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Performance evaluation of a rapid, universal and automation amenable sepsis diagnostic workflow that identifies pathogens and determines their antimicrobial resistance directly from whole blood

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Introduction

Fast and accurate diagnosis of bloodstream infections (BSIs) is critical for guiding an appropriate treatment regimen in patients, who face hourly-increasing mortality risk due to sepsis[1]. The current gold standard technique is blood culture, and it takes 15 to 72 hours for pathogen identification (ID) and an additional 8 to 15 hours for antimicrobial susceptibility (AST). Apart from the long turnaround time, blood culture is prone to false positives and negatives. Most of the nucleic acid-based methods in the market also require blood culture as a starting sample and/or are limited in identifying pre-defined set of pathogens and antimicrobial resistance (AMR) genes[2]. Hence, the availability of a rapid, pathogen agnostic diagnostic for sepsis which detects pathogens and associated AMR directly from whole blood in a clinically actionable turnaround time, remains a major unmet need in managing critically ill patients.

Aim

Towards advancing the field of sepsis diagnostics, we have developed a cutting-edge approach called **ASPIRE#**: **A**gnostic **S**epsis **P**athogen **I**dentification and **RE**sistance determination. This workflow is rapid, pathogen agnostic, quantitative, automation amenable, utilizes nanopore sequencing for real-time analysis and can report pathogen ID and AMR determinants, directly from whole blood, within 6 to 8 hours. The workflow is illustrated in Figure 1. The workflow identifies pathogens by sequencing key genetic targets amplified from pathogen DNA extracted from less than 5 mL of whole blood.

Method

In this method, we use a proprietary multiplex amplification strategy, wherein the PCR primer sets were selected to target highly conserved, short genomic regions flanking systematic phylogenetic variation from a very large variety of bacteria. The method includes ID and AMR multiplex amplification modules. Amplicon sequencing of these selected genetic fragments, from ID and AMR modules, enables differentiation of thousands of organisms, including complex mixtures. Multiple primer sets are multiplexed for bacterial and fungal identification together in one multiplex module. Bacterial ribosomal RNA (rRNA) loci are used for extremely broad and coarse differentiation of most bacteria, while narrower and less broadly conserved loci are targeted to members of bacterial groups less differentiable by rRNA loci alone (e.g. *tufB* to resolve species of *Staphylococcus* or *valS* to resolve members of the Enterobacteriaceae—Figure 2). Additionally, optional multiplex assay modules are designed to identify a wide variety of AMR markers.

As part of the sample preparation, we have included an enrichment method that significantly enriches the target pathogen DNA by depleting the background human genomic DNA and blood-based inhibitors. Using this enrichment method, we were able to deplete nearly 98% of human genomic DNA which enables the ASPIRE workflow to sensitively detect pathogens (Figure 3).

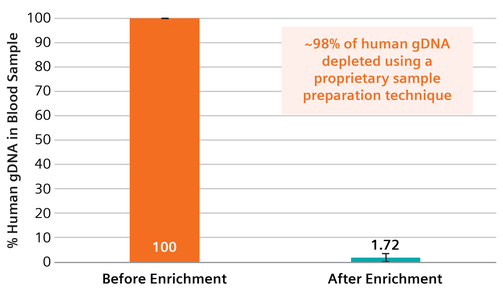

Figure 3. ASPIRE workflow enables direct detection of pathogens from whole blood using proprietary sample preparation method that depletes human genomic DNA without compromising microbial recovery.

For the contrived sample tests, we used <5 mL whole blood samples spiked with microorganisms at 5 to 100 CFU/mL, processed to enrich and extract the target DNA, amplify it with the multiplexed PCRs using ID and AMR modules and sequencing the prepared amplicon libraries on Oxford Nanopore Technologies' MinION device.

For clinical sensitivity tests, we procured blood culture positives, negatives and uncultured whole blood clinical samples from Discovery Life Sciences (DLS). These samples were processed like contrived samples via ASPIRE workflow. The clinical sensitivity testing has been done in a blinded way with no known prior information about the pathogen present in the sample.

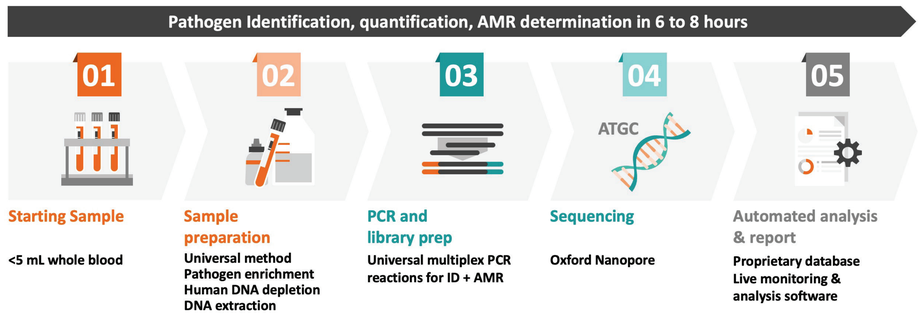

Figure 1. Overview of ASPIRE workflow. The workflow is rapid, pathogen agnostic and automation amenable.

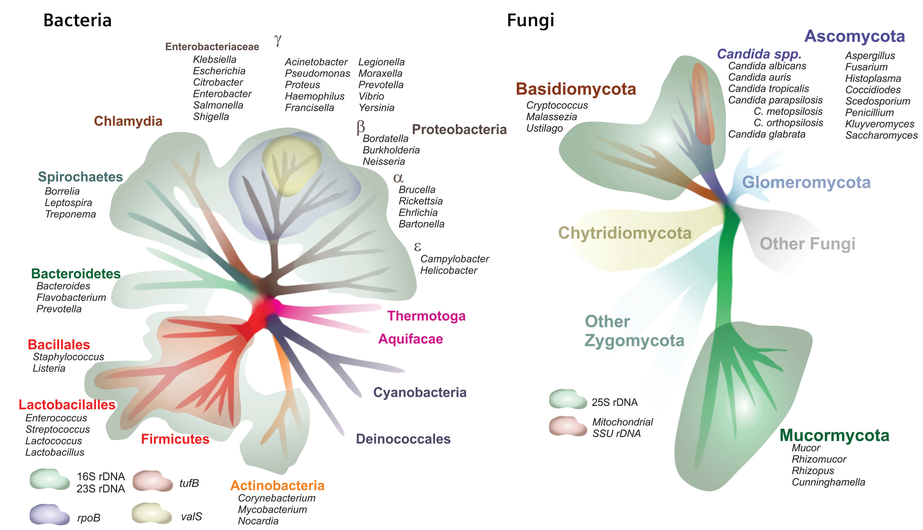

Figure 2. Broad Phylogenetic targeting of bacteria and fungi by a minimal multiplex of ASPIRE PCR primer sets.

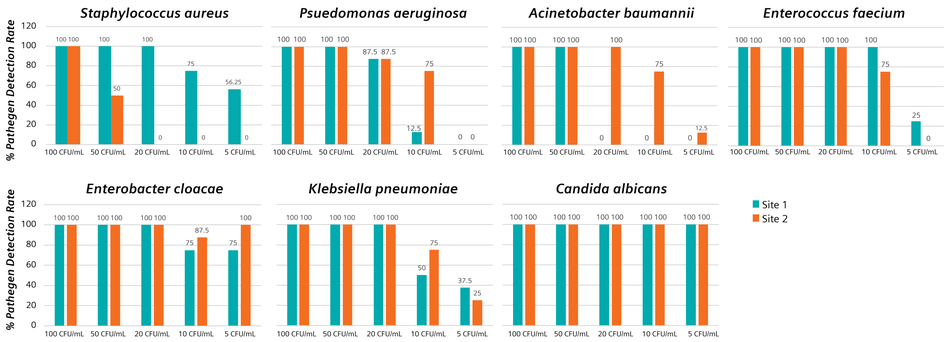

Figure 4. Analytical Sensitivity assessment with ESKAPE bacteria and Candida albicans tested across two multiple teams, sites 1 and 2.

Table 1. Clinical sensitivity and specificity with sub-target blood volumes consisting of 58 *blood culture samples* (50 positive, 8 negative) and 31 *uncultured whole blood patient samples* (19 positive and 12 negative) to stress-test the assay with non-ideal blood volumes.

Match / MM	Blood Culture clinical sample*		Culture Total	Whole blood clinical sample*		Clinical Total	Grand Total
	Negative	Positive		Negative	Positive		
FN	0	0	0	0	2	2	2
FP	4	0	4	0	0	0	4
MM	0	3	3	0	3	3	6
TP	4	47	51	12	14	26	77
Total	**8**	**50**	**58**	**12**	**19**	**31**	**89**

MM-Mismatch, FN- False Negative, FP-False Positive, TP-True Positive
**Volumes used in this assessment is less than recommended starting volume for ASPIRE workflow.*

Results

We evaluated the analytical sensitivity of ASPIRE workflow using < 5 mL contrived blood samples (n=112 at two separate testing sites) spiked with typical BSI bacteria and fungi at 5 to 100 CFU/mL. We used eight sequencing run replicates for 20, 10 and 5 CFU/mL assessment and four sequencing run replicates for 50 and 100 CFU/mL assessment. This assessment was done simultaneously at two collaborator sites to assess for reproducibility. The results outlined in Figure 4 indicate that the ASPIRE workflow has an analytical sensitivity of 5–10 CFU/mL.

As part of a preliminary clinical study, a collection of 89 blinded clinical blood samples were purchased from DLS and tested at site 2 with the pathogen ID and AMR (β-lactamases, methicillin, vancomycin, and colistin) assay modules. Due to limited availability of original blood draw material that had not been subject to blood culture, 19 original positive uncultured ***whole blood*** patient samples, 12 confirmed negative *uncultured whole blood patient samples* and/or *healthy donor whole blood samples*, and 58 ***blood culture*** samples (50 positives and 8 negatives) were tested. The sample volume available for this study was less than the intended sample volume designed for ASPIRE workflow. Therefore, sensitivity was compromised for *uncultured whole blood patient samples* utilizing less than the intended assay target volume. Also, specificity was compromised for *cultured blood samples* due to microbial DNA in the culture despite the lack of an identified cultured organism(s) by the sample provider. The clinical sensitivity study results are outlined in Tables 1 and 2, which indicates more than 80% concordance with blood culture results, both with uncultured whole blood patient samples and blood culture samples. Due to limited sample volumes, we could not perform the discrepancy analysis to validate ASPIRE workflow findings.

Table 2. Performance of ASPIRE workflow with sub volume clinical samples.

Sample Type	% Total Agreement (n)	Sensitivity (n)	Specificity (n)
Blood Culture*	87.9 (58)	94 (50)	50 (8)
Whole Blood*	83.9 (31)	73.7 (19)	100 (12)
All	86.5 (89)	88.4 (69)	80 (20)

**Volumes used in this assessment is less than recommended starting volume for ASPIRE workflow.*

Conclusions

- As shown in Figure 5, ASPIRE is a novel, cutting-edge, rapid, automation amenable and universal sepsis diagnostic workflow that can identify pathogens and characterize their AMR directly from whole blood.

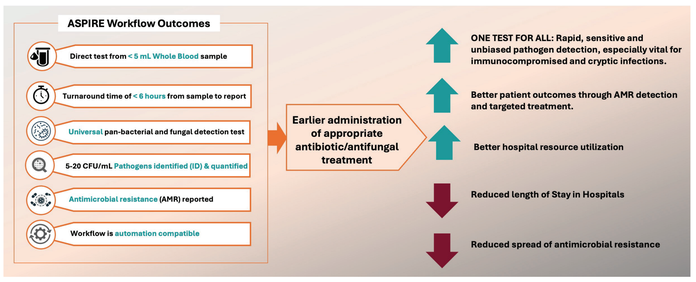

Figure 5. Key Workflow Outcomes. ASPIRE workflow may enable earlier administration of appropriate therapy by rapid identification of infection causing pathogens and characterizing their antimicrobial and antifungal resistances.

- We were able to demonstrate detection of 5 to 10 CFU/mL for ESKAPE bacteria and *Candida* using the ASPIRE workflow.
- Clinical studies showed substantial concordance between blood cultures and ASPIRE workflows at 87.8% and a high sensitivity of 94% when tested with 58 blood culture samples. Also, with direct whole blood samples, we observed ~83% clinical concordance. We anticipate a better clinical assay performance with the intended starting blood volume.

References

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#The products/features mentioned here are in development; future availability cannot be guaranteed.

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