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Laboratory Evaluation of the SeptiCyte RAPID Host Response Assay In Differentiating Infection-Positive Versus Infection-Negative Systemic Inflammation Patients Suspected of Sepsis

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ABSTRACT

Background: Early diagnosis of sepsis is critical for timely treatment but remains challenging. A rapid host response assay, SeptiCyte, was recently approved by the FDA for aiding early differentiation of infection-positive from infection-negative systemic inflammation in patients suspected of sepsis. This test is a fully automated reverse transcription polymerase chain reaction (RT-qPCR) assay that quantifies changes in the expression of two host immune response genes and results are available in about 1 hour. The present study aimed to evaluate the SeptiCyte assay performance in a clinical setting at a large medical center. **Method:** Eighty-two patients admitted to the University Hospitals Cleveland Medical Center with suspected sepsis from July to September 2022 were included in the study. SeptiCyte samples were obtained at the time of initial evaluation. In addition, healthy donor blood samples and extracted RNA samples were also utilized for assay validation. The SeptiCyte assay was performed according to the manufacturer's instructions using multi-chambered fluidic cartridges and the Idylla System (Immunexpress, Seattle, WA). The assay result was reported as the SeptiScore (range 0-15), a calculated ratio of the expression of two host immune response genes, and the SeptiCyte band (ranging from 1 to 4, with 1 indicating the lowest risk of sepsis and 4 indicating the highest risk). The assay cutoff values are 5.0, 6.2 and 7.4 respectively for the band 2, 3 and 4. **Results:** Intra- and inter-assay precision was 0.2-6.2% and 0-3.2%, respectively. % Recovery at the cutoff for band 3 (SeptiScore 6.0) was 102.4% with a CV of 2.4%. Method comparison with the Immunexpress lab demonstrated equivalent performance, as evidenced by a slope of 0.99 and an intercept of 0.27 by Deming regression. SeptiScore showed a weak positive correlation with CRP (Correlation Coefficient 0.389, $p<0.01$) but no association with procalcitonin (n=76), lactate (n=78), international normalized ratio (INR), platelet, neutrophil, lymphocyte, or white blood cell (WBC) count (n=82). CRP (n=71) median concentration in patients with band 4 (16.8) was significantly higher than in patients who were band 1 (3.6). The SeptiScore of blood culture positive cases (n=22) was 7.2 ± 2.0 (mean ± SD) and that of pathogen negative non-sepsis (by sepsis retrospective review of event only, n=10) cases was 5.6 ± 1.6 ($p<0.05$). In addition, the median SeptiScore was notably higher in patients with viral infection compared to those without infection (9.2 vs. 6.2, $p<0.05$). Further analysis showed 92% (11/12) of patients with hematologic cancer were categorized as band 4 including one non-sepsis case per the retrospective review. SeptiScore was similar between patients with non-hematologic cancer and those without malignancy ($p>0.05$). **Conclusion:** The SeptiCyte RAPID assay showed good analytical performance. SeptiScore represents an independent factor to quantify host response in patients suspected of sepsis. It may add value in aiding differentiation between infection-positive from infection-negative systemic inflammation with promising results in patients with viral infections or those with positive blood cultures. The negative predictive value of low SeptiScore within band 1 or 2 may need further investigation.

INTRODUCTION

- Sepsis claims 11 million lives each year worldwide. Timely treatment is critical for survival, but early diagnosis remains challenging due to the lack of sensitive and specific tests.
- Sepsis-3: life-threatening organ dysfunction from a dysregulated host response to infection. Biomarkers identifying abnormal host responses to infection may provide an early diagnosis approach.
- The SeptiCyte RAPID assay was cleared by the FDA in November 2021 for aiding the differentiation of infective (sepsis) from infection-negative systemic inflammation in patients suspected of sepsis on their first day of ICU admission.
- This assay measures mRNA transcripts for host immune biomarkers PLA2G7 and PLAC8 using an automated RT-qPCR method and results are available in about 1 hour. The present study aimed to evaluate the SeptiCyte assay performance in a clinical setting at a large medical center.

METHODS

Analytical evaluation of the Septicyte rapid assay

- Blood samples from sepsis patients and healthy donors and extracted RNA samples were used in the analytical assay evaluation.
- Assay accuracy was evaluated by comparing with the manufacturer lab using 20 samples across ther reportable range.

Subjects and SeptiCyte test

- 82 patients admitted to the University Hospitals Cleveland Medical Center with suspected sepsis from July to September 2022 were included in the study.
- 2.5 ml of blood from each patient was collected into the PAXgene RNA tube on their first day of ICU. Samples were stored at room temperature and assayed per the manufacturer's instruction within 72 hours of collection.
- Patients received standard care and Septicyte results were evaluated by a retrospective chart review.

RESULTS

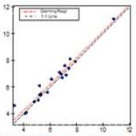
1. Evaluation of analytical performance

Table 1. Analytical performance characteristics of the SeptiCyte Rapid Test

	Mean SeptiScore	SeptiCyte band level	Total CV	Intra-assay	Inter-assay
Precision (n=10)					
Control RNA-Low	2.9	Band 1	6.2%	6.2%	0.0%
Control RNA- High	6.7	Band 3	3.4%	52.2%	2.6%
Donor blood sample	4.8	Band 1	3.2%	0.2%	3.2%

Verification of accuracy and precision at the cutoff level: The cutoff for band 3 (SeptiScore 6.0) w as verified using an RNA sample. Manufacture defined value: 6.30. Measured mean: 6.5. Recovery:102.4%; CV: 2.4%.

Method comparison (n=20)



X: **Immunexpress SeptiScore; Y: In-house testing SeptiScore**

R	0.9717
Bias (%)	*0.21 (3.5%)*
Slope (95% conf)	*0.991 (0.875 to 1.08)*
Intercept (95%conf)	*0.27 (-0.47 to 1.01)*

2. Study cohort

Table 2. Demographic and clinical characteristics of the study cohort (n=82)

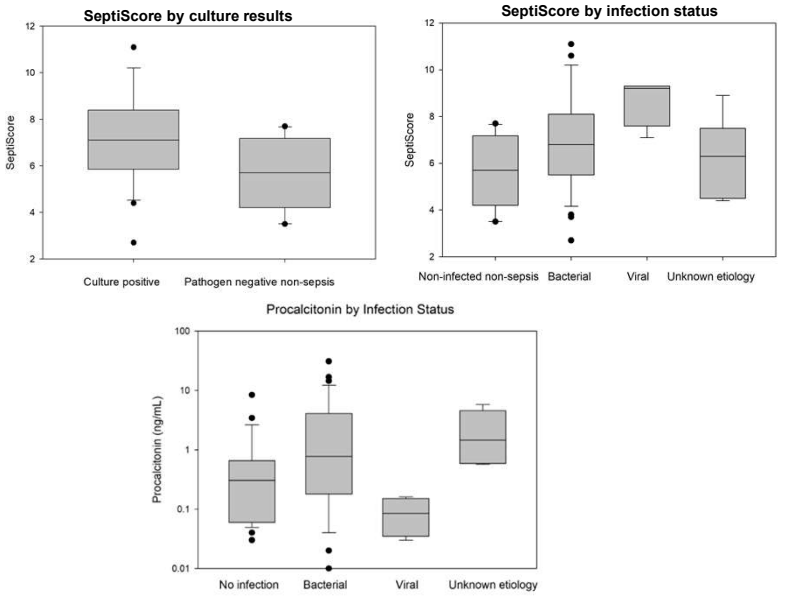
Sex	Female: 36; Male: 46
Age in years	Mean: 64.0; SD: 18.3
White cell count median (IQR)	10.60 (6.5-18.3) ×10⁹/L
Lymphocyte count median (IQR)	0.81 (0.51-1.48) ×10⁹/L
Neutrophil count median (IQR)	8.84 (4.32-14.47) ×10⁹/L
C-Reactive Protein median (IQR)	9.96 (5.89-17.23) mg/dL
Procalcitonin median (IQR)	0.60 (0.11-3.12) ng/mL
Culture Results	
Blood culture positive	**22**
Bacterial	18
Bacterial and fungal	1
Bacterial and viral	2
Fungal	1
Other body fluid culture positive	**15**
Bacterial	12
Bacterial and fungal	2
Fungal	1
Culture negative	**45**
Clinical infection unknown etiology	7
Viral (SARS-CoV-2)	4
Inconclusive	4
No evidence of infection	30
Malignancy diseases	
No malignancy	49
Hematologic cancer	12
Non-hematologic malignancy	21

3. Correlation between SeptiCyte and other laboratory results

Table 3. Correlation between SeptiScore and other lab results

	Vs. SeptiScore	
	Correlation Coefficient	p value
CRP	0.389	0.000607
Lactate	0.0397	0.728
Procalcitonin	0.0589	0.606
Lymphocytes	0.131	0.269
Neutrophils	0.124	0.0795
White blood cell count	0.195	0.285
INR	-0.174	0.238
Platelet count	-0.127	0.255

4. SeptiScore and procalcitonin concentration by culture results and infection status



Top left panel: The median SeptiScore was significantly higher in blood culture-positive patients than in culture-negative non-sepsis patients ($p<0.05$). Top right panel: SeptiScore was significantly higher in patients with viral infection than in non-infected patients ($p=0.023$). Bottom panel: Procalcitonin concentrations were significantly higher in patients with bacterial infection ($p=0.020$) and infection with unknown etiology ($p=0.005$) when compared with patients without evidence of infection. Procalcitonin concentrations were similar between patients with a viral infection and without evidence of infection ($p>0.05$).

CONCLUSIONS

The SeptiCyte RAPID assay demonstrated acceptable analytical performance in our study. SeptiScore generated from the assay did not show any strong correlation with the existing commonly used markers and represents an independent parameter to quantify host response in patients suspected of sepsis. It may add value in aiding differentiation between infection-positive from infection-negative systemic inflammation with promising results in patients with viral infections or those with positive blood cultures. Further large-scale studies are needed in evaluating the assay's real-life sensitivity and specificity and impact on sepsis patient management.

Acknowledgment

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