Fever without apparent focus of infection	7	**5.9**
Febrile neutropenia	5	**4.2**
Primary bacteremia	4	**3.4**
Intra-abdominal infections	3	**2.5**
Endocarditis	3	**2.5**
Spontaneous Bacterial Peritonitis	3	**2.5**
Pelvic infections	1	**0.85**

SD: standard deviation
*Contaminated blood culture. ** A significant isolate vs. Contamination was defined according to the algorithm proposed by Beekman *et al*[20]

Table 4. Distribution of positive and negative blood cultures in both techniques.

Result in blood cultures obtained using the unique blood culture collection method	Result in blood cultures obtained using the standard blood collection method		Total
	Negative	Positive	
Negative	69	1	70
Positive	3	40	43
Total	72	41	113

associated with these infections, followed by *Staphylococcus haemolyticus* and *Staphylococcus hominis*[25]. In a study by[22], some isolates of these microorganisms were considered clinically significant when recovered from more than three of the six culture bottles collected. However, as this number of bottles is not routinely used in our setting, we applied the algorithm proposed by [20] which incorporates persistence of isolation over time and the patient's clinical condition to determine pathogenicity.

In our study, among the seven CoNS isolates, four were classified as pathogenic based on clinical correlation and microbiological criteria, including recovery in two or more bottles obtained through the standard technique (three venipunctures) and in more than two bottles using the single-collection method. Consistent with these findings, several studies have shown that blood culture collection through a single venipuncture may reduce the risk of contamination[10]. In addition to its role in the interpretation of isolates, contamination should be considered an integral component of the overall performance of blood culture collection strategies. From a clinical perspective, contamination is not merely a methodological limitation but a relevant outcome, as it may lead to unnecessary antibiotic use, additional diagnostic procedures and increased healthcare costs. In our study, the lower contamination rate observed with the single venipuncture strategy suggests a potential practical advantage; however, this finding should be interpreted cautiously and warrants further evaluation in studies specifically designed to assess contamination as a primary outcome.

From an operational perspective, the single venipuncture strategy offers several advantages, including reduced procedure time, decreased patient discomfort, and lower resource utilization. It may also facilitate more timely clinical decision-making; however, this study did not directly evaluate downstream clinical outcomes, such as time to antibiotic administration or therapeutic modifications; therefore, these potential benefits should be interpreted cautiously[1,2]. Although cost analysis was not a primary objective of this study, the single blood culture collection approach was associated with lower procedural costs due to reduced resource utilization. Compared with the standard technique, an estimated cost reduction of approximately USD 1.48 per procedure (2020 values) was observed, primarily driven by decreased use of consumables, including sterile gloves and gauze. In addition, this estimate does not account for potential savings in nursing time, as the single-venipuncture approach eliminates the need for multiple punctures and the 20–30-minute intervals between collections; including these factors would result in a greater overall cost reduction.

An additional methodological consideration relates to the sampling designs. Although the within-patient approach and shared venipuncture strategy improved comparability and reduced variability, it does not fully reflect the two independent sampling procedures as performed in routine clinical practice.

This may have influenced the observed agreement between the two strategies and should be considered when interpreting the results and their applicability to real-world settings.

This study had several limitations. First, the absence of an independent reference standard constrains the interpretation of traditional diagnostic accuracy measures, such as sensitivity and specificity; accordingly, agreement-based metrics may provide a more appropriate assessment of the performance. Second, the standard practice in our institution—and in many hospitals in Colombia—consists of collecting three blood culture bottles (30 mL in total), which differs from the international recommendations of two or three sets (40–60 mL). Given that blood culture yield is strongly influenced by total blood volume, number of bottles, and local collection practices, the generalizability of our findings to settings using different protocols or higher blood volumes may be limited. Therefore, our results are most applicable to centers with similar sampling approaches.

In addition, prior antibiotic use in a subset of patients represented a potential source of heterogeneity. Previous antimicrobial exposure may reduce blood culture yield and affect bacteremia detection, thereby influencing the comparative performance of the two strategies. Due to sample size constraints, stratified analyses according to prior antibiotic exposure were not performed; therefore, this factor should be considered when interpreting the results. The absence of an indeterminate category may have led to forced classification in borderline cases, which could introduce some degree of classification bias.

Therefore, our findings should be interpreted as evidence of comparable diagnostic yield and agreement between the two sampling strategies, rather than definitive validation of one method over the other. Future multicenter studies, including larger blood volumes and alternative methodological approaches, are needed to confirm these findings. Additionally, cost-effectiveness analyses should be conducted to further evaluate the potential advantages and their impact on clinical outcomes should be further evaluated in future studies.

Table 5. Diagnostic performance characteristics of unique blood culture compared to the standard technique (multiple venipunctures).

Diagnostic performance measures	Calculation	Value	CI 95	
			LI	LS
Prevalence	Pr(A)	36.0%	27.0%	45.9%
Sensitivity	Pr(+|A)	97.6%	87.1%	99.9%
Specificity	Pr(-|N)	95.8%	88.3%	99.1%
Area under the ROC curve	(Sen. + Spec.)/2	0.967	0.934	1
Likelihood ratio (+)	Pr(+A)/ Pr(+N)	23.4	7.73	71
Likelihood ratio (-)	Pr(-A)/Pr(-N)	0.0255	0.0037	0.176
Odds Ratio	LR(+)/LR(-)	920	109	.
Positive predictive value	Pr(A|+)	93%	80.90%	98.50%
Negative predictive value	Pr(N|-)	98.60%	92.30%	100%

(LI: Limit inferior; LS: Limit superior; CI: Confidence interval.)

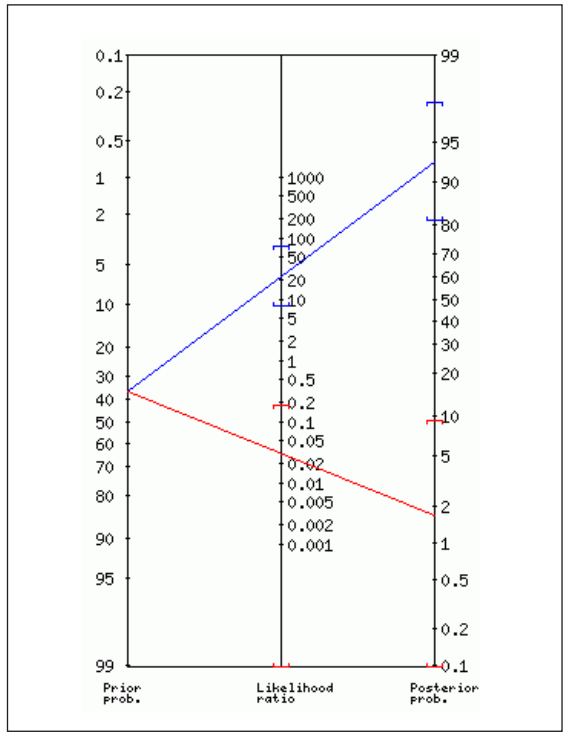

Figure 2. Post-test probability after obtaining a result from a blood culture collected using the unique blood culture collection method.

In conclusion, the single blood culture technique (30 mL) may represent a feasible and less invasive alternative, showing comparable diagnostic yield and high agreement with the standard multiple venipuncture method in patients with sepsis. These findings should be interpreted as a comparison between sampling strategies rather than as definitive evidence of diagnostic accuracy, given the absence of an independent gold standard and the non-independence of both the approaches. From a clinical perspective, this strategy may offer operational advantages, including reduced collection time, lower resource utilization, and improved patient comfort, without apparent compromise in detection performance. Further multicenter studies with larger blood volumes and alternative methodological approaches are required to confirm these findings, and their impact on clinical outcomes should be evaluated in future studies before broader implementation.

Ethical considerations

Protection of persons and animals. The study was approved by the Ethics and Research Committee of Fundación Cardioinfantil-Instituto de Cardiología. Written informed consent was obtained from all participants.

Protection of vulnerable populations. No vulnerable populations were specifically included in this study.

Confidentiality. The authors followed institutional protocols regarding access to clinical information. All data were anonymized prior to analysis.

Privacy. No patient-identifying information is included in this manuscript.

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Conflict of interest. The authors declare no conflicts of interest.

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Statement on the use of artificial intelligence. ChatGPT (OpenAI, GPT-5.5) was used exclusively for language editing and improvement of manuscript readability. No artificial intelligence tool was used for study design, data analysis, interpretation of results, or generation of scientific content. The authors reviewed all revisions and took full responsibility for the manuscript

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