SIEMENS Healthineers

A-108

Comprehensive Performance Evaluation of a Rapid, Universal, Sepsis Diagnostic Workflow that identifies Pathogens and characterizes their Antimicrobial Resistance

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Background

Bloodstream infections (BSIs) require rapid and precise diagnosis and antimicrobial resistance (AMR) characterization to guide timely treatment, which is essential to initiate appropriate therapy in patients with sepsis mortality increases with every passing hour of delay[1]. The current gold standard, blood culture-based workflow requires approximately 15–72 hours for pathogen identification (ID), followed by an additional 8–15 hours for antimicrobial susceptibility testing (AST), with limitations in sensitivity and specificity[2,3,4]. Existing molecular methods are often culture-dependent or panel-restricted, highlighting the need for a rapid, unbiased diagnostic approach capable of directly detecting pathogens and antimicrobial resistance (AMR) directly from whole blood.

Towards advancing the field of sepsis diagnostics, we have developed a cutting-edge approach called ASPIRE: Agnostic Sepsis Pathogen Identification and REsistance determination. This workflow is rapid, agnostic, quantitative, automation amenable that can report pathogen ID and AMR determinants directly from whole blood within 6 to 8 hours using nanopore sequencing. The workflow is illustrated in Figure 1.

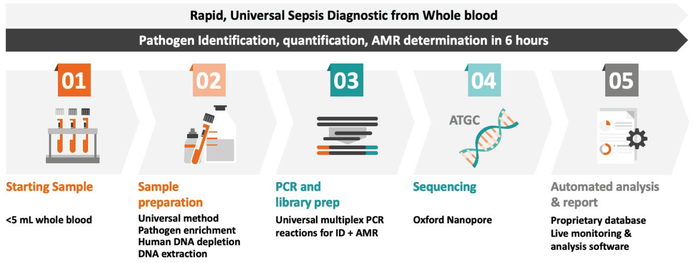

Figure 1. Overview of ASPIRE workflow. The workflow is rapid, pathogen agnostic and automation amenable.

Methods

ASPIRE uses whole blood with sample preparation, universal lysis, pathogen DNA enrichment, unique multiplexing strategy with separate multiplex PCRs for pathogen and AMR detection, automated nanopore sequencing, and real-time analysis using proprietary NanoSepsID software. This interface can be used to manually launch a sample analysis or to start a sequencing run where analysis will be fully automated in sync with sequencing. (Figure 2). The workflow includes one pathogen ID module and three AMR modules.

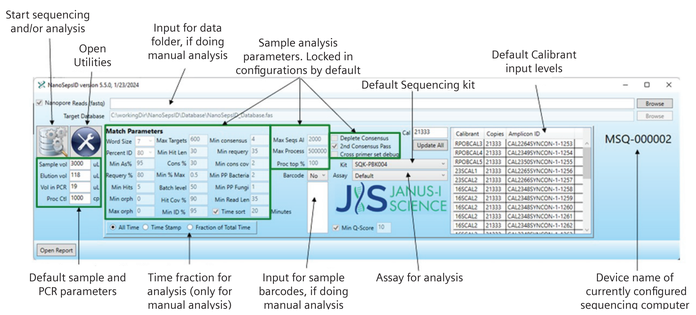

Figure 2. Primary graphical interface for NanoSepsID. This is the window that comes up on program launch.

- AMR performance was evaluated using whole blood samples spiked with genomic DNA from 19 clinical bacterial isolates with characterized resistance signatures.
- Analytical sensitivity was assessed using contrived whole blood samples (n=50) spiked with ESKAPE pathogens and *Candida albicans* at 5–100 CFU/mL across two operators and two sites.
- Clinical evaluation included 177 prospectively collected samples tested in parallel with blood culture; 20 samples remain under discrepancy resolution.
- Breadth of coverage (BoC) was assessed using 19 additional bacterial isolates tested at low concentration in whole blood.

References

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Acknowledgements: *This work was supported by National Institute of Allergy and Infectious Disease (NIAID) and National Institutes of Health (NIH) through Contract No. 75N93023C00025.*

ASPIRE is currently under development; it is not for sale in the U.S.A. Its future availability cannot be guaranteed.

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Published by Siemens Healthcare Diagnostics Inc.
07-2026

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Results

AMR Detection (Table 1):

- ASPIRE accurately and simultaneously identified multiple AMR determinants across multidrug-resistant isolates.
- All targets were correctly identified except the IMP-13 variant.

Table 1. Summary of AMR isolate testing.

#	Species Expected (Ground truth)	Species Detected with ASPIRE	Bla genes as communicated	AMR Markers detected
1	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	OXA-488¶, PDC-35¶, VIM-2	blaVIM, aph(3')-Iib, sul1, gyrA T83I, parC S80L
2	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	OXA-4, OXA-486¶, PDC-3¶, VIM-2	blaOXA-1 Group, blaVIM
3	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	OXA-1184, OXA-488¶, PDC-35¶	blaOXA-10 Group, aac(6')-Ib, aph(3')-Iib, sul1, gyrA T83I, parC S80L
4	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	KPC-2, OXA-904¶, PDC-3¶	blaKPC-2, aph(3')-Iib, gyrA+parC (wt)
5	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	KPC-5, OXA-2, OXA-50¶, PDC-103¶	blaKPC-2§, blaOXA-2 Group, aph(3')-IIb
6	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	KPC, TEM, SHV	blaKPC-2, blaKPC-160, blaTEM (wt), blaSHV (wt), aac(6')-Ib, ant(2'')-Ia, ant(3'')-Ia, dfrA12, sul1, sul2, P. aeruginosa D87N
7	*K. oxytoca*¥	*Klebsiella michiganensis*¥	SHV	blaSHV (L35Q, G238S, E240L), aac(3)-IIg, aac(6')-Ib, ant(3'')-Ia, aph(3')-IIb, sull, sul2, gyrA S83T
8	*Escherichia coli*	*Escherichia coli*	CTX-M-15, TEM	blaCTX-M-8/9/25 Group, TEM (wt), aac(3)-IId, aph(6)-Id, dfrA17, sul1, sul2
9	*Escherichia coli*	*Escherichia coli*	CMY, CTX-M-15	blaCTX-M-15/1 Group, blaCMY, aac(3)-IIe, aac(6')-Ib, aph(6)-Id, blaOXA-1 Group, dfrA17, sul1, sul2, gyrA S83L (mix with wt), *P. aeruginosa* D87N
10	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	Imp-13*	ant(2'')-Ia, aph(3')-Iib, blaSHV L35Q, sull, *P. aeruginosa* D87N
11	*Acinetobacter baumannii*	*Acinetobacter baumannii*	ADC¶, OXA-69	blaOXA-51 Group, ant(2'')-Ia, ant(3'')-Iia, sul2, gyrA S83L, parC S80L
12	*Acinetobacter baumannii*	*Acinetobacter baumannii*	ADC¶, OXA-69, TEM	blaOXA-51 Group, blaTEM (wt), aac(3)-Ia, ant(3'')-Iia, aph(3')-VI, aph(6)-Id, sul1, gyrA S83L, parC S80L
13	*Acinetobacter baumannii*	*Acinetobacter baumannii*	ADC¶, OXA-69, OXA-23	blaOXA-23 Group, blaOXA-51 Group, aac(3)-Ia, aadA6, ant(3'')-Iia, sul1, gyrA S83L, parC S80L
14	*Acinetobacter baumannii*	*Acinetobacter baumannii*	ADC¶, OXA-59¶	blaOXA-51 Group, blaOXA-58 Group, blaOXA-23 Group, ant(2'')-Ia, ant(3'')-Ia, ant(3'')-IIa, aph(3')-VI, aph(6)-Id, blaPER, sul1, sul2
15	*Acinetobacter baumannii*	*Acinetobacter baumanii*	ADC¶, PER, OXA-69	blaPER, blaOXA-51 Group, ant(2'')-Ia, ant(3'')-IIa, aph(6)-Id, gyrA S83L
16	*Citrobacter freundii*€	*Citrobacter braakii*€	CTX-M-15, KPC-3, NDM-1, OXA-1, SHV-12, TEM-1	blaCTX-M-15/1 Group, blaKPC-3, blaNDM, blaOXA-1 Group, blaSHV (L35Q, G238S, E240K), blaTEM (wt), aac(6')-Ib, ant(3'')-Ia, aph(6)-Id, dfrA12, sul1
17	*Klebsiella aerogenes*	*Klebsiella aerogenes*	blaKPC-3, blaNDM-1, blaSHV-12, blaTEM-1A	blaKPC-3, blaNDM, blaSHV (L35Q, G238S, E240K), blaTEM (wt), blaKPC-3, blaNDM, aac(6')-Ib
18	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*	OXA-396¶, PDC-3¶, PME-1¶	ant(2'')-Ia, aph(6)-Id, sul1, gyrA T83I, parC S80L

**IMP primers have several mismatches in blaIMP-13 – covering blaIMP-13 and several other blaIMP variants robustly will require supplementing with additional primers*
¥ Isolate labeled as Klebsiella oxytoca was detected as Klebsiella michiganensis (from the K. oxytoca complex)
€ Citrobacter braakii (a member of the C. freundii complex) is often identified as Citrobacter freundii in culture testing
§ KPC-2 and KPC-5 are 99.9% identical
¶ Not in target list
RED = Listed in truth key, but not detected in ASPIRE workflow, BLUE = Listed in truth key data and detected in ASPIRE workflow

Analytical sensitivity (Figure 3):

- ASPIRE detected ESKAPE pathogens and *Candida albicans* at 5–10 CFU/mL
- Improved sensitivity at lower CFU with increased the sample volume from 3 mL to 4.5 mL to enable the detection of 5 CFU/mL of *Enterococcus faecium*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The results are summarized in Figure 3.

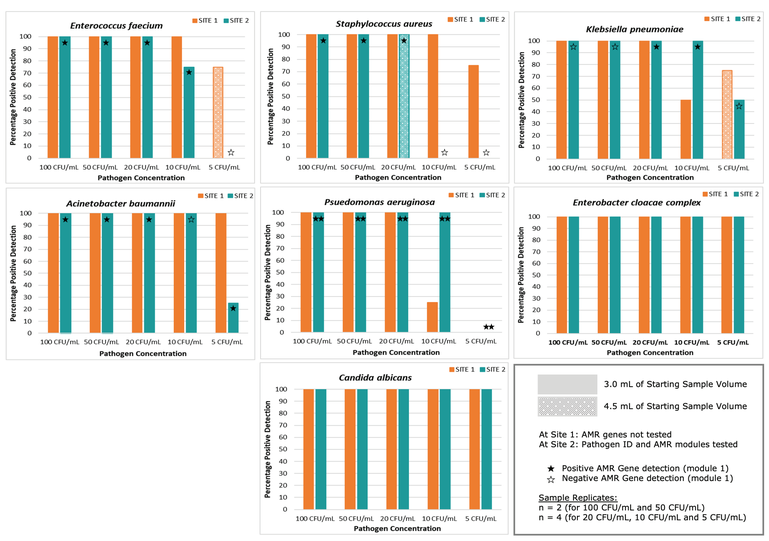


Figure 3. Analytical Sensitivity

Analytical sensitivity of the ASPIRE workflow was tested with all ESKAPE pathogens along with *Candida albicans*. Pathogens were tested at duplicates for 100 CFU/mL and 50 CFU/mL where as 20 CFU/mL, 10 CFU/mL and 5 CFU/mL had quadruplicates. The starting sample volume for the developed 6 hours ASPIRE workflow is 3.0 mL with an exception of 4.5 mL for *Enterococcus faecium* and *Klebsiella pneumoniae* at 5 CFU/mL at Site 1 and *Staphylococcus aureus* at 20 CFU/mL at Site 2 to improve positive detection rate. While Site 1 analyzed for Pathogen ID only, Site analyzed for both Pathogen ID & AMR module 1. The Asterix denotes for presence or absence of AMR gene(s) detections

Clinical sensitivity (Figure 4):

- Preliminary results demonstrated 45% concordance with blood culture, with ongoing discrepancy resolution.
- Sensitivity was 56% and specificity was 45%.

Investigative method (ASPIRE)	Reference method- Blood culture (BC): VA (BC) + (n=29)	VA (BC) – (144)	Total (n=173) +4 samples PCR inhibited
ASPIRE + (Agreement)	10 (TP) (Microbe identified by BC and ASPIRE are same)	76 (FP) (Microbes identified by ASPIRE alone)	86
ASPIRE – (Agreement)	8 (FN) (No microbe detected)	68 (TN) (No microbe detected)	76
ASPIRE + (Disagreement)	11 (Microbe identified by BC and ASPIRE are different)	N/A	11
Total	29	144	173

Confusion Matrix

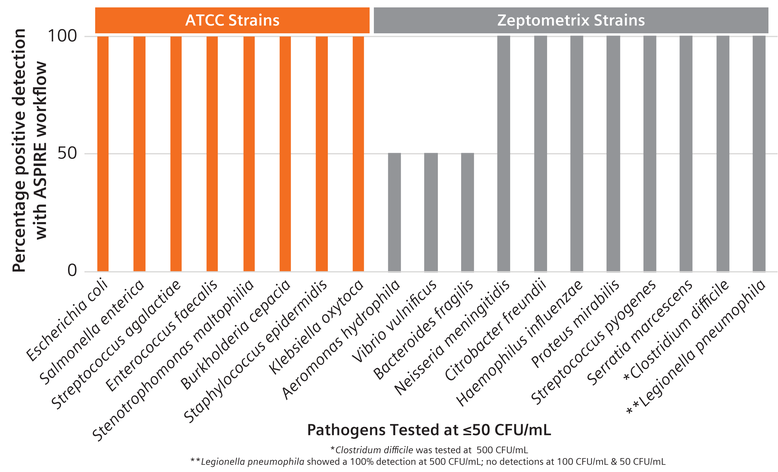
	Actually Positive (1)	Actually Negative (0)	
Predicted Positive (1)	True Positives (TPs)	False Positives (FPs)	Sensitivity $\frac{TP}{(TP+FN)}$
Predicted Negative (0)	False Negatives (FNs)	True Negatives (TNs)	Specificity $\frac{TN}{(TN+FP)}$

Clinical Performance	
Sensitivity	56%
Specificity	47.2%
% agreement	45%

Figure 4. Clinical performance of ASPIRE workflow tested with 177 samples, verified by Blood culture method which is the refence method. Out of 177, 29 samples were blood culture positive, and 144 samples were blood culture negative.

Breadth of Coverage (Figure 5):

- 100% detection across 19 bacterial species, supporting broad organism coverage.
- This indicates a broad coverage of the ASPIRE workflow and attests for the unbiased and universal detection capability of the workflow.



**Clostridium difficile* was tested at 500 CFU/mL
***Legionella pneumophila* showed a 100% detection at 500 CFU/mL; no detections at 100 CFU/mL & 50 CFU/mL

Figure 5. Breadth of Coverage

Study was conducted with 19 pathogens (Gram +ve/-ve) in duplicates at ≤50 CFU/mL concentration. Three pathogens showed 50% positive detection rate while rest of the pathogens had a 100% positive detection rate when processed with 3 mL of starting sample volume in the 6 hours of developed ASPIRE workflow. The pathogens were sourced from ATCC and titred based on culturing method or pre-titred Zeptometrix stocks were procured.

Conclusion

- ASPIRE is a novel, rapid, and universal whole blood sepsis diagnostic workflow for direct pathogen ID and AMR detection with a TAT of ~6 hours.
- The ASPIRE workflow demonstrated broad organism coverage, low-level detection of bacteria and fungi, and successful detection of most targeted AMR determinants.
- Preliminary clinical data highlight contamination control as a key area for further optimization.
- The workflow can detect 5–10 CFU/mL of bacteria and fungi from 3.0–4.5 mL of Whole blood sample. In addition to identifying pathogens, the workflow can also detect antimicrobial resistance signatures from the pathogens.
- The low clinical sensitivity of the workflow is mainly due to the contaminants seen- either presented by samples themselves or introduced during the workflow.