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Development of pre-analytics for a Rapid, Integrated Sepsis Diagnostic and Prognostic Workflow combining Pathogen Detection with Host Immune Response Profiling

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Introduction

Sepsis is a life-threatening syndrome caused by a dysregulated immune response to infection and remains a major global health burden, contributing to approximately 20% of deaths worldwide[1]. Early identification of both the infecting pathogen and illness severity is critical, particularly in austere and military medicine settings where timely treatment or transport decisions are essential. However, the current gold standard, blood culture, requires 0.5 to 5 days for pathogen identification and antimicrobial susceptibility testing, delaying clinical decision-making[2,3,4]. No commercially available integrated solution currently combines pathogen detection with sepsis severity assessment. To address this gap, we developed INSPIRE (INtegrated Sepsis Pathogen identification and Immune Response Evaluation), a 6-hour nanopore sequencing–based diagnostic and prognostic workflow for direct whole-blood analysis (Figure 1). INSPIRE enables unbiased pathogen identification, antimicrobial resistance (AMR) detection, and mortality risk prediction using universally amplified DNA signatures and relative gene expression profiling of 18 clinically relevant host mRNAs[5], including three reference genes.

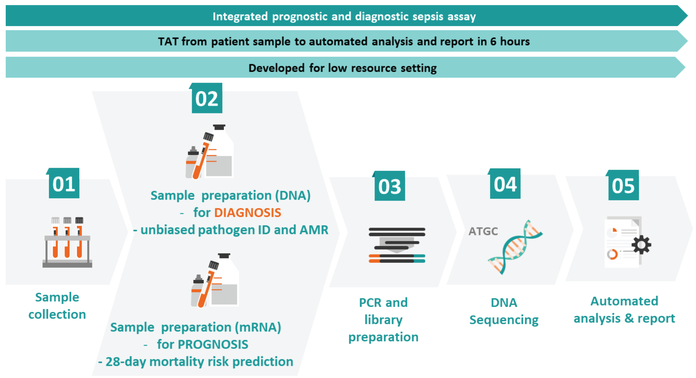


Figure 1. Schematic representation of the overview of INSPIRE workflow.

Methods

Nucleic acid purification and processing

Host mRNA was enriched from PAXgene blood samples using an optimized capture probe-based method targeting 18 transcripts. The 18-mRNA panel was identified in an earlier study and described by Chenoweth et al. 2024. Pathogen DNA was recovered from EDTA whole blood using a universal lysis method compatible with bacterial and fungal pathogens, followed by capture probe-based purification. DNA and RNA eluates were amplified by multiplex PCR. Primer sets for pathogen identification were designed to target short, highly conserved genomic regions flanking systematic phylogenetic variation across a broad range of bacterial and fungal species. In case of host mRNA conserved exon-exon junction was used to design PCR primers. Amplicons were purified using AMPure beads, prepared for nanopore sequencing and run on ONT flow cells.

Software and algorithms

The real-time analysis of this integrated sequencing run was done using "NanoSepsIDx" software, and reads were mapped against the proprietary database to identify pathogen/s and its AMR. In addition, a sepsis severity risk score for the same patient sample is determined by the integrated "Predict_d28" classifier algorithm that uses a weighted fuzzy-logic approach to predict mortality based on sepsis endotypes.

Integrated workflow for pathogen identification and risk prediction

Whole blood samples collected in EDTA tubes were spiked with Staphylococcus aureus or Enterococcus faecium at a final concentration of 50 CFU/mL. These contrived EDTA samples were processed in parallel with matched PAXgene whole blood samples for host mRNA purification. Pathogen DNA was purified using the capture probe-based method, whereas host mRNA was purified using either the capture probe-based method or the standard silica-based method as a control. A total of 10 sample sets were processed to evaluate the integrated workflow using the capture probe–based method for both pathogen identification and risk prediction, as well as the combined approach of capture probe-based pathogen identification and silica-based host mRNA purification for risk prediction. In this preliminary workflow format, the data is continuously captured, analyzed and reports are generated at 5 different time points of 15, 30, 60, 120, and 180 minutes.

Results

Sequencing summary results from four healthy donors showed that the capture probe–based mRNA purification method yielded results comparable to those obtained with the standard silica-based total RNA purification method, followed by sequencing. Samples purified using the capture probe–based method were sequenced on the same Oxford Nanopore Technologies (ONT) flow cell as total RNA. Each healthy donor sample was processed in duplicates using the capture probe assay, along with one standard sample, and the results are summarized in Table 1.

Table 1. Sequencing summary results for four healthy donors comparing the capture probe–based mRNA purification method with the standard silica-based total RNA purification method used for template preparation.

	Sample 1			Sample 2			Sample 3			Sample 4		
Sample	CP Rep 1	CP Rep 2	Stan-dard	CP Rep 1	CP Rep 2	Stan-dard	CP Rep 1	CP Rep 2	Stan-dard	CP Rep 1	CP Rep 2	Stan-dard
28-day mortality risk assessment:	Low	Low	Low	Low	Low	Low	Low	Low	Low	–	–	Low
28-day mortality risk weight:	0.069	0.098	0.069	0.046	0.06	0.116	0.053	0.083	0.142	–	–	0.122

Note: CP: capture probe-based mRNA purification. Standard: Total RNA purified using silica columns.

Interday and intraday repeatability of the capture probe-based mRNA purification method was evaluated using four healthy donor samples processed in duplicate across three independent days and analyzed by nanopore sequencing. Replicates from each donor were subsequently analyzed within a single sequencing run. Overall, the results demonstrated consistent performance across both intraday and interday conditions, supporting the repeatability of the capture probe–based mRNA purification workflow.

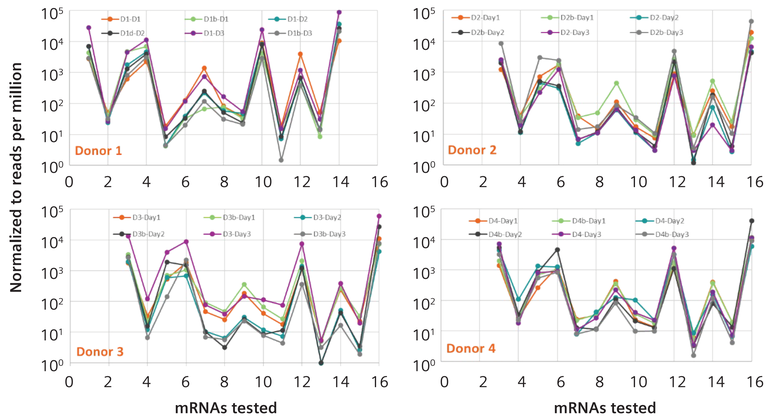
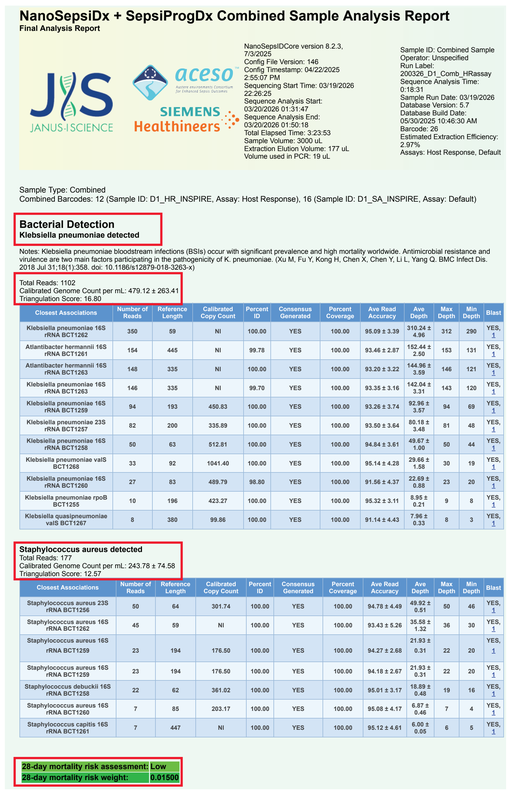


Figure 2. mRNA read counts normalized to the reference mRNA read count, plotted on a logarithmic scale on the y-axis. The x-axis represents the 15 different mRNAs[1] analyzed in the study. The four panels present results from four different healthy donors. The similar expression patterns observed across donors indicate consistent mRNA profiling and support the repeatability of the capture probe–based purification and sequencing workflow.

Sequencing generated a compiled report that included pathogen ID, AMR gene detection, and sepsis risk prediction. AMR detection results are not shown, as the strains used to spike the samples did not contain resistance genes. Detection of AMR genes was evaluated and confirmed in a separate study.



NanoSepsiDx + SepsiProgDx Combined Sample Analysis Report

Final Analysis Report

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NanoSepsiDCore version 8.2.3, 7/3/2025
Config File Version: 146
Config Timestamp: 04/22/2025 2:55:07 PM
Sequencing Start Time: 03/19/2026 22:26:25
Sequence Analysis Start: 03/20/2026 01:31:47
Sequence Analysis End: 03/20/2026 01:50:18
Total Elapsed Time: 3:23:53
Sample Volume: 3000 uL
Extraction Elution Volume: 177 uL
Volume used in PCR: 19 uL

Sample ID: Combined Sample
Operator: Unspecified
Run Label: 200326_D1_Comb_HRassay
Sequence Analysis Time: 0:18:31
Sample Run Date: 03/19/2026
Database Version: 5.7
Database Build Date: 05/30/2025 10:46:30 AM
Barcode: 26
Estimated Extraction Efficiency: 2.97%
Assays: Host Response, Default

Sample Type: Combined
Combined Barcodes: 12 (Sample ID: D1_HR_INSPIRE, Assay: Host Response), 16 (Sample ID: D1_SA_INSPIRE, Assay: Default)

Bacterial Detection
Klebsiella pneumoniae detected

Notes: Klebsiella pneumoniae bloodstream infections (BSIs) occur with significant prevalence and high mortality worldwide. Antimicrobial resistance and virulence are two main factors participating in the pathogenicity of K. pneumoniae. (Xu M, Fu Y, Kong H, Chen X, Chen Y, Li L, Yang Q. BMC Infect Dis. 2018 Jul 31;18(1):358. doi: 10.1186/s12879-018-3263-x)

Total Reads: 1102
Calibrated Genome Count per mL: 479.12 ± 263.41
Triangulation Score: 16.80

Closest Associations	Number of Reads	Reference Length	Calibrated Copy Count	Percent ID	Consensus Generated	Percent Coverage	Ave Read Accuracy	Ave Depth	Max Depth	Min Depth	Blast
Klebsiella pneumoniae 16S rRNA BCT1262	350	59	NI	100.00	YES	100.00	95.09 ± 3.39	310.24 ± 4.96	312	290	YES, 1
Atlantibacter hermannii 16S rRNA BCT1261	154	445	NI	99.78	YES	100.00	93.46 ± 2.87	152.44 ± 2.50	153	131	YES, 1
Atlantibacter hermannii 16S rRNA BCT1263	148	335	NI	100.00	YES	100.00	93.20 ± 3.22	144.96 ± 3.09	146	121	YES, 1
Klebsiella pneumoniae 16S rRNA BCT1263	146	335	NI	99.70	YES	100.00	93.35 ± 3.16	142.04 ± 3.31	143	120	YES, 1
Klebsiella pneumoniae 16S rRNA BCT1259	94	193	450.83	100.00	YES	100.00	93.26 ± 3.74	92.96 ± 3.57	94	69	YES, 1
Klebsiella pneumoniae 23S rRNA BCT1257	82	200	335.89	100.00	YES	100.00	93.50 ± 3.64	80.18 ± 3.48	81	48	YES, 1
Klebsiella pneumoniae 16S rRNA BCT1258	50	63	512.81	100.00	YES	100.00	94.84 ± 3.61	49.67 ± 1.00	50	44	YES, 1
Klebsiella pneumoniae valS BCT1268	33	92	1041.40	100.00	YES	100.00	95.14 ± 4.28	29.66 ± 1.58	30	19	YES, 1
Klebsiella pneumoniae 16S rRNA BCT1260	27	83	489.79	98.80	YES	100.00	91.56 ± 4.37	22.69 ± 0.88	23	20	YES, 1
Klebsiella pneumoniae rpoB BCT1255	10	196	423.27	100.00	YES	100.00	95.32 ± 3.11	8.95 ± 0.21	9	8	YES, 1
Klebsiella quasipneumoniae valS BCT1267	8	380	99.86	100.00	YES	100.00	91.14 ± 4.43	7.96 ± 0.33	8	3	YES, 1

Staphylococcus aureus detected
Total Reads: 177
Calibrated Genome Count per mL: 243.78 ± 74.58
Triangulation Score: 12.57

Closest Associations	Number of Reads	Reference Length	Calibrated Copy Count	Percent ID	Consensus Generated	Percent Coverage	Ave Read Accuracy	Ave Depth	Max Depth	Min Depth	Blast
Staphylococcus aureus 23S rRNA BCT1256	50	64	301.74	100.00	YES	100.00	94.78 ± 4.49	49.92 ± 0.51	50	46	YES, 1
Staphylococcus aureus 16S rRNA BCT1262	45	59	NI	100.00	YES	100.00	93.43 ± 5.26	36.58 ± 1.32	36	30	YES, 1
Staphylococcus aureus 16S rRNA BCT1259	23	194	176.50	100.00	YES	100.00	94.27 ± 2.68	21.93 ± 0.31	22	20	YES, 1
Staphylococcus aureus 16S rRNA BCT1259	23	194	176.50	100.00	YES	100.00	94.18 ± 2.67	21.93 ± 0.31	22	20	YES, 1
Staphylococcus debuckii 16S rRNA BCT1258	22	62	361.02	100.00	YES	100.00	95.01 ± 3.17	18.89 ± 0.48	19	16	YES, 1
Staphylococcus aureus 16S rRNA BCT1260	7	85	203.17	100.00	YES	100.00	95.08 ± 4.17	6.87 ± 0.46	7	4	YES, 1
Staphylococcus capitis 16S rRNA BCT1261	7	447	NI	100.00	YES	100.00	95.12 ± 4.61	6.00 ± 0.00	6	5	YES, 1

28-day mortality risk assessment: Low
28-day mortality risk weight: 0.01500

Figure 3. Example of the preliminary report format generated by the integrated workflow. The report includes the identified pathogen, calibrated pathogen genome count per mL, antimicrobial resistance (AMR) gene detection, and sepsis severity risk prediction.

Table 2. Summary of the results obtained from the integrated sequencing run for pathogen identification and sepsis risk prediction

EDTA whole blood samples spiked with *Staphylococcus aureus*						
		15′	30′	60′	120′	180′
Sample 1	Pathogen ID	+	+	+	+	+
	CP-RP	–	Low	Low	Low	Low
	Pathogen ID	+	+	+	+	+
	SB-RP	Low	Low	Low	Low	Low
Sample 2	Pathogen ID	ND	ND	+	+	+
	CP-RP	–	–	Low	Low	Low
	Pathogen ID	+	+	+	+	+
	SB-RP	Low	Low	Low	Low	Low
Sample 3	Pathogen ID	+	+	+	+	+
	CP-RP	–	Low	Low	Low	Low
	Pathogen ID	+	+	+	+	+
	SB-RP	Low	Low	Low	Low	Low
Sample 4	Pathogen ID	+	+	+	+	+
	CP-RP	–	–	Low	Low	Low
	Pathogen ID	+	+	+	+	+
	SB-RP	Low	Low	Low	Low	Low
Sample 5	Pathogen ID	+	+	+	+	+
	CP-RP	–	–	–	Low	Low
	Pathogen ID	+	+	+	+	+
	SB-RP	Low	Low	Low	Low	Low
EDTA whole blood samples spiked with *Enterococcus faecium*						
		15′	30′	60′	120′	180′
Sample 1	Pathogen ID	+	+	+	+	+
	CP-RP	–	Low	Low	Low	Low
	Pathogen ID	+	+	+	+	+
	SB-RP	Low	Low	Low	Low	Low
Sample 2	Pathogen ID	ND	ND	+	+	+
	CP-RP	–	–	Low	Low	Low
	Pathogen ID	+	+	+	+	+
	SB-RP	Low	Low	Low	Low	Low
Sample 3	Pathogen ID	+	+	+	+	+
	CP-RP	–	Low	Low	Low	Low
	Pathogen ID	+	+	+	+	+
	SB-RP	Low	Low	Low	Low	Low
Sample 4	Pathogen ID	+	+	+	+	+
	CP-RP	–	–	Low	Low	Low
	Pathogen ID	+	+	+	+	+
	SB-RP	Low	Low	Low	Low	Low
Sample 5	Pathogen ID	+	+	+	+	+
	CP-RP	–	–	–	Low	Low
	Pathogen ID	+	+	+	+	+
	SB-RP	Low	Low	Low	Low	Low

Note: CP-RP- Capture probe-based mRNA purification – Risk prediction. SB-RP- Silica based RNA purification – Risk prediction. Pathogen ID- Pathogen identification

Conclusion

The INSPIRE assay is a rapid and novel integrated diagnostic and prognostic workflow that enables direct analysis from whole blood. By combining pathogen identification with host mRNA profiling, the assay can identify infecting pathogen(s), detect antimicrobial resistance (AMR) determinants, and stratify patients based on risk of severe outcomes. This integrated approach has the potential to support earlier clinical decision-making, improve prioritization of high-risk patients, and enhance sepsis management, particularly in time-critical and resource-limited settings.

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