Likelihood of blood culture positivity using SeptiCyte RAPID

Krupa Navalkar[1], Alyse Wheelock[2], Melissa Gregory[2], Danielle Clark[2], Hannah Kibuuka[3], Stephen Okello[3], Sharon Atukunda[3], Abdullah Wailagala[4], Peter Waitt[4], Francis Kakooza[4], George Oduro[5], Nehkonti Adams[6], Maximilian Dietrich[7], Maik von der Forst[7], Marcus J. Schultz[8,9], Neil R. Aggarwal[10], Jared A. Greenberg[11], Thomas D. Yager[1], Richard B. Brandon[1*]

[1] Immunexpress Inc., Seattle, WA 98109, USA; krupa.n@immunexpress.com (K.N.); thomas.y@immunexpress.com (T.D.Y.); Richard.b@immunexpress.com (R.B.B.)

[2] Austere Environments Consortium for Enhanced Sepsis Outcomes (ACESO), Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, MD, USA; AWheelock@aceso-sepsis.org (A.W.); DClark@aceso-sepsis.org (D.C.); Melissa.gregory2@nih.gov (M.G.)

[3] Makerere University Walter Reed Program, Kampala, Uganda; hkibuuka@muwrp.org (H.K.); sokello@muwrp.org (S.O.); satukunda@muwrp.org (S.A.)

[4] Makerere University Infectious Diseases Institute, Kampala, Uganda; awailagala@idi.co.ug (A.Wa.); PWaitt@idi.co.ug (P.W.); fkakooza@idi.co.ug (F.K.)

[5] Komfo Anokye Teaching Hospital, Kumasi, Ghana; gdoduro@hotmail.com (G.O.)

[6] Infectious Diseases Directorate, Naval Medical Research Center, Silver Spring, MD, USA; nehkonti.adams.mil@health.mil (N.A.)

[7] Heidelberg University, Medical Faculty Heidelberg, Department of Anesthesiology, Im Neuenheimer Feld 420, 69120 Heidelberg, Germany; Maximilian.dietrich@med.uni-heidelberg.de (M.D.); Maik.Forst@med.uni-heidelberg.de (M.v.d.F.)

[8] Division of Cardiothoracic and Vascular Anesthesia and Intensive Care Medicine, Department of Anesthesia, General Intensive Care, and Pain Management, Medical University of Vienna, Vienna, Austria; marcus.j.schultz@gmail.com (M.J.S.)

[9] Nuffield Department of Medicine, University of Oxford, Oxford, UK

[10] Division of Pulmonary Sciences and Critical Care Medicine, University of Colorado School of Medicine, Anschutz Medical Campus, Denver, CO 80045, USA; neil.aggarwal@cuanschutz.edu (N.A.)

[11] Rush Medical College and Rush University Medical Center, Chicago, IL 60612, USA; Jared_Greenberg@rush.edu (J.G.)

* Correspondence: Richard.b@immunexpress.com; Tel.: +61 400 612 684

Abstract: Early diagnosis and identification of causative pathogens using blood culture in patients suspected of Blood Stream Infection (BSI) and sepsis are critical for improving patient outcomes through early and more targeted treatment. **Methods:** Post-hoc retrospective analysis of indiviudal patient data from three prospective clinical studies, conducted in North America, Europe and Africa, to investigate the association between SeptiCyte RAPID test results (SeptiScores) and blood culture results. **Hypothesis:** that a significant correlation exists between elevated SeptiScores and positive blood culture results, and between low SeptiScores and negative blood culture results. **Results:** As the SeptiScore increases, the relative probabilty of a septic patient being BC(+) as opposed to BC(-) also increases. A non-linear positive correlation is observed. Below SeptiScores of 10, the ratio of probabilities of BC(+) sepsis / BC(-) sepsis is <1 while above SeptiScores of 10 this ratio is >1. Thus, septic patients with SeptiScores >10 have a higher probability of being BC(+) compared to BC(-). **Conclusions:** Elevated SeptiScores, obtained before blood culture results, are indicative of increased blood culture positivity. This may have clinical utility, particularly in resource limited settings, as an aid for improving the efficiency of blood culture practice, for instance by informing patient selection and interpretation of blood culture results.

Keywords: sepsis, blood culture, blood stream infection, SeptiCyte RAPID, SeptiScore

1. Introduction

Bloodstream infections (BSI) are a major cause of morbidity and mortality in both hospital and community settings worldwide [1-3]. Early diagnosis and identification of causative pathogens in patients suspected of BSI and sepsis are critical for improving patient outcomes through early and more targeted treatment. This unmet need is most acute in poorly resourced environments, which include not just healthcare settings throughout the lower and middle income countries (LMICs), but also pandemic and mass-casualty scenarios, and prolonged field care settings during military operations.

A lack of early BSI diagnosis has led to more patients receiving inappropriate empirical therapy and to an increase in overall mortality and antimicrobial resistance [4]. Diagnosis of BSI largely relies on culture-based methods that have evolved little in decades [5]. Some challenges associated with traditional culture-based methods for diagnosing BSI include lack of timeliness [6], reduced sensitivity associated with prior antibiotic use (false negatives), and contamination (false positives).

Empirical data indicates that >90% of all blood cultures (BC) taken are negative [7-9], and only ~50% of patients retrospectively diagnosed with sepsis have positive blood culture results [10]. Of those blood cultures that are positive, a contamination frequency as high as 16-23% has been reported [9]. Blood culture contamination is known to lead to increased pharmacy and laboratory costs, increased length of hospital stays, and unnecessary antibiotic use [9, 11]. In addition, the interpretation of blood culture results -pathogen or contaminant? - is not always straightforward, and may depend on the background and experience of the clinician [13-16].

Thus, shortcomings in clinical practice related to testing for BSI are evident and have been noted previously [16]. Various recommendations have been proposed aimed at decreasing the frequency of unnecessary blood cultures, for taking blood cultures of optimum volumes at optimum times, and for optimized phlebotomy techniques to decrease the frequency of contaminants [17]. The suggestion has also been made to take blood cultures only when certain leading indicators are positive [Fabre et al. 2020] [18]. On this point, temperature spikes alone appear to be insufficient as a predictor of when blood cultures should be taken [Riedel 2008 and references therein] [19 and references therein].

SeptiCyte RAPID is an FDA-cleared, host-immune-response test with a one-hour turnaround time that provides a likelihood of sepsis on a scale of 0 - 15 (SeptiScore) (Balk et al., 2024a, 2024b). [20, 21] Results fall into one of four discrete bands with higher SeptiScores indicating higher likelihoods of sepsis. In this study, we tested the hypothesis that a significant correlation exists between elevated SeptiScores and positive blood culture results, and between low SeptiScores and negative blood culture results. If this hypothesis were confirmed, it would support the proposition that SeptiScores could be used to improve the efficiency of blood culture practice, such as by helping to guide patient selection for initial or repeat blood culturing.

2. Materials and Methods

2.1. Study Cohorts

Patients were derived from three independent obervational clinical studies, herein called "510k", "SeptAsTERS" and "UGANDA". The design of these clinical studies has been described in detail elsewhere (respectively Miller et al., 2018; von der Forst et al., 2024; Blair et al., 2023) [22-24]. **Figure 1** shows a flow diagram that describes the selection of patients included in this study. For the 510k and SeptAsTERS studies, diagnoses of sepsis or the non-infectious systemic inflammatory response syndrome (SIRS) were made using a method called Retrospective Physician Diagnosis (RPD) [22]. For the UGANDA study, sepsis / SIRS calls were made on the basis of clinical assessment by attending physicians at multiple time points.

2.1.1 The "510k" study

The "510k" study, conducted at multiple sites in the USA and Europe, enrolled a total of 419 patients from the intensive care unit (ICU) who displayed SIRS and were suspected of sepsis. By consensus RPD, 154 (36.8%) of these patients were determined to have sepsis, and 224 (53.5%) were determined to have SIRS. There were 41 patients (9.8% of total) who could not be classified unambiguously as sepsis or SIRS. These were considered "indeterminates" and are not included in the present analysis. Blood cultures were ordered on the basis of clinical judgement and accordingly were only taken if deemed necessary by the care team. Of the 154 patients classified as septic, 81 had blood cultures taken, and of these 48 (59.3%) were blood culture positive (see Figure 1). The comparator for classification as SIRS or sepsis in this study was consensus clinical adjudication by an external 3-member panel of expert clinicians. The adjudication process has been described in detail in the online data supplement Part 3, in Miller et al., 2018 [22]. The RPD process generally conformed to the conceptual framework provided by the Sepsis-2 definition (Levy et al., 2003) [25].

2.1.2 The "SeptAsTERS" study

The "SeptAsTERS" study, conducted at a single hospital in Germany, enrolled 57 post-surgical ICU patients with SIRS and showing signs of clinical deterioration. A total of 32 (56.1%) were retrospectively determined to have sepsis, and 25 (43.9%) were determined to have SIRS (see Table 1). Blood cultures were ordered on the basis of clinical judgement and accordingly were only taken if

deemed necessary by the care team. Of the 32 septic patients, 15 (46.8%) were blood culture positive. The comparator for classification as SIRS or sepsis in this study was a post-hoc assessment of all patients by three independent intensive care professionals, who were not involved in the study. The process followed a model similar to the one described in the online data supplement Part 3 by Miller et al. [2018] [22] based on FDA Guidance and on publications by Klein Klouwenberg et al. [26, 27]. Details of the post-hoc assessment process are provided in von der Forst et al. (2024) [23]. As organ dysfunction parameters were taken into account, the assessment can be viewed as having been performed under the Sepsis-3 conceptual framework [28].

2.1.3 The "UGANDA" study

The "UGANDA" study, performed at Fort Portal Regional Referral Hospital, Kabarole District, Western Uganda and ACESO Uganda (Makerere University Walter Reed Program and Infectious Disease Institute), Kampala, Uganda, comprises a subset of a larger study which is described in Blair et al. (2023) [23]. The 167 adult patients considered here presented initially to hospital with clinical signs of SIRS and a suspicion of sepsis, and ultimately were recruited from the emergency department (ED) and from medical wards (as opposed to the ICU as was the case for patients in the 510k and SeptAsTERS studies). Patients were excluded if aged <18, weighed less than 40kg, or were on chemotherapy, asplenic, immunosuppressed or pregnant. As dictated by protocol, blood cultures were taken for every patient. The comparator for classification as SIRS or sepsis in this study was based on the sepsis-2 criteria (Levy et al., 2003) [25]. Patient evaluation was conducted by the on-site care team at multiple timepoints (screening, 0h, 6h, 24h, and in some cases 72h). Of the 167 patients enrolled in this study, 134 (80.2%) were evaluated as sepsis / severe sepsis at presentation, and 3 (1.8%), although evaluated as sepsis/severe sepsis at presentation, were later retrospectively adjudicated as SIRS by an independent clinician. A total of 30 patients (18.0%) could not be classified as SIRS or sepsis, and therefore were designated "indeterminate" and not included in subsequent analyses.

2.1.4 The Ghanian supplement

To supplement the number of SIRS patients of African origin, five (5) additional SIRS patients from Ghana were included in the study. These patients were selected from "discovery cohort" participants in "An Observational Study of Sepsis in Kumasi, Ghana", protocol number NMRC.2016.0004-GHA [29, 30]. These patients had been previously adjudicated by the external physician panel and thought ultimately to have non-infectious etiologies of their presentations (i.e. not sepsis, ergo SIRS by the definition used in the present work). Aggregated clinical information from these five patients is included in Supplement S1, but as individual patient clinical values were not available, the patients could not be included in the demographic and clinical data of Table 1. Their SeptiScores, however, were individually available and are included in all figures.

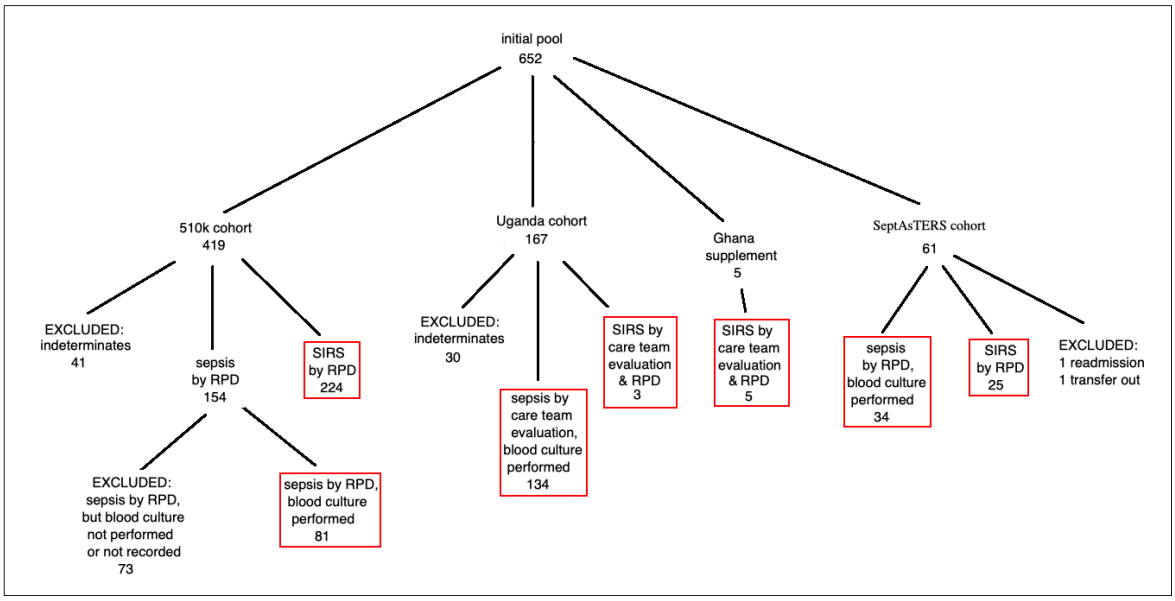


Figure 1. Flow Diagram of Patients Included in This Study. The initial pool from which the final patient selection was made consisted of 652 patients from three prior studies. The final pool consisted of 506 patients (red boxes). Patients were excluded from consideration for either of three reasons: 1) if they fell under protocol exclusion criteria (2 from SeptAsTERS), 2) if they were deemed "indeterminate" (i.e. could not be confidently diagnosed as either SIRS or sepsis; 41 patients from 510k and 30 patients from Uganda), or 3) if they were diagnosed as sepsis but blood cultures were either not performed or not recorded (73 patients from the 510k cohort).

2.2. SeptiCyte RAPID

SeptiCyte RAPID is a cartridge-based reverse transcription – quantitative polymerase chain reaction (RT-qPCR) test that quantifies the relative amounts of two mRNA transripts (PLAC8, PLA2G7) from human blood samples. The test has regulatory clearances for use in adults in the USA, Europe and Australia. The test was validated and shown to display consistent performance in a heterogeneous patient population of 419 ICU patients from the Netherlands and U.S.A. (Balk et al., 2024a, 2024b) [20, 21]. The validation cohort included immunosuppressed patients, and patients with low white cell counts. SeptiCyte RAPID is run on the Idylla hardware platform, using version 1.2 of the test specific software (Biocartis NV, Mechelen, Belgium). The test is performed by pipetting 0.9 mL of PAXgene-stabilized blood (corresponding to 0.24 mL of drawn blood), or 0.24 mL of EDTA anti-coagulated whole blood, into a custom cartridge which performs all assay steps including sample extraction/purification and RT-qPCR for the detection and relative quantification of the PLAC8 and PLA2G7 mRNA transcripts. The SeptiCyte RAPID test results are calculated and presented automatically through a software-generated report, which includes a quantitative score (SeptiScore, range 0–15, see **Figure 2**). The SeptiScore is calculated as the difference between the RT-qPCR Cq values for PLA2G7 and PLAC8, and is positively correlated with sepsis probability. The test has a hands-on time of ~2 minutes and a turnaround time of ~1 hour.

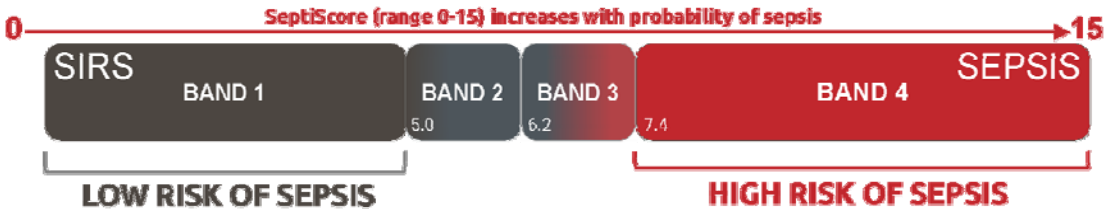


Figure 2. SeptiCyte RAPID Reporting SeptiCyte RAPID provides a SeptiScore ranging from 0 to 15 and falling into one of four "SeptiScore Bands" of increasing sepsis probability. Figure adapted from Balk et al. (2024a) [20].

2.3. Statistical analysis

As this is a post-hoc retrospective analysis of data from three independent studies, it has not been possible to define a 'primary endpoint'. Instead, we test the hypothesis that a significant correlation exists between elevated SeptiScores and positive blood culture results, and between low SeptiScores and negative blood culture results.

2.3.1. Conventional Statistical Tests

Conventional statistical tests were mainly conducted with the R 'stats' package [31]. For simple two-group comparisons, p-values were calculated using a proportions test, and for multiple-group comparisons, p-values were calculatedwith Pearson's chi-squared test (Table 1). Point bi-serial correlations were calculated using the R package 'ltm' [32].

2.3.2. Receiver Operating Characteristic (ROC) Curve Analysis

Receiver Operating Characteristic (ROC) curve analysis with calculation of area under curve (AUC) values [33] was performed using the pROC package in R [34]. Confidence intervals for AUC were calculated by method of DeLong et al. [35] as implemented in pROC [34]. A conventional interpretation of ROC curves was used, in which AUC 0.70-0.80 was considered acceptable, 0.80-0.90 was considered good, and AUC 0.90-1.00 was considered excellent (see e.g. Table 4 of Elkahwagy & Kiriacos, 2024) [36].

2.3.3 SeptiScore Binary Cutoffs

Figure 2 presents the accepted 4-band framework for interpreting SeptiCyte RAPID test results. We also performed binary analyses, in which a single cutoff at a predefined SeptiScore boundary (5.0, 6.2, 7.4, 11.4) is used to generate 2x2 tables and sensitivity / specificity tables. This is most useful for understanding the behavior of the SeptiCyte RAPID test at extreme values of the SeptiScore, such as in a high sensitivity regime with binary cutoff = 5.0, or a high specificity regime with binary cutoff = 11.4.

2.3.4. Probability Ratio Analysis

This study was conducted under the hypothesis that a significant correlation exists between elevated SeptiScores and positive blood culture results, and between low SeptiScores and negative blood culture results. A prediction that follows from this hypothesis is that the ratio of probabilities of BC(+) sepsis / BC(-) sepsis should increase in a monotonic fashion with increasing SeptiScore. This prediction was tested quantitatively, in the following way.

From the pooled data, the numbers of BC(+) sepsis, BC(-) sepsis, and SIRS were counted in eleven adjacent SeptiScore intervals: 0-4, 4.1-5, 5.1-6, 6.1-7, 7.1-8, 8.1-9, 9.1-10, 10.1-11, 11.1-12, 12.1-13, 13.1-15. In each interval the probabilities of BC(+) sepsis and BC(-) sepsis were then calculated as follows:

$$\Pr(\text{BC(+) sepsis}) = N(\text{BC(+) sepsis}) / [\, N(\text{BC(+) sepsis}) + N(\text{SIRS}) \,]$$
$$\Pr(\text{BC(-) sepsis}) = N(\text{BC(-) sepsis}) / [\, N(\text{BC(-) sepsis}) + N(\text{SIRS}) \,]$$

The ratio R of these two quantities was computed and plotted as a function of SeptiScore. A monotonic increase in this ratio is indicated by $R_{i+1} / R_i > 1$ where i is the index variable for the intervals defined above. To test if a trend of monotonic increase in R_{i+1} / R_i was statistically significant, the Jonckheere-Terpstra Test [37] was used, with a web applet available at www.metricgate.com

3. Results

3.1. Patient Demographic, Clinical and Laboratory Characteristics

Table 1 presents the demographic and clinical characteristics of the patients analyzed in the present study. As described in the flow diagram of Figure 1, a total of 652 patients suspected of sepsis were originally considered, comprising 419, 167 and 61 patients from the 510k, UGANDA and SeptAsTERS studies respectively. An extra 5 patients from a Ghanian cohort were added, to supplement the number of available SIRS having a strictly African origin. For 41/419 (9.8%) of patients from the 510k study and 30/167 (18.0%) of patients from the UGANDA study, no unambiguous call of sepsis or SIRS could be made; these patients were therefore deemed "indeterminate" and excluded from further analysis. Of the 322 patients deemed septic, 73 were excluded because blood cultures were either not taken or blood culture results were not recorded (73/322 = 22.7%). A total of 504 patients ultimately were analyzed in the present study (red boxes in Figure 1), comprising 247 with sepsis (consensus or site adjudicated) and available blood culture results, and 257 with SIRS (consensus or adjudicated).

Besides the obvious difference in race/ethnicity compositions between the Ugandan, 510k and SeptAsTERS cohorts, there were slight but statistically significant imbalances in the male/female ratio in both the 510k and SeptAsTERS cohorts ($p < 0.04$ each). A statistically significant difference ($p < 0.001$) was observed between the three studies for the sequential organ failure assessment (SOFA) score. Patients from the Uganda study had a lower median SOFA score (median 2) vs. patients from the 510k study (median 5) or the SeptAsTERS study (median 5).

Patient laboratory characteristics, based on sepsis / SIRS calls and blood culture status, for the three studies are presented in Supplements S2-S5. Across all three studies, a total of 85 septic patients had positive blood cultures with similar proportions of Gram-positive and Gram-negative bacterial isolates. The 510k study had six patients with mixed culture results, in which both Gram-positive and Gram-negative bacteria were isolated. The median C-reactive protein (CRP) level was elevated in all three studies and did not differentiate sepsis from SIRS patients. CRP was highest in the SeptAsTERS study which consisted exclusively of post-abdominal surgery patients. White blood cell (WBC) counts varied widely, from 300 to 57,000 cells / uL (510k study), 2,000 to 75,000 cells/uL (UGANDA study), and 1,300 to 63,000 cells/uL (SeptAsTERS study). In each study, the highest WBC counts were observed in the sepsis group, as opposed to the SIRS group. Median

SeptiScores for patients with positive blood cultures fell in SeptiScore Band 4 (>7.4) for all three studies.

Table 1. Patient Demographics and Clinical Parameters. Abbreviations: SOFA, sequential organ failure assessment (score); M, male, F, female; ND, not determined. A two-sample proportions test was used to estimate p-values for the sex ratio in each study. Pearson's chi-squared test was used to estimate the p-value for comparing SOFA score distributions between the three studies.

	Clinical Study			p-value
Patient characteristic	**510k**	**UGANDA**	**SeptAsTERS**	
Total number	305	137*	59	ND
Age, median (range)	58 (18-90)	46 (18-86) [missing: 12]	66 (30-84)	0.072
Sex (M/F)	172 (56%) M 133 (44%) F (p = 0.037)	65 (47%) M 72 (53%) F	38 (64%) M 21 (36%) F (p = 0.038)	0.064
Race / Ethnicity White African or African heritage Asian Hispanic Other/Unknown	 189 (61.9%) 82 (27%) 14 (4.6%) 16 (5.2%) 4 (1.3%)	 0 137 (100%) 0 0 0	 59 (100%) 0 0 0 0	p < 0.00001
SOFA, median (observed range) [number and % missing values]	5 (0-20) [missing values: 34/305 = 11.1%]	2 (1-10) [no missing values]	5 (1-18) [no missing values]	<0.001
Sepsis	81 [1]	134 [2]	34 [1]	ND
SIRS	224	3	25	ND

[1] Patients classified as septic by consensus RPD, for whom blood culture results were available

[2] Patients classified as sepsis / severe sepsis / septic shock by the on-site care team at multiple timepoints during the patient's hospital stay (screening, 0h, 6h, 24h and in some cases 72 h). Data included from 0h timepoint only.

*5 SIRS patients from the Ghanian cohort are not included in this table, because only aggregate clinical data was available. See Supplement S1.

3.2. Performance of SeptiCyte RAPID Compared to Blood Culture

We stratified patients on the basis of sepsis / SIRS status, blood culture result, and SeptiScore (**Figure 3**). In this figure, the four SeptiScore bands are separated by vertical black lines where Band 1 indicates lowest risk of sepsis and Band 4 indicates highest risk of sepsis. Panel A (red points) shows the distribution of SeptiScores (scale of 0-15) for 85 blood culture positive patients diagnosed with sepsis. An important feature to note are that most (66/85 = 78%) of the patients fell in SeptiScore Band 4, with some patients displaying very high SeptiScores. Further detail on the microbiology results for the 5 patients with highest SeptiScores (12.9-15.0) is given in Supplement S6.

A second important feature to note about Panel A is that none of the BC(+) sepsis patients fell in SeptiScore Band 1. There were, however, eight (8/85 = 9.4%) such patients with SeptiScores in Band 2. These would be considered "false negatives" for sepsis if evaluated solely on the basis of their relatively low SeptiScores. The eight patients were spread across all three studies. Further detail on the microbiology results for these patients can be found in Supplement S7. Both Gram-negative and Gram-positive bacteria were isolated. Four of the patients (# 1,4,6,8 in Table S7) received antibiotics before the blood sample was drawn for SeptiScore measurement, which might explain their low SeptiScores. Four patients (# 1,2,5,6 in Table S7) displayed long blood culture times to positivity (3d, 4d, 9d, 10d) arguing for contamination; these might be better placed in the sepsis BC(-) category of Panel B (below). However, patient #3 (*S. anginosus* from central catheter, time to positivity 1 day) and Patient # 7 (*K. pneumoniae*, time to positivity 41 hours) were not obvious contaminants, were not pre-treated with antibiotics, and fell below the 10th percentile of the SeptiScore cumulative distribution for BC(+) sepsis (see Fig 3D below), and therefore should be flagged for further investigation.

Panel B (blue points) shows the distribution of SeptiScores for 162 sepsis patients that had blood cultures ordered with negative results, or that were positive for cultures other than from blood. Of the 162 patients retrospectively diagnosed with sepsis and with negative blood cultures, 126 (77.8%) had SeptiScores in Bands 3 or 4. Some of the BC(-) sepsis patients had quite extreme Band 4 SeptiScores; five patients with highest SeptiScores (12.1-13.1) are detailed in Supplement S8.

Another point to note is that 10 BC(-) sepsis patients had Band 1 SeptiScores. These comprise 6.2% of the 162 patients in the BC(-) sepsis category. Further detail on these patients can be found in Supplement S9. Patients of the types represented in this table would seem to require further detailed investigation, because if they were truly septic, they would have been missed by an alert system based on either blood culture or SeptiCyte RAPID.

Panel C shows the distribution of SeptiScores for SIRS patients that were blood culture negative (grey dots) and for five blood culture positive SIRS patients (red points). Of note is the high proportion of SIRS patients in Bands 1 and 2 (n=175/ 263 = 66.5%). Further detail on the microbiology results for the five BC(+) SIRS patients can be found in Supplement S10. The bacteria isolated from the three BC(+) SIRS patients with lowest SeptiScores were considered by the RPD panelists to be not clinically relevant and possibly contaminants: *Bacillus spp.* (SeptiScore 4.9); *Fusobacterium* (8 days to positivity, no antibiotics given, SeptiScore 5.4); *coagulase negative Staphylococcus aureus* (6 days to positivity, no antibiotics given, SeptiScore 5.4).

There were also some BC(-) patients deemed SIRS, but with Band 4 SeptiScores. These are represented

by the rightmost grey points in Panel C. Further detail on a selection of these patients is provided in Supplement 11.

Panel D presents a quantification of the data from Panels A, B, C in terms of cumulative distributions of SeptiScores. There is clearly an offset of the distributions in the direction SIRS < sepsis BC(-) < sepsis BC(+).

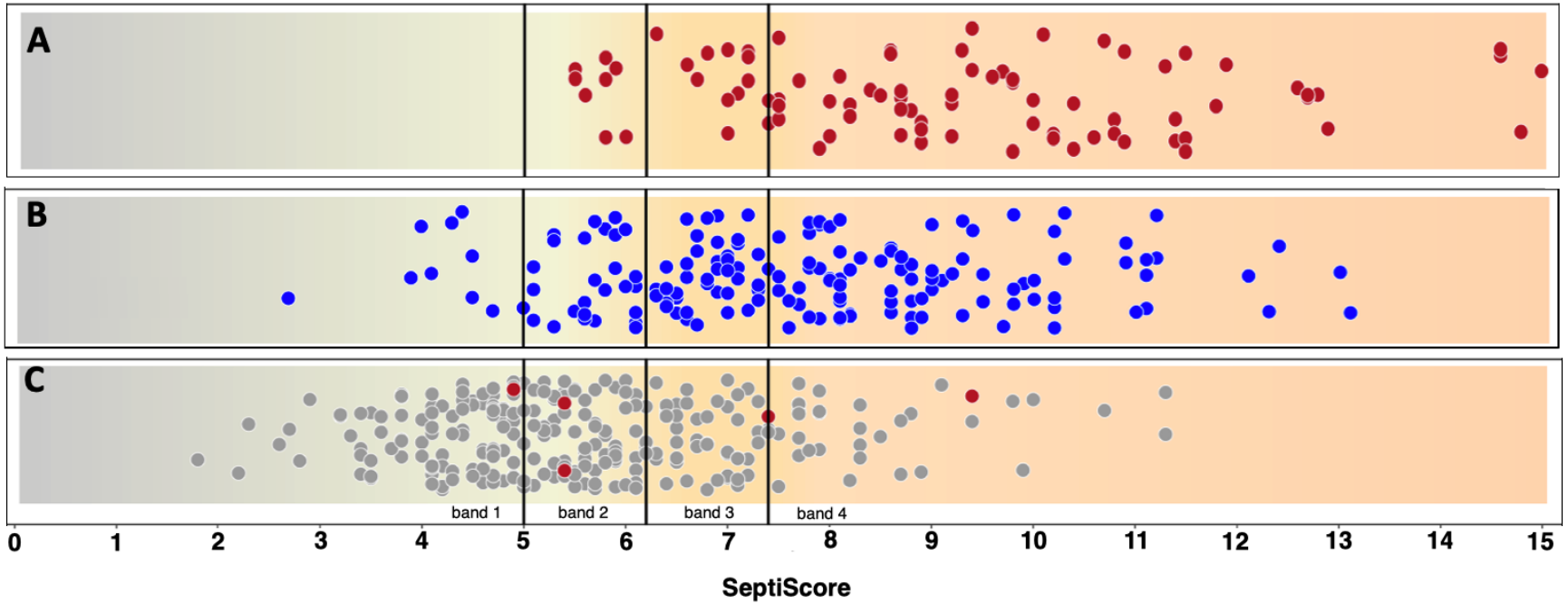
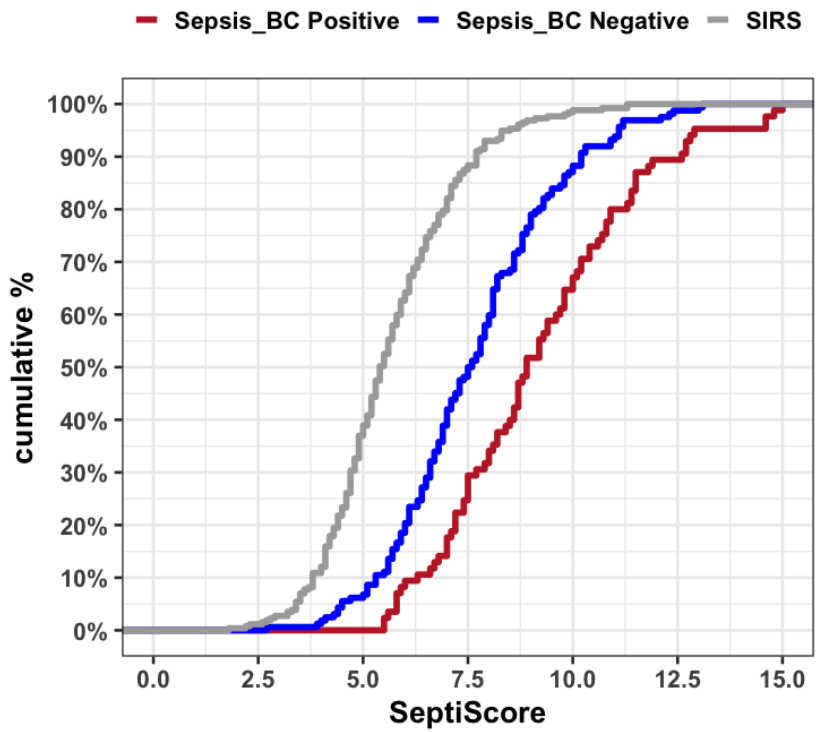


Figure 3. Distribution of Patients Based on RPD (sepsis / SIRS), Blood Culture Status and SeptiScore. Patients from all three cohorts are included. For A, B and C, SeptiScore (range 0-15) is shown on the horizontal axis, with the boundaries between adjacent SeptiScore bands indicated by vertical black lines at values 5.0, 6.2 and 7.4. **(A)** patients retrospectively diagnosed as sepsis with positive blood cultures (red points, n=85). **(B)** patients retrospectively diagnosed as sepsis with negative blood culture results (blue points, n=162) of which 126 (77.8%) fall into SeptiScore bands 3 and 4. **(C)** patients retrospectively diagnosed with SIRS that were blood culture negative (grey points, n=257). Also shown in this panel are three patients retrospectively diagnosed as SIRS, with positive blood cultures (red points, n=5). **(D)** Quantification of data from Panels A, B, C in terms of cumulative SeptiScore distributions.

D



Based on the data presented in **Figure 3**, we next evaluated the diagnostic performance of SeptiCyte RAPID for differentiating patients that were retrospectively diagnosed septic and blood culture positive, from those retrospectively diagnosed with SIRS in a patient population initially suspected of sepsis. **Table 2** summarizes the results of this evaluation, according to SeptiScore Band. Of the 348 patients considered in this table, 85 were both septic and blood culture positive, and 263 were determined to have SIRS. Patients with a SeptiScore falling between 7.4 and 15 (Band 4) had a positive likelihood ratio (LR+) of 5.88 for blood culture positive sepsis (as compared to SIRS). In contrast, patients with a SeptiScore falling between 0 and 4.9 (Band 1) had an LR+ of 0. Further detail, including LR+ calculations, are provided in Supplement S12.

Note that the LR+ values for BC(+) sepsis depend upon the composition of the group to which BC(+) sepsis is being compared. In our analysis, the comparator is the BC(-) SIRS group [patients of Fig 3c], consistent with the Intended Use of the SeptiCyte RAPID test (discrimination of sepsis vs. SIRS). Understandably, the LR+ values for BC(+) sepsis would be decreased if the BC(-) comparator group were expanded to include BC(-) sepsis patients [i.e. Fig 3b patients mixed in with Fig 3c patients]. However, in practice this would not be a meaningful comparison, because clinicians would be expected to use SeptiCyte to discriminate between sepsis and SIRS in accordance with the test's Intended Use, not to discriminate between BC(+) sepsis and BC(-) sepsis.

Table 2. Diagnostic performance of SeptiCyte RAPID, for BC(+) sepsis versus SIRS, in terms of Positive Likelihood Ratio (LR+). For this table calculations were based on 85 patients that were blood culture positive and–diagnosed with sepsis, and 263 patients that were diagnosed with SIRS (including 3 SIRS patients with positive blood cultures). Patients with a SeptiScore falling between 7.4 and 15 (Band 4) had an LR+ of 5.88 for blood culture positive sepsis relative to SIRS. Patients with a SeptiScore falling between 0 and 4.9 (Band 1) had an LR+ of 0 for blood culture positive sepsis relative to SIRS.

SeptiCyte RAPID Band	Sepsis and BC(+)	SIRS	Total Patients	LR+
Band 4 (7.4 – 15)	66	34	100	5.88
Band 3 (6.2 – 7.3)	11	50	61	0.66
Band 2 (5.0 – 6.1)	8	78	86	0.31
Band 1 (0 – 4.9)	0	95	95	0
Total	**85**	**257**	**348**	

We next performed additional analyses of SeptiCyte RAPID performance, including calculations of area under the receiver operating characteristic curve (ROC AUC), sensitivity, specificity and

Youden's index, using data from the combined three study cohorts. **Figure 4A** reproduces the data of **Figure 2** in the form of box-whisker plots. **Figure 4B** presents three ROC curves for SeptiCyte RAPID performance, for sepsis vs. SIRS discrimination, depending on the septic patients's blood culture results. The red curve represents BC(+) sepsis versus SIRS AUC = 0.91). The blue curve represents BC(-) sepsis versus SIRS (AUC = 0.79). Finally the black curve represents sepsis irrespective of blood culture results, versus SIRS (AUC = 0.83). **Figure 4C** shows sensitivity (blue) and specificity (red) curves as a function of the SeptiScore, for the BC(+) versus SIRS patients. The two curves cross at SeptiScore of 7.0 (Youden's index). Band boundaries at 5.0, 6.2, 7.4 are shown as vertical black lines. **Figure 4D** presents a plot of 11 incremental calculations of the relative probabilities of BC(+) sepsis / BC(-) sepsis, as a function of SeptiScore. As the SeptiScore increases, the relative probabilty of a septic patient being BC(+) as opposed to BC(-) also increases. The correlation between increasing SeptiScore and increasing probability ratio is statistically significant according to the Jonckheere-Terpstra trend test [37] as indicated by test statistic value J = 52.5 and p-value: 9.92×10^{-5}. At a SeptiScore of 10, the ratio of probabilities increases above 1 (dotted line in figure). That is, septic patients with SeptiScores >10 have a higher probability of being BC(+) compared to BC(-).

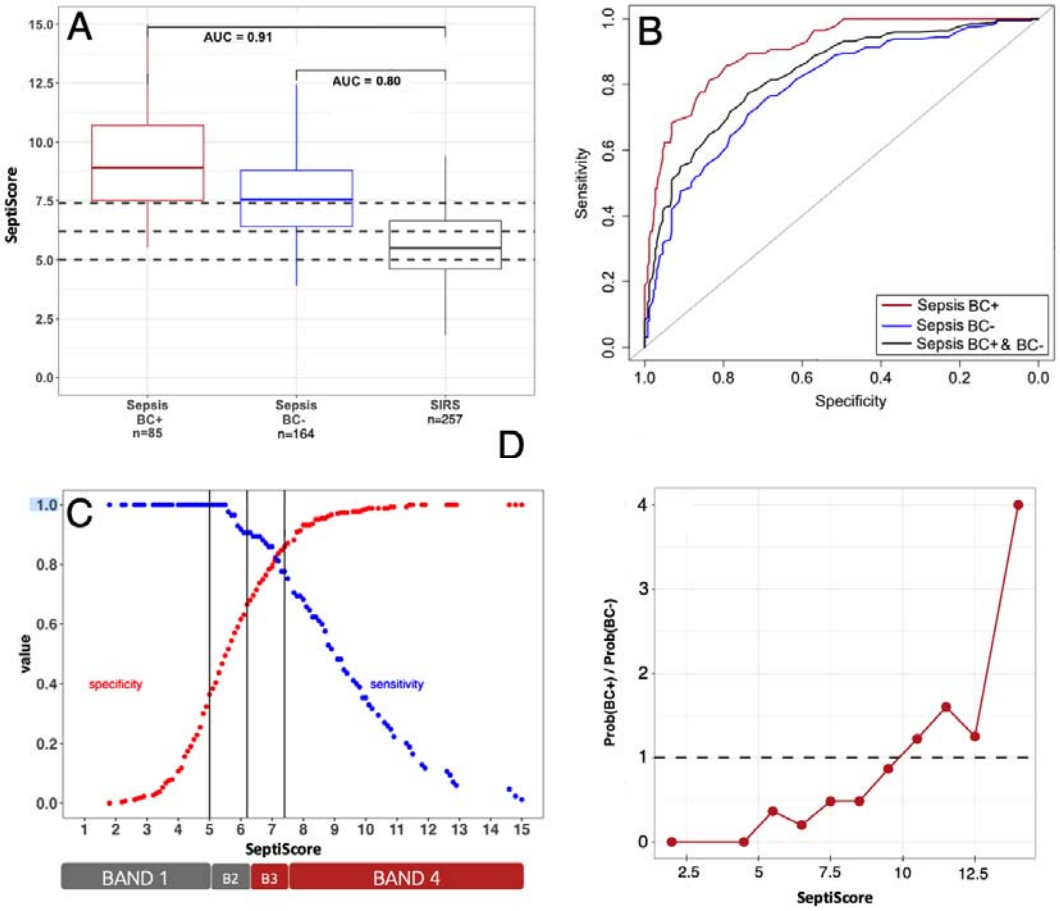
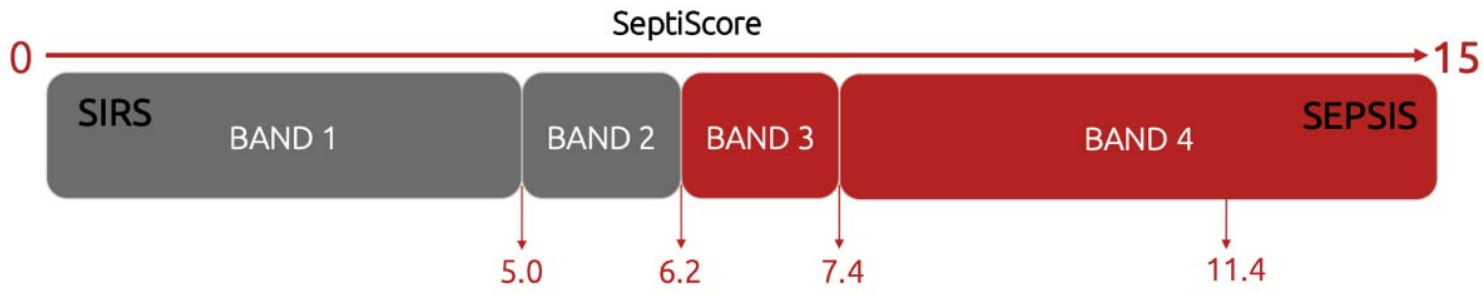


Figure 4. SeptiCyte Performance in Blood Culture Positive Sepsis Patients. (A) Box and whisker plot showing performance of SeptiCyte RAPID when comparing 85 consensus sepsis patients that were either BC(+) or BC(-) to 257 SIRS patients. Data compiled from all three studies. From ROC analysis (Panel B) the calculated AUC for BC(+) sepsis vs. SIRS was 0.91 and the calculated AUC for BC(-) sepsis vs. SIRS was 0.80. **(B)** SeptiCyte RAPID ROC curves for BC(+) sepsis vs. SIRS (red, AUC 0.91, 95% CI 0.87-0.94), for BC(-) sepsis vs. SIRS (blue, AUC = 0.80, 95% CI: 0.75-0.84), and for pooled sepsis (irrespective of blood culture results) vs. SIRS (black, AUC = 0.84, 95% CI: 0.80-0.87). **(C)**

Binarized model in which sensitivity (blue) and specificity (red) are plotted as a function of cutoff SeptiScore for dataset consisting of BC(+) sepsis (N=85) vs. SIRS (N=257). Binary cutoffs at 5.0, 6.2, 7.4 are shown as vertical black lines. **(D)** The ratio of probabilities for BC(+) sepsis versus BC(-) sepsis, as a function of SeptiScore. The data points in this panel are calculated from the numbers of BC(+) sepsis, BC(-) sepsis, and SIRS in the following eleven SeptiScore intervals: 0-4, 4.1-5, 5.1-6, 6.1-7, 7.1-8, 8.1-9, 9.1-10, 10.1-11, 11.1-12, 12.1-13, 13.1-15, and are plotted at the interval midpoints. For septic patients, increasing SeptiScore correlates positively with an increase in relative probability of being BC(+) as opposed to BC(-). The dotted line indicates a probability ratio of 1.0. For SeptiScores above 10 the probability of a septic patient being being BC(+) is higher than that of being BC(-).

Figure 5 presents sensitivity and specificity calculations for BC(+) sepsis and BC(-) sepsis versus SIRS, for binarized models with single cutoffs at the predefined SeptiScore Band boundary (5.0, 6.2, 7.4) and also at 11.4. This figure is derived from the more complete ROC curve analysis of **Figure 4B**. For a binary cutoff at SeptiScore 5.0 the sensitivity was 100%, and for a binary cutoff at SeptiScore 11.4 the specificity was 100%. That is, based on the patients considered in this study, no patients with SeptiScores less than 5.0 were retrospectively BC(+) sepsis, and no patients with SeptiScores greater than 11.4 were retrospectively SIRS.



	Binary cutoff at 5.0		Binary cutoff at 6.2		Binary cutoff at 7.4		Binary cutoff at 11.4	
comparison	sensitivity	specificity	sensitivity	specificity	sensitivity	specificity	sensitivity	specificity
BC(+) sepsis vs SIRS	100%	37.0%	90.6%	67.3%	77.7%	87.0%	18.8%	100%
BC(-) sepsis vs SIRS	94.0%	37.0%	77.0%	67.0%	52.0%	86.8%	3.0%	100%
all sepsis vs SIRS	96.0%	37.0%	82.0%	67.0%	61.0%	87.0%	8.4%	100%

Figure 5. Sensitivity and Specificity of SeptiCyte RAPID for Sepsis BC(+) or Sepsis BC(-) Versus SIRS. This figure presents binarized classification models, w cutoffs at the predefined SeptiScore boundaries (5.0, 6.2, 7.4, 11.4). The tabular values show calculated sensitivity and specificity for BC(+) sepsis and BC(-) sepsis SIRS for each SeptiCyte RAPID band boundary, and for a cutoff at 11.4 at which value SeptiCyte RAPID has 100% specificity (no patients were retrospectiv called SIRS below this SeptiScore).

4. Discussion

The current reference standard for diagnosing BSI is blood culture. However, blood culture practice and interpretation of results have a number of limitations including low positive rates, contamination and a lack of timeliness. Downstream implications from these limitations include unnecessary antibiotic use, an increase in overall mortality and antimicrobial resistance, increased pharmacy and laboratory costs, and length of hospital stays (Butler 2023, Fabre 2025, Dempsey 2019) [4, 9, 11]. A number of interventions as part of blood culture diagnostic stewardship have been described including careful patient selection and efforts to keep contamination rates less than or equal to a target rate of 1% of all blood cultures taken (Fabre 2020, Schinkel 2023, Doern 2019, CLSI M47) [38-41]. Therefore, it can be argued that blood culture practice is currently suboptimal and there is a need for improved BC stewardship (Fabre, 2025) [9].

In this study, we conducted a post-hoc retrospective analysis of patients suspected of sepsis, representing diverse populations across multiple continents (North America, Europe, Africa). We have shown a positive correlation between sepsis likelihood, measured using SeptiCyte RAPID, and likelihood of blood culture positivity. From our study of 504 patients, of which 16.9% (n=85) were blood culture positive, our results showed that patients with SeptiScores greater than 10 had a higher probability of being BC(+) sepsis than BC(-) sepsis. Further, patients with SeptiScores less than 5.0 had a 0% probability of being BC(+) sepsis (**Figure 4C**). That is, positive blood cultures in patients with SeptiScores less than 5.0 are likely to be either a contaminant or not relevant to a sepsis diagnosis. SeptiCyte RAPID has a one-hour turnaround time and therefore precedes blood culture results by at least 12 hours. These results suggest that SeptiScore may aid blood culture practice by guiding patient selection based on likelihood of being blood culture positive. Further, SeptiScore may aid in interpretation of positive blood culture results with respect to likelihood of contamination or relevance to a sepsis diagnosis.

Patients with SeptiScores greater than 11.4 had 100% probability of being sepsis (either BC(+) sepsis or BC(-)), and over 77% of patients retrospectively diagnosed with sepsis and with negative blood culture results had band 3 and 4 SeptiScores. Thus SeptiScores also tend to be elevated in cases of apparent non-bacteremic sepsis. It is recognized that some septic events may not be bacteremic in nature, and may instead be due to localized (non-bloodborne) bacterial infections, or to infections from other types of pathogens (viral, fungal, parasitic) [Chang et al. 2024; Lee e al. 2024; Xu et al., 2024] [42-44].

Overall blood culture positive rate in our study was 16.9%, which is higher than that recently reported in the USA. Fabre (2025) [9], in a study involving 362,327 blood cultures conducted between 2019 and 2020 and in 19 USA hospitals reported blood culture positive rates from 5.6% to 7.3% depending upon hospital location (ICU, ward) and whether the patients were surgical or medical. The higher positive rate in our study may relate to unintentional bias in patient selection, since the patients in all three cohorts examined here were enrolled on the basis of being critically ill. In the Fabre study (2025) [9] the contamination rate was 1.5% of all blood cultures taken which equates to ~24% contamination rate for positive cultures. The overall contamination rate in our study could not be accurately calculated because a blood culture was not taken from every patient. However, out of a total of 85 blood culture positive results there were 3 retrospectively diagnosed with SIRS and with low SeptiScores and considered to be either contaminants or not relevant to a sepsis diagnosis (3.5% of all positive cultures).

The ROC AUC for using SeptiScore to discriminate SIRS vs. (BC)+ sepsis was 0.91 (see **Figure 4A**). The calculated Likelihood Ratio (LR) for blood culture positivity for SeptiCyte RAPID results in Band 4 was 5.88. An important point is that there were no blood culture positive patients retrospectively diagnosed with sepsis that had a Band 1 SeptiScore (<5) (LR = 0). Another point to note is that SeptiCyte RAPID results are available within one hour and in the same time frame as WBC data. Such a timeframe is significantly shorter than reported median times to blood culture positivity of 15.7 hours (Lambregts et al., 2019) [6]. That is, SeptiCyte RAPID results are available at least 12 hours prior to gaining any blood culture results and within the 3-hour period recommended by the Surviving Sepsis Campaign (Evans et al., 2021) [45] for administration of antibiotics for patients suspected of sepsis (without shock). Based on these results, possible considerations with respect to blood culture diagnostic stewardship are suggested in Table 3.

Table 3: Possible Clinical Utility of SeptiCyte RAPID for Blood Culture Practice

		No Prior Blood Culture Result	Previous Blood Culture Result	
			Positive	**Negative**
SeptiScore	**High**	• Take initial and follow-up BC sets • Increased efforts to isolate pathogen • Use syndromic panels • Look for possible source	• Repeat SeptiCyte RAPID after 48-72 hours if symptomatic or deteriorating • Repeat blood cultures to rule out metastatic spread	• Increased efforts to isolate pathogen • Take additional blood culture sets • Use syndromic panels • Look for possible source
	Low	• Consider alternate diagnoses • Repeat SeptiCyte RAPID if patient deteriorating	• Rule out contamination • Repeat SeptiCyte RAPID. If, upon repeat, a high SeptiScore obtained, then repeat blood cultures. If another low SeptiScore obtained, then consider alternate diagnosis	• Low likelihood of follow up blood cultures being positive unless patient deteriorates • Consider alternate diagnosis

For patients with high SeptiScores (>7.4, Band 4) and positive blood culture results, SeptiCyte RAPID could be repeated to confirm a diagnosis and to rule out metastatic spread. For patients with high SeptiScores and negative blood culture results, more intense efforts could be made to identify a causative pathogen to include the use of multiple blood culture sets and molecular diagnostic syndromic panels. High SeptiScores may be an indication the AB treatment is not effective.

For patients with low SeptiScores (<5.0, Band 1) and positive blood culture results, contamination could be considered along with a diagnosis other than sepsis or BSI. For patients with low SeptiScores and negative blood culture results, alternative diagnoses (other than sepsis) should be considered.

Further clinical utility for SeptiCyte RAPID could lie in determining whether follow-up blood cultures (FUBC) are needed. It is likely that additional blood cultures will be drawn in patients that continue to deteriorate. FUBC are generally collected after the occurrence of a positive blood culture with a known pathogen that matches the clinical presentation. The aim is to determine the presence of persistent bacteraemia or document the clearance of known bacteraemia. FUBCs are suggested in the management of *Staphylococcus aureus* bacteraemia, endocarditis, and candidemia (Liu 2011, Baddour 2015, Pappas 2016 [46-48]. However, there is controversy as to the utility of FUBC in patients with Gram-negative BSI (Ong et al., 2024). [49] SeptiCyte RAPID results obtained within 1-2 hours of testing could provide guidance on whether FUBCs are necessary and perhaps even determine whether a patient had a persistent bacteraemia or had cleared a known bacteraemia.

Eight sepsis patients with positive blood culture results had SeptiScores falling in Band 2 (5-6.2) (**Figure 3A**). These would be considered "false negatives" for sepsis if evaluated solely on the basis of their relatively low SeptiScores. Closer examination of the microbiology results for these eight patients showed that four had prior antibiotic exposure, and four had long time-to-positivity (3, 4, 9 and 10 days), which may indicate contamination. Such results suggest that many factors need to be taken into consideration when interpreting the clinical relevance of SeptiCyte RAPID and blood culture results, including the timing of blood draws, effect of antibiotics, time to positivity, organism isolated, potential for contamination, other clinical parameters and laboratory results, and patient trajectory (whether the patient is getting better or worse).

Our study has several limitations. First, despite enrolling over 500 patients, there were only 85 septic patients with confirmed positive blood cultures (85/506 = 16.8% positivity), which may limit the generality of conclusions drawn. However, the study included multiple geographic sites (North America, Europe, Africa) and enrolled patients with diverse race / ethnicities and co-morbidities (including patients with HIV, tuberculosis and malaria), and with WBC counts ranging from neutropenic to above normal. Similar blood culture positivity rates, isolated pathogens and SeptiScore distributions for each of the three separate studies suggests that our results and conclusions could be applied more broadly. It should be recognized, though, that the African cohort was recruited and assessed with a relatively well-controlled clinical trial, which may be atypical of the usual level of care available in this LMIC setting. An in-depth comparison of results from the three geographic areas (North America, Europe, Africa) is ongoing and will be presented in a future publication. A second limitation was that in the 510k study, blood cultures were taken at the discretion of the attending clinician. That is, not every enrolled patient had a blood culture taken. Patients with complicated diagnoses such as pneumonia with possible sepsis may not always have triggered blood culture orders [50], and patients with low-level bacteremia may not have undergone extensive enough blood sampling [51], and so may have been 'missed'. Calculations of blood culture positivity and contamination rates are therefore not necessarily accurate for a population of patients merely *suspected* of sepsis. Third, exact timing for blood culture and SeptiCyte RAPID sampling was not known for all patients. Therefore, the influence of antibiotics on blood culture and SeptiCyte RAPID results could not be established with certainty, nor could an exact correlation between blood culture and SeptiCyte RAPID results be determined. A fourth limitation was that the Uganda study relied upon multiple clinical assessments by the on-site care team in real time, but did not have RPDs performed by a panel of external physicians. Thus calculations of performance of SeptiCyte RAPID for differentiating BC(+) sepsis from (true)

SIRS are subject to greater uncertainty for the Uganda study, than for the 510k and SeptAsTERS studies. Fifth, there may have been some selection bias, for example under the decision to exclude patients with "indeterminate" diagnoses instead of forcing these patients into the sepsis or SIRS categories (Figure 1). Finally, SeptiCyte RAPID provides a likelihood of sepsis based on a SeptiScore range of 0-15 and is pathogen agnostic. In future prospective usage of the test, high SeptiScores could be due to a viral or parasitic infection rather than a bacterial or fungal infection as detected by culture. Therefore, caution would need to be taken with respect to interpreting high SeptiScores which indicate higher likelihood of sepsis rather than just culture-positive bacterial or fungal infection.

5. Conclusions

SeptiCyte RAPID test results (SeptiScores) are positively correlated with the likelihood of blood culture positivity. This finding could facilitate diagnostic stewardship with respect to patient selection and interpretation of positive blood culture results. Further work could involve determining the generalisability of these results, including whether SeptiScores correlate to the likelihood of different types of infection (e.g. Staphylococcus, endocarditis, Gram negative BSI, viruses, fungi, malaria). Ultimately the clinical utility of SeptiCyte RAPID for diagnostic stewardship will be established through interventional studies and measurement of impact on patient outcomes, hospital costs, and antibiotic stewardship.

Supplementary Materials: Supplements S1-S12 have been combined into a single pdf file, which is available for download.

Author Contributions: Conceptualization, R.B.B., T.D.Y., K.N.; methodology, A.W., K.N.; software, K.N; validation, A.W., M.D., M. vdF., K.N.; formal analysis, A.W., M.D., M.vdF., K.N., T.D.Y., R.B.B.; investigation, A.W., M.G., S.A., M.D., M.vdF., K.N., R.B.B.; resources, D.C., M.D., M.vdF., R.B.B.; data curation, K.N.; writing—original draft preparation, R.B.B., T.D.Y.; writing—review and editing, A.W., M.G., D.C., H.K., S.O., S.A., A.Wa. P.W., F.K., G.O., N.A., M.D., M.vdF., M.J.S., J.A.G., N.R.A., N]K.N., T.D.Y., R.B.B.; visualization, K.N., T.D.Y.; supervision, D.C., R.B.B.; project administration, D.C., M.D., M.vdF., R.B.B.; funding acquisition, D.C., N.A., M.D., M.vdF., R.B.B. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: All research reported in this study was conducted in accordance with the Declaration of Helsinki, and in accordance with relevant guidelines and regulations. Informed Consent was obtained from all subjects or their legal representatives. The SeptAsTERS component of this study was approved by the Ethics Committee of the Medical Faculty of Heidelberg University (S-118/2021) and was registered in the German Clinical Trials Register (DRKS00024891) prior to enrolment. The Uganda component of this study was approved under IRB NMRC.2016.0004-GHA and NMRC.2017.0001. The Ghanaian supplement to this study was conducted under protocol number NMRC.2016.0004-GHA ("An Observational Study of Sepsis in Kumasi, Ghana") and approved by the Komfo Anokye Teaching Hospital IRB and the Naval Medical Research Center (NMRC) IRB prior to enrolment, in compliance with all applicable US Federal regulations governing the protection of human subjects. The 510k component of this study was conducted under the following approvals. Ethics approval for the MARS trial was given by the medical ethics committee of AMC, Amsterdam (approval # 10-056C, 16 June 2010). Ethics approvals for the VENUS trial were given by the relevant Institutional Review Boards as follows: Intermountain Medical Center/Latter Day Saints Hospital (approval # 1024931, 21 January 2014); Johns Hopkins Hospital (approval # IRB00087839, 28 January 2016); Rush University Medical Center (approval #

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study, or from their legal representatives.

Data Availability Statement: The datasets used and/or analyzed during the current study are Available from the corresponding authors upon reasonable request.

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Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of variance
AUC	area under [ROC] curve
BC	blood culture(s)
BSI	bloodstream infection
CI	Confidence interval
Cq	Threshold-crossing cycle (in qPCR)
CRP	C-reactive protein
ED	Emergency department
F	female
FDA	Food and Drug Administration (USA)
FUBC	follow-up blood culture
ICU	Intensive care unit
LMIC	Lower and middle income country

LR+	Positive likelihood ratio
M	male
mL	Milliliter
ND	Not determined
PLA2G7	Phospholipase A2 group VII (mRNA transcript)
PLAC8	Placenta-associated 8 (mRNA transcript)
ROC	receiver operating characteristic (curve)
RPD	Retrospective physician diagnosis
RT-qPCR	reverse transcription – quantitative polymerase chain reaction
SIRS	systemic inflammatory response syndrome
SOFA	Sequential organ failure assessment (score)
WBC	white blood cell

References

1. Ziegler, M.J.; Pellegrini, D.C.; Safdar, N. Attributable mortality of central line associated bloodstream infection: Systematic review and meta-analysis. *Infection* **2015**, *43*, 29–36.

2. Reunes, S.; Rombaut, V.; Vogelaers, D.; Brusselaers, N.; Lizy, C.; Cankurtaran, M.; Labeau, S.; Petrovic, M.; Blot, S. Risk factors and mortality for nosocomial bloodstream infections in elderly patients. *Eur. J. Intern. Med.* **2011**, *22*, e39–e44.

3. Stewart, A.G.; Simos, P.; Sivabalan, P.; Escolà-Vergé, L.; Garnham, K.; Isler, B., on behalf of the ESGBIES Study Group. An Update on Recent Clinical Trial Data in Bloodstream Infection. *Antibiotics* **2024**, *13*, 1035.

4. Butler, M.S.; Henderson, I.R.; Capon, R.J.; Blaskovich, M.A.T. Antibiotics in the clinical pipeline as of December 2022.*J. Antibiotics* **2023**, *76*, 431–473.

5. Opal, S.M.; Wittebole X. Biomarkers of Infection and Sepsis. *Crit. Care Clin.* **2020**; 36, 11–22.

6. Lambregts, M.M.C.; Bernards, A.T.; Beek, M.T.; van der Visser, L.G.; de Boer, M.G. Time to positivity of blood cultures supports early re-evaluation of empiric broad-spectrum antimicrobial therapy. *PLoS ONE* **2019**, 14:e0208819.

7. Coburn, B.; Morris, A.M.; Tomlinson, G.; Detsky, A.S. Does This Adult Patient With Suspected Bacteremia Require Blood Cultures? *JAMA* **2012**; 308, 502–511.

8. Chou, E.H.; Mann, S.; Hsu, T.-C.; Hsu, W.-T.; Liu, C.C.-Y.; Bhakta, T.; Hassani, D.M.; Lee, C.C. Incidence, trends, and outcomes of infection sites among hospitalizations of sepsis: A nationwide study. *PLoS ONE* **2020**, 15:e0227752.

9. Fabre, V.; Hsu, Y.-J.; Carroll, K.C.; Salinas, A.B.; Gadala, A.; Bower, C.; Boyd, S.; Degnan, K.O.; Dhaubhadel, P.; Diekema, D.J.; et al. Blood Culture Use in Medical and Surgical Intensive Care Units and Wards. *JAMA Netw. Open* **2025**; 8:e2454738.

10. Mellhammar, L.; Kahn, F.; Whitlow, C.; Kander, T.; Christensson, B.; Linder, A. Bacteremic sepsis leads to higher mortality when adjusting for confounders with propensity score matching. *Sci. Rep.* **2021**, 11:6972.

11. Dempsey, C.; Skoglund, E.; Muldrew, K.L.; Garey, K.W. Economic health care costs of blood culture contamination: A systematic review. *Am. J. Infec. Control* **2019**, 47, 963–967.

12. Roger, P.; Strzelecki, A.-C.; Dautezac, V.; Hennet, M.-A.; Borredon, G.; Brisou, P.; Girard, D.; Assi, A. Diagnostic uncertainties in patients with bacteraemia: impact on antibiotic prescriptions and outcome. *J. Antimicrob. Chemother.* **2024,** 80, 238-246.

13. Donner, L.M.; Campbell, W.S.; Lyden, E.; Van Schooneveld, T.C. Assessment of Rapid-Blood-Culture-Identification Result Interpretation and Antibiotic Prescribing Practices. *J. Clin. Microbiol.* **2017**, 55, 1496-1507.

14. Balıkçı, A.; Belas, Z.; Eren Topkaya, A. Kan Kültürü Pozitifliği: Etken ya da Kontaminasyon mu? [Blood culture positivity: is it pathogen or contaminant?]. *Mikrobiyol. Bul. [Turkey]* **2013**, 47, 135-140. doi: 10.5578/mb.4181.

15. Hossain, B.; Islam, M.S.; Rahman, A.; Marzan, M.; Rafiqullah, I.; Connor, N.E.; Hasanuzzaman, M.; Islam, M.; Hamer, D.H.; Hibberd, P.L.; Saha, S.K. Understanding Bacterial Isolates in Blood Culture and Approaches Used to Define Bacteria as Contaminants: A Literature Review. *Pediatr. Infect. Dis. J.* **2016**, 35, S45-51.

16. Lamy, B.; Dargère, S.; Arendrup, M.C.; Parienti, J.J.; Tattevin, P. How to Optimize the Use of Blood Cultures for the Diagnosis of Bloodstream Infections? A State-of-the Art. *Front. Microbiol.* **2016**, 7, 697.

17. Baron, E.J.; Miller, J.M.; Weinstein, M.P.; Richter, S.S.; Gilligan, P.H.; Thomson, R.B. Jr.; Bourbeau, P.; Carroll, K.C.; Kehl, S.C.; Dunne, W.M., et al. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)(a). *Clin. Infect. Dis.* **2013**, 57, e22-e121.

18. Fabre, V.; Sharara, S.L.; Salinas, A.B.; Carroll, K.C.; Desai, S.; Cosgrove, S.E. Does This Patient Need Blood Cultures? A Scoping Review of Indications for Blood Cultures in Adult Nonneutropenic Inpatients. *Clin. Infect. Dis.* **2020**, 71, 1339-1347.

19. Riedel, S.; Bourbeau, P.; Swartz, B.; Brecher, S.; Carrol, K. C.; Stamper, P. D.; et al. Timing of specimen collection for blood cultures from febrile patients with bacteremia. *J. Clin. Microbiol.* **2008**, 46, 1381–1385.

20. Balk, R.; Esper, A.M.; Martin, G.S.; Miller, R.R., III; Lopansri, B.K.; Burke, J.P.; Levy, M.; Opal, S.; Rothman, R.E.; D'Alessio, F.R.; et al. Validation of SeptiCyte RAPID to Discriminate Sepsis from Non-Infectious Systemic Inflammation. *J. Clin. Med*. **2024**, 13, 1194.

21. Balk, R.; Esper, A.M.; Martin, G.S.; Miller, R.R., III; Lopansri, B.K.; Burke, J.P.; Levy, M.; Rothman, R.E.; D'Alessio, F.R.; Sidhaye, V.K., et al. Rapid and Robust Identification of Sepsis Using SeptiCyte RAPID in a Heterogeneous Patient Population. *J. Clin. Med*. **2024**, 13, 6044.

22. Miller, R. R., 3rd.; Lopansri, B. K.; Burke, J. P.; Levy, M.; Opal, S.; Rothman, R. E.; D'Alessio, F. R.; Sidhaye, V. K.; Aggarwal, N. R.; Balk, R., et al. Validation of a Host Response Assay, SeptiCyte LAB, for Discriminating Sepsis from Systemic Inflammatory Response Syndrome in the ICU. *Am. J. Resp. Crit. Care*. **2018**, 198, 903–913.

23. von der Forst, M.; Back, L.; Tourelle, K. M.; Gruneberg, D.; Weigand, M. A.; Schmitt, F. C. F.; Dietrich, M. SeptAsTERS- SeptiCyte RAPID as assessment tool for early recognition of sepsis – a prospective observational study. *Infection* **2024** Nov 22; 1–13.

24. Blair P.W.; Okello S.; Wailagala A.; Ayebare R.R.; Olebo D.F.; Kayiira M.; Kemigisha S.M.; Kayondo W.; Gregory M.; Koehler J.W.; et al. Long-term Mortality Among Hospitalized Adults with Sepsis in Uganda: a Prospective Cohort Study. medRxiv, posted September 15, 2023. doi: https://doi.org/10.1101/2023.09.14.23295526

25. Levy, M. M.; Fink, M. P.; Marshall, J. C.; Abraham, E.; Angus, D.; Cook, D.; Cohen, J.; Opal, S. M.; Vincent, J. L.; Ramsay, G.; SCCM/ESICM/ACCP/ATS/SIS. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit. Care Med.* **2003**, 31, 1250-1256.

26. Klein Klouwenberg, P. M.; Ong, D. S.; Bos, L. D.; de Beer, F. M.; van Hooijdonk, R. T.; Huson, M. A.; Straat, M.; van Vught, L. A.; Wieske, L.; Horn, J.; et al. Interobserver agreement of centers for disease control and prevention criteria for classifying infections in critically ill patients. *Crit. Care Med.* **2013**, 41, 2373–2378.

27. Klein Klouwenberg, P. M.; van Mourik, M. S.; Ong, D. S.; Horn, J.; Schultz, M. J.; Cremer, O. L.; Bonten, M. J.; MARS Consortium. Electronic implementation of a novel surveillance paradigm for ventilator-associated events. Feasibility and validation. *Am. J. Respir. Crit. Care Med.* **2014**, *189*, 947–955.

28. Shankar-Hari, M.; Phillips, G. S.; Levy, M. L.; Seymour, C. W.; Liu, V. X.; Deutschman, C. S.; Angus, D. C.; Rubenfeld, G. D.; Singer, M.; Sepsis Definitions Task Force. Developing a New Definition and Assessing New Clinical Criteria for Septic Shock: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*. **2016**, 315, 775-787.

29. Blair, P.W.; Mehta, R.; Oppong, C.K.; Tin, S.; Ko, E.; Tsalik, E.L.; Chenoweth, J.; Rozo, M.; Adams, N.; Beckett, C.; et al. Screening tools for predicting mortality of adults with suspected sepsis: an international sepsis cohort validation study. *BMJ Open* **2023**, 13, e067840.

30. Chenoweth, J.G.; Colantuoni, C.; Striegel, D.A.; Genzor, P.; Brandsma, J.; Blair, P.W.; Krishnan, S.; Chiyka, E.; Fazli, M.; Mehta, R.; et al. Gene expression signatures in blood from a West African sepsis cohort define host response phenotypes. *Nat. Commun.* **2024**, 15, 4606.

31. R Core Team. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria, 2024; ISBN 3-900051-07-0. Available online: http://www.R-project.org/ (accessed on 1 September 2024).

32. Rizopoulos, D. ltm: An R package for Latent Variable Modeling and Item Response Theory Analyses. *J. Stat. Softw.* **2006**, 17, 1–25.

33. Hanley, J.A.; McNeil, B.J. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* **1982**, 143, 29–36.

34. Robin, X.; Turck, N.; Hainard, A.; Tiberti, N.; Lisacek, F.; Sanchez, J.-C.; Mu□ller, M. pROC: An open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinform.* **2011**, 12, 77.

35. Delong, E.R.; Delong, D.M.; Clarke-Pearson, D.L. Comparing the Areas under Two or More Correlated Receiver Operating Characteristic Curves: A Nonparametric Approach. *Biometrics* **1988**, 44, 837–845.

36. Elkahwagy, D.M.A.S.; Kiriacos, C.J.; Mansour, M. Logistic regression and other statistical tools in diagnostic biomarker studies. *Clin. Transl. Oncol*. **2024**, 26, 2172-2180. Erratum in: *Clin. Transl. Oncol.* **2024**, 26, 2393.

37. Ali, A.; Rasheed, A.; Siddiqui, A. A.; Naseer, M.; Wasim, S.; Akhtar, W. Non-Parametric Test for Ordered Medians: The Jonckheere Terpstra Test. *Int. J. Stats. Med. Res.* **2015**, 4, 203–207.

38. Fabre, V.; Sharara, S. L.; Salinas, A. B.; Carroll, K. C.; Desai, S.; Cosgrove, S. E. Does This Patient Need Blood Cultures? A Scoping Review of Indications for Blood Cultures in Adult Nonneutropenic Inpatients. *Clin. Infect. Dis.* **2020**, 71, 1339–1347.

39. Schinkel, M.; Boerman, A.; Carroll, K.; Cosgrove, S. E.; Hsu, Y. J.; Klein, E.; Nanayakkara, P.; Schade, R.; Wiersinga, W. J.; Fabre, V. Impact of blood culture contamination on antibiotic use, resource utilization, and clinical outcomes: a retrospective cohort study in Dutch and US hospitals. *Open Forum Infect. Dis*. **2023**, 11, ofad644.

40. Doern, G. V.; Carroll, K. C.; Diekema, D. J.; Garey, K. W.; Rupp, M. E.; Weinstein, M. P.; Sexton, D. J. (2019). Practical guidance for clinical microbiology laboratories:a comprehensive update on the problem of blood culture contamination and a discussion of methods for addressing the problem. *Clin. Microbiol. Rev*. **2019**, 33, e00009-19.

41. CLSI. *Principles and Procedures for Blood Cultures*, 2nd ed. Clinical and Laboratory Standards Institute; 2022: CLSI guideline M47.

42. Lee R.; Al Rifaie R.; Subedi K.; Coletti C. Comparative Analysis of Bacteremic and Non-bacteremic Sepsis: A Retrospective Study. *Cureus* **2024**, 16, e76418.

43. Chang, Y.; Oh, J. H.; Oh, D. K.; Lee, S. Y.; Hyun, D. G.; Park, M. H.; Lim, C. M.; Korean Sepsis Alliance (KSA) investigators (2024). Culture-negative sepsis may be a different entity from culture-positive sepsis: a prospective nationwide multicenter cohort study. *Crit. Care* **2024**, 28, 385.

44. Xu, J.Q.; Zhang, W.Y.; Fu, J.J.; Fang, X.Z.; Gao, C.G.; Li, C.; Yao, L.; Li, Q.L.; Yang, X.B.; Ren, L.H., et al. Viral sepsis: diagnosis, clinical features, pathogenesis, and clinical considerations. *Mil. Med. Res.* **2024**, 11, 78.

45. Evans, L. ; Rhodes, A. ; Alhazzani, W., et al. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. *Crit. Care Med*. **2021**, 49, e1063–e1143.

46. Liu, C.; Bayer, A.; Cosgrove, S. E.; Daum, R. S.; Fridkin, S. K.; Gorwitz, R. J.; Kaplan, S. L.; Karchmer, A. W.; Levine, D. P.; Murray, B. E.; et al. (2011). Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant Staphylococcus aureus infections in adults and children. *Clin. Infect. Dis.* **2011**, 52, e18e55.

47. Baddour, L. M.; Wilson, W. R.; Bayer, A. S.; Fowler, V. G.; Jr, Tleyjeh, I. M.; Rybak, M. J.; Barsic, B.; Lockhart, P. B.; Gewitz, M. H.; Levison, M. E.; et al. Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association. *Circulation* **2015**, 132, 1435e86.

48. Pappas, P. G.; Kauffman, C. A.; Andes, D. R.; Clancy, C. J.; Marr, K. A.; Ostrosky-Zeichner, L.; Reboli, A. C.; Schuster, M. G.; Vazquez, J. A.; Walsh, T. J.; et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. *Clin. Infect. Dis* **2016**, 62, e1e50.

49. Ong, S. W. X.; Luo, J.; Fridman, D. J.; Lee, S. M.; Johnstone, J.; Schwartz, K. L.; Diong, C.; Patel, S. N.; MacFadden, D.; Langford, B.; et al. Follow-up blood cultures do not reduce mortality in hospitalized patients with Gram-negative bloodstream infection: a retrospective population-wide cohort study. *Clin. Microbiol. Infect*. **2024**, 30, 890–898.

50. Kim, T. W.; Lee, S. U.; Park, B.; Jeon, K.; Park, S.; Suh, G. Y.; Oh, D. K.; Lee, S. Y.; Park, M. H.; Lee, H.; et al. Clinical effects of bacteremia in sepsis patients with community-acquired pneumonia. *BMC Infect. Dis*. **2023**, 23, 887.

51. Lee, A.; Mirrett, S.; Reller, L.B.; Weinstein, M.P. Detection of bloodstream infections in adults: how many blood cultures are needed? *J. Clin. Microbiol.* **2007**, 45, 3546-3548.