Direct Detection of Bloodstream Pathogens from Whole Blood using the LiDia-SEQ™ Platform: The First, NGS-Based Sample-to-Result Platform

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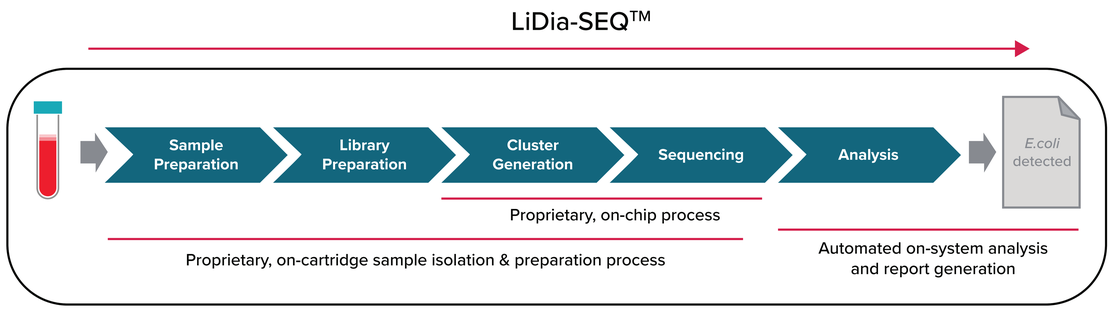
INTRODUCTION

Blood stream infections (BSI) or Sepsis is a life-threatening condition that affects more than 48 million people worldwide and contributes to approximately 11 million deaths per year. Current standard of care methods like blood culture can take days for results to be available. Sequencing to detect and identify a bloodstream pathogen is a manual process, with highly variable times to result. DNAe has developed a sample-to-result, NGS-based, BSI/AMR Test on our prototype LiDia-SEQ™ platform, that detects bacterial targets, with select antimicrobial resistance markers (AMR), and fungal targets directly from whole blood. The LiDia-SEQ™ platform consists of the system, test kits, and software.



DNAe's LiDia-SEQ™ automated NGS workflow

DNAe's LiDia-SEQ™ system test has automated the following steps: sample prep, library prep, clonal amplification, sequencing, and bioinformatic analysis to generate the test result. The entire workflow is completed in hours in a fully automated system. In this study, DNAe has evaluated the performance of its BSI/AMR test on low-level spiked blood samples.

MATERIALS & METHODS

Materials The DNAe BSI/AMR test uses proprietary sample preparation, library preparation, and sequencing reagents. For orthogonal comparison purposes, duplicate samples were also run using a NEBNext® Ultra™ II DNA Library Prep Kit from Illumina for library preparation and MiniSeq™ Mid Output Kit (300 cycles) for sequencing. Organisms were purchased or obtained from multiple banks.

Methods All samples were prepared using 4mL of whole blood spiked at either 30, 10, or 3 CFU/mL with a range of bacterial and fungal strains or un-spiked for negative controls. All samples were run automatically on the LiDia-SEQ™ system, utilizing BSI/AMR test. This involved loading a blood sample tube into a LiDia-SEQ™ BSI/AMR test cartridge, and then into the LiDia-SEQ™ system. The system interfaces with the cartridge to transfer the sample from the blood tube before lysing the pathogens, binding specific probes to the target DNA, binding the probes on proprietary beads, washing away inhibitors, and concentrating the targets. The system amplified the eluates and controlled the number of target copies using DNAe's proprietary copy control method. The system clustered the targets on a flowcell and sequenced using DNAe's patented ISFET technology, which detects protons released during nucleotide incorporation. The sequencing results were analyzed through DNAe's analysis pipeline to assign test results. For comparison purposes, all results were also compared to a test that used an orthogonal sequencing method, specifically: DNAe's sample and library preparation steps directly from DNAe LiDia-SEQ™, followed by Illumina Library prep for sequencing on the Illumina MiniSeq®, then analysis using the DNAe assay caller pipeline.

RESULTS

DNAe's automated instrument and BSI/AMR test detected spiked organisms at concentrations as low as 10 CFU/mL and 3 CFU/mL (Table 1). Testing is in-progress on additional organisms at lower concentration.

CONCLUSION & DISCUSSION

The results presented highlight the performance of the LiDia-SEQ™ BSI/AMR Test on the prototype LiDia-SEQ™ system to accurately identify a wide range (>16 organisms) of bacteria (with selected AMRs) and fungal pathogens down to clinically relevant concentrations of 3-10 CFU/mL.

LiDia-SEQ™'s ability to detect a comprehensive panel of pathogens and resistance genes directly from whole blood, in a fully automated workflow, underscores the LiDia-SEQ™ Platform's potential as a transformative tool for point-of-need BSI diagnostics.

Table 1: Spiked Samples tested at 30, 10 or 3 CFU/mL with DNAe BSI/AMR test workflow on fully automated LiDia-SEQ™ system.

Spiked Sample and Strain	Spike Level	DNAe BSI/AMR Test Result	DNAe AMR Detection
Acinetobacter baumannii CDC AR-0045	30 CFU/mL	*Acinetobacter baumannii*	OXA-51,OXA-23, TEM
	10 CFU/mL	*Acinetobacter baumannii*	OXA-51,OXA-23, TEM
Aerococcus viridans 11563	30 CFU/mL	*Aerococcus* Genus	
Candida albicans 66027	10 CFU/mL	*Candida albicans*	
	3 CFU/mL	*Candida albicans*	
Candida auris CDC AR-0381	10 CFU/mL	*Candidozyma auris*	
	3 CFU/mL	*Candidozyma auris*	
Citrobacter braakii 51113	30 CFU/mL	*Enterobacteriaceae* Family	
Enterobacter cloacae CDC AR-501	30 CFU/mL	*Enterobacter hormaechei*	TEM
	10 CFU/mL	*Enterobacter hormaechei*	TEM
Enterococcus faecium BAA-2318	30 CFU/mL	*Enterococcus faecium*	vanA
	10 CFU/mL	*Enterococcus faecium*	vanA
	3 CFU/mL	*Enterococcus faecium*	vanA
Escherichia coli NCTC 13353	30 CFU/mL	*Escherichia coli*	CTX-M group 1
	10 CFU/mL	*Escherichia coli*	CTX-M group 1
	3 CFU/mL	*Escherichia coli*	CTX-M group 1
Haemophilus influenzae 49247	10 CFU/mL	*Haemophilus influenzae*	
Klebsiella pneumoniae BAA-1898	30 CFU/mL	*Klebsiella pneumoniae*	KPC, TEM
	10 CFU/mL	*Klebsiella pneumoniae*	KPC, TEM
	3 CFU/mL	*Klebsiella pneumoniae*	KPC, TEM
Morganella morganii 25830	30 CFU/mL	*Morganella morganii*	
Proteus mirabilis CDC AR-0159	30 CFU/mL	*Proteus mirabilis*	NDM
	10 CFU/mL	*Proteus mirabilis*	NDM
Pseudomonas aeruginosa CDC-AR-0092	30 CFU/mL	*Pseudomonas* Genus	
Salmonella enterica CDC AR-0919	30 CFU/mL	*Salmonella enterica*	
Serratia marcescens CDC AR-0517	30 CFU/mL	*Serratia marcescens*	CTX-M group 1, TEM
	10 CFU/mL	*Serratia* Genus	CTX-M group 1, TEM
Shigella sonnei 29930	30 CFU/mL	*Escherichia/Shigella* species	
	10 CFU/mL	*Escherichia/Shigella* species	
Staphylococcus aureus BAA-2094	10 CFU/mL	*Staphylococcus aureus*	mecA
	3 CFU/mL	*Staphylococcus aureus*	mecA
Streptococcus pneumoniae 51916	10 CFU/mL	*Streptococcus* species	
Negative Control		Negative Control*	

*Common contaminates: *Bradyrhizobium spp.,Pseudomonas fluorescens., Comamonas spp., Cutibacterium acnes, Saccharomyces cerevisiae, and Staphylococcus epidermidis*

References: Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study. Rudd, Kristina E, et al. The Lancet 2020, Volume 395, Issue 10219, 200 – 211
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