Direct Detection of Bloodstream Pathogens from Whole Blood Using the Workflow from the DNAe BSI/AMR Test: The First NGS Sample-To-Result Solution

M. Wisniewski, C. Massire, S. Poplawski, D. Morley, D. Wooldridge, M. Dudas, G. Worsley, S. Vaughn, A. Komoroczi, F. Gazio, I. Jones, F. Matthews, S. Narayanan, and S. Reed

DNAe London, United Kingdom and Carlsbad, California, USA

CONTACT INFORMATION
Michele Wisniewski
michele.wisniewski@dnae.com

INTRODUCTION

Sepsis is a significant life-threatening condition with 48.9 million cases per year leading to 11 million deaths worldwide[1]. Patient outcomes are directly related to the timely diagnosis and identification of the pathogen and administration of the appropriate antibiotic treatment. However, traditional standard of care methods like blood culture are labor intensive, slow and take days for actionable results, and limited to detection of organisms that can grow in blood culture. See Figure 1. DNAe is developing the first rapid, sample-to-result, next-generation sequencing (NGS) solution, the DNAe bloodstream infections/antimicrobial resistance (BSI/AMR Test), for identification of bacterial and fungal targets directly from whole blood within a work shift compared to days for the current standard of care.

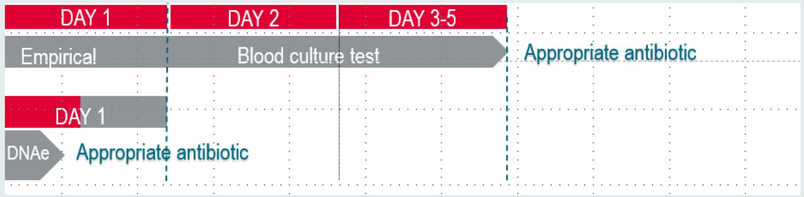

Figure 1: Typical timeline for pathogen identification in sepsis cases using current standard of care methods

The BSI/AMR Test can detect a comprehensive range of bacterial and fungal targets with select antibiotic resistance markers. The test workflow includes sample preparation, library preparation, cluster generation, sequencing, and bioinformatic analysis to generate a clinically actionable test result. The test is designed to work on DNAe's proprietary sample-to-result NGS platform using the LiDia-SEQ™ platform. The entire workflow is completed within a work shift. See Figure 2. In this study, DNAe have evaluated the initial performance of the DNAe BSI/AMR Test workflow on low-level spikes, blanks, and clinical samples.

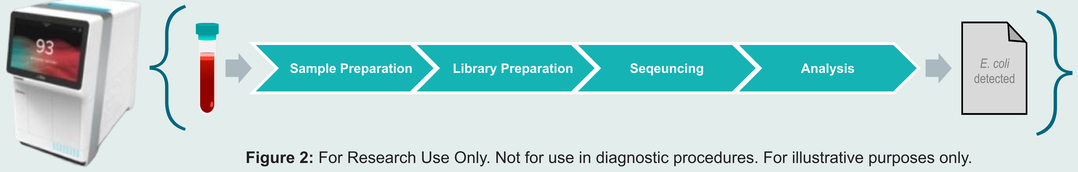

Figure 2: For Research Use Only. Not for use in diagnostic procedures. For illustrative purposes only.

MATERIALS AND METHODS

Materials: The DNAe BSI/AMR Test uses proprietary sample preparation, library preparation, and sequencing reagents. A NEBNext® Ultra™ II DNA Library Prep Kit from Illumina for library preparation and MiniSeq™ Mid Output Kit (300 cycles) for sequencing was used for the orthogonal baselining study. Organisms were purchased or obtained from multiple banks as shown in Table 1 below. Clinical samples were obtained through a research clinical study using UCSD Hillcrest and CALM Lab sites.

Organism	Strain	Culture Collection
Acinetobacter Baumanii	CDC AR-0045	CDC AR Isolate Bank
Candida auris	66027	ATCC
Candida albicans	CDC AR-0381	CDC AR Isolate Bank
Enterobacter cloacae	CDC AR-501	CDC AR Isolate Bank
Enterococcus faesium	BAA-2318	ATCC
Escherichia coli	NCTC 13463	NCTC
Psuedomonas aeruginosa	CDC AR-0092	CDC AR Isolate Bank
Salmonella enterica	CDC AR-0919	CDC AR Isolate Bank
Staphylococcus aureus	BAA-2094	ATCC

Table 1: Source of Spike Organisms

Methods: Spiked samples and no template controls were prepared by using 3 mL whole blood and spiked at 100 CFU/mL 10 CFU/mL and 3 CFU/mL with qualified bacterial and fungal strains. Samples for clinical studies used de-identified clinical samples which included the standard of care results and isolated organisms. DNAe BSI/AMR Test Method: All samples were processed through the BSI/AMR Test workflow on the bench or on LiDia-SEQ™ Platform by lysing the sample, binding the probes to a magnetic bead, washing inhibitors, and concentrating the targets. The eluate was amplified, and the copies of the targets were controlled by DNAe's proprietary copy control method. The targets were clustered on our semiconductor flowcell and were sequenced using DNAe's patented ISFET technology which rapidly detects protons released during nucleotide addition. DNAe's proprietary analysis pipeline, the DNAe Assay Caller, was then used to analyse the sequencing data to generate test results. Orthogonal Test Method: All DNAe results were compared to an orthogonal test method that used the DNAe sample preparation, library preparation and data analysis steps combined with an orthogonal sequencing method using a library prep kit to add adaptors. The orthogonal sequencing data was analyzed using the DNAe Assay Caller to generate the Orthogonal test result.

RESULTS

The DNAe BSI/AMR Test workflow successfully detected all spiked samples for all 10 tested organisms down to ≤ 3CFU/mL (Table 2). The blank samples detected all the correct internal controls and had minimal or low-level contaminants that were used to threshold the analysis. All 10 clinical samples were successfully tested by the BSI/AMR Test workflow. The test successfully detected organisms at ≤3 CFU/mL from the clinical samples and had 90% positive percent agreement (PPA) with the standard of care/clinical isolate results. All the clinical samples were also tested with the orthogonal testing method and there was 100% PPA between the test result of the BSI/AMR Test and the orthogonal test method (Table 3). Lastly, spiked whole blood samples were tested by DNAe BSI/AMR Test on the LiDia-SEQ™ platform and the test successfully detected the spiked organisms and reported a genus/species level call (Table 4).

Spiked Samples	CFU Spike	Number of Replicates	DNAe BSI/AMR Test Result
Acinetobacter baumanii	3	1	*Acinetobacter baumanii*
	10	1	*Acinetobacter baumanii*
Candida albicans	3	2	*Candida genus*
	10	2	*Candida genus*
	100	2	*Candida genus*
Candida auris	3	1	*Candida auris*
	10	2	*Candida auris*
	100	2	*Candida auris*
Enterobacter cloacae	3	1	*Enterobacter genus*
	10	1	*Enterobacter genus*
	100	2	*Enterobacter genus*
Enterobacter faecium	3	2	*Enterobacter faecium*
	10	2	*Enterobacter faecium*
Enterobacter cloacae	3	1	*Enterobacter genus*
	10	1	*Enterobacter genus*
	100	2	*Enterobacter genus*
Pseudomonas aeruginosa	3	1	*Pseudomonas genus*
	10	1	*Pseudomonas genus*
	100	1	*Pseudomonas genus*
Salmonella enterica	3	2	*Salmonella enterica*
	10	2	*Salmonella enterica*
	100	2	*Salmonella enterica*
Staphylococcus aureus	3	2	*Staphylococcus aureus*
	10	2	*Staphylococcus aureus*
	100	2	*Staphylococcus aureus*

Table 2: Spiked Samples tested at three concentration (3, 10, 100 CFU/mL) with the DNAe BSI/AMR Test workflow

Blood Culture Result (Clinical Isolate)	DNAe BSI/AMR Test Result (DNAe Sequencing)	Orthogonal Test Result (Illumina Sequencing)
Escherichia coli	*Escherichia coli w/ gyr A, gyr B, CTX-MG 1*	*Escherichia coli w/ gyr A, CTX-MG 1*
Staphylococcus aureus (MRSA)	*Staphylococcus aureus* (MRSA) w/ mecA, gyrB	*Staphylococcus aureus* (MRSA) w/ mecA, gyrB
Enterobacter cloacae	*Enterobacter cloacae/ludwigii*	*Enterobacter ludwigii w/ gyrA*
Citrobacter freundii	*Citrobacter freundii w/ gyB*	*Citrobacter freundii w/ gyB*
Aerococcus viridans	*Bartonella guintana*	*Bartonella guintana*
Enterococcus faecium	*Enterococcus faecalis w/ vanA*	*Enterococcus faecalis w/ vanA*
Enterococcus faecalis	*Enterococcus faecalis*	*Enterococcus faecalis*
Morganella morganii	*Morganella morganii*	*Morganella morganii*
Staphylococcus aureus (MRSA)	*Staphylococcus aureus* (MRSA) w/ mecA, gyrB	*Staphylococcus aureus* (MRSA) w/ mecA, gyrB
Escherichia coli	*Escherichia coli w/ gyrA, gyrB, CTX-MG 1*	*Escherichia coli w/ gyrA, gyrB, CTX-MG1*

Table 3: Clinical Samples tested by DNAe BSI/AMR Test Workflow and Orthogonal test method

Spiked Sample	DNAe BSI/AMR Test Result (Run on LiDia-SEQ Platform)	Orthogonal Test Result (Illumina Sequencing)
Escherichia coli	*Escherichia coli**	*Escherichia coli***
Staphylococcus aureus (MRSA)	*Staphylococcus aureus (MRSA)**	*Staphylococcus aureus (MRSA)***
Candida albicans	*Candida albicans**	*Candida albicans***
Negative Control	*Negative Control**	*Negative Control***

** Also detected at a lower level in the background: Bradyrhizobium spp., Comamonas spp., and Pseudomonas spp.*
***Also detected at a lower level in the background: Bradyrhizobium spp. and Pseudomonas fluorescens.*

Table 4: Spiked Samples tested at 50CFU/mL by the DNAe BSI/AMR Test on the LiDia-SEQ™ platform

CONCLUSION & DISCUSSION

The DNAe BSI/AMR Test workflow can comprehensively detect bacterial and fungal targets directly from whole blood at low, clinically relevant levels of detection. These represent the first performance data of the LiDia-SEQ™ BSI/AMR Test and demonstrate the potential of the technology to deliver clinically actionable results within a work shift compared to days with standard-of-care methods.

REFERENCES

1. 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

Acknowledgement

This project has been supported in whole or in part with federal funds from the Department of Health and Human Services; Administration for Strategic Preparedness and Response; Biomedical Advanced Research and Development Authority (BARDA), under contract number HHSO100201600017C.