

**S5 Data** for McHugh et al., “A Molecular Host Response Assay to Discriminate Between Sepsis and Infection-Negative Systemic Inflammation in Critically Ill Patients: Discovery and Validation in Independent Cohorts”

## **Disease Severity as a Potential Confounding Variable**

### **1. Objective**

The objective was to investigate whether severity of disease, as measured by APACHE IV score or SOFA score, might be a confounding variable in using *SeptiCyte Lab* to discriminate between cases (sepsis) and controls (infection-negative systemic inflammation).

### **2. Method**

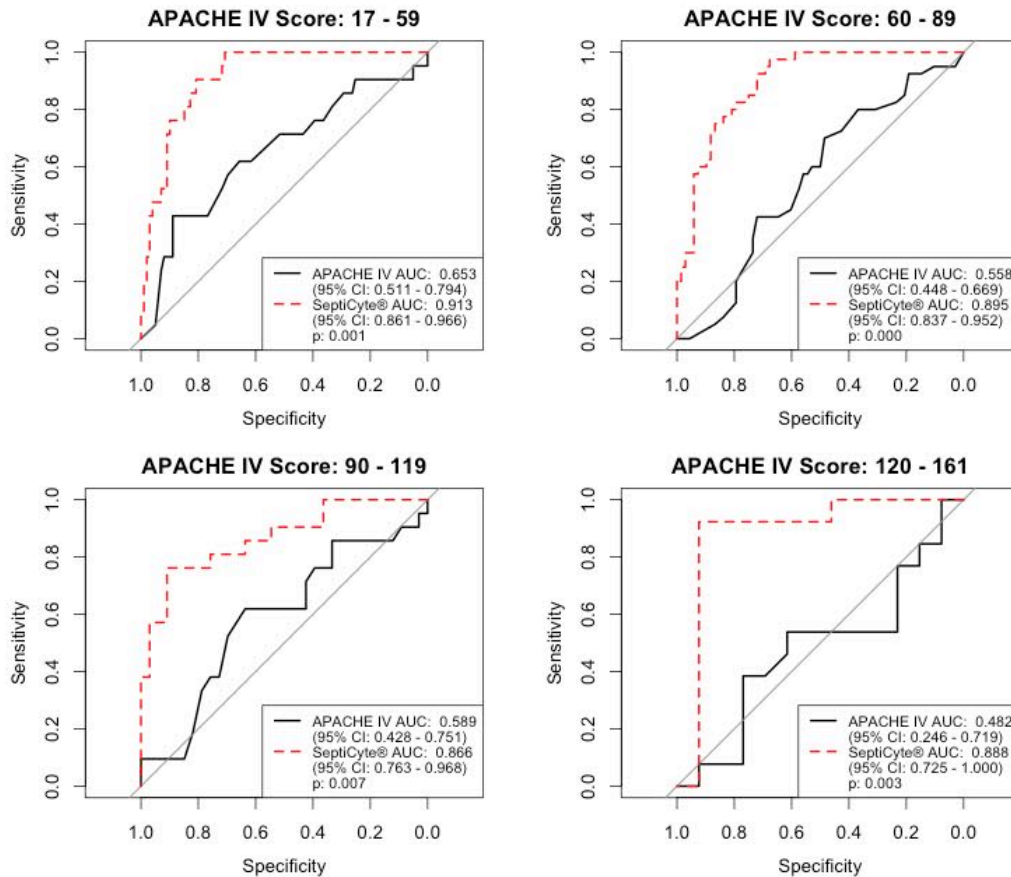
*Analysis #1:* The entire patient pool (Validation Cohorts 1+2+3+4+5, excluding patients classified with an infection likelihood of possible; n=306) was subjected to ROC curve analysis, using either *SeptiCyte Lab* score, SOFA score, or APACHE IV score as a classifier.

*Analysis #2:* The entire patient pool (Validation Cohorts 1+2+3+4+5, excluding patients classified with an infection likelihood of possible; n=306) was stratified into “bins” on the basis of SOFA score or APACHE IV score. *SeptiCyte Lab* was then applied to the separate “bins” and the resultant ROC curves were pairwise compared.

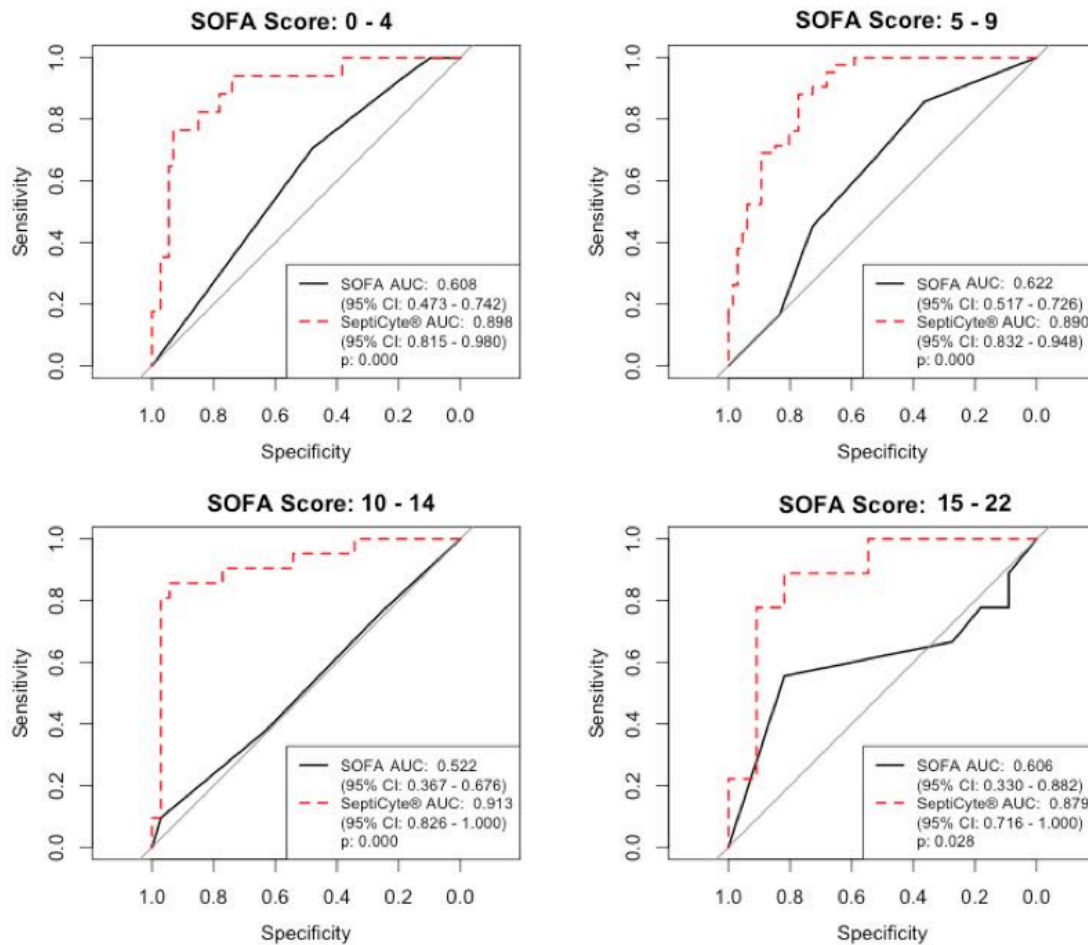
### 3. Results

*Analysis #1:* Comparative ROC curve analyses on the pooled data (Validation Cohorts 1+2+3+4+5, excluding patients classified with an infection likelihood of possible; n=306) have been presented in **Figure 5, Panel A** in the main text.

*Analysis #2:* Results of ROC curve analysis on subsets of the combined dataset, stratified on either APACHE IV Score or SOFA Score, are presented below in **Figure 1** (APACHE IV) and **Figure 2** (SOFA).



**Figure 1:** ROC plots for SeptiCyt Lab, after stratifying patients by APACHE IV score



**Figure 2:** ROC plots for SeptiCyte Lab, after stratifying patients by SOFA score

The AUCs for the various bins are summarized in **Table 1** (APACHE IV Score) and **Table 2** (SOFA Score).

**Table 1:** Stratification of patients into APACHE IV Bins, followed by ROC curve analysis using SeptiCyte Lab.

Apache IV bin	Composition	AUC
17-59	99 controls, 21 cases	0.913 (95% CI: 0.861-0.966)
60-89	68 controls, 40 cases	0.895 (95% CI: 0.837-0.952)
90-119	33 controls, 21 cases	0.866 (95% CI: 0.763-0.968)
120-161	13 controls, 13 cases	0.888 (95% CI: 0.725-1.000)

**Table 2:** Stratification of patients into SOFA Bins, followed by ROC curve analysis using SeptiCyte Lab.

SOFA bin	Composition	Septicyte AUC
0-4	73 controls, 17 cases	0.898 (95% CI: 0.815-0.980)
5-9	66 controls, 42 cases	0.890 (95% CI: 0.832-0.948)
10-14	35 controls, 21 cases	0.913 (95% CI: 0.826-1.000)
15-22	11 controls, 9 cases	0.879 (95% CI: 0.716-1.000)

The p-values for comparison across bins are given in **Table 3** (for APACHE IV Score) and **Table 4** (for SOFA Score). We found no significant difference between SeptiCyte Lab ROC curves across the different “bins”.

**Table 3:** p values for pairwise comparison of SeptiCyte Lab ROC curves, after stratifying patients by APACHE IV score

APACHE IV Score	17-59	60-89	90-119	120-161
17-59	1.00	0.30	0.56	0.23
60-89	0.30	1.00	0.76	0.57
90-119	0.56	0.76	1.00	0.47
120-161	0.23	0.57	0.47	1.00

**Table 4:** p values for pairwise comparison of SeptiCyte Lab ROC curves, after stratifying patients by SOFA score

SOFA Score	0-4	5-9	10-14	15-22
0-4	1.00	0.87	0.41	0.99
5-9	0.87	1.00	0.30	0.92
10-14	0.41	0.30	1.00	0.60
15-22	0.99	0.92	0.60	1.00