An assay for the detection of sepsis pathogens directly from whole blood samples

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Context: DNAe has developed a unique sequencing workflow to make it compatible with a sample-to-result cartridge – to make it suitable for a rapid, automated, and fully-integrated test, for use at the point of need. In this study, the assay and analysis software (automated when in end-use), are described and tested on samples manually from end to end to benchmark it and to demonstrate how the analysis performs at intermediate steps.

BACKGROUND

Bloodstream Infection (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[1]. Timely identification of the responsible pathogen and potential antimicrobial drug resistance (AMR) is paramount for administration of proper treatment and patient survival. Current standard of care relies on blood culture methods and remains a manual process, with highly variable time to result.

DNAe has developed a direct from whole-blood sample-to-result, NGS-based, BSI/AMR assay. Using DNAe's assay workflow and sequencing technology we have achieved a quasi-universal breadth of coverage of over ~7,400 bacterial and ~800 fungal species and 26 common AMR markers (Figure 1). Here we demonstrate the performance of our proprietary assay (manual format), and specifically the (DNAe) sequencing and analysis pipeline components of the workflow.

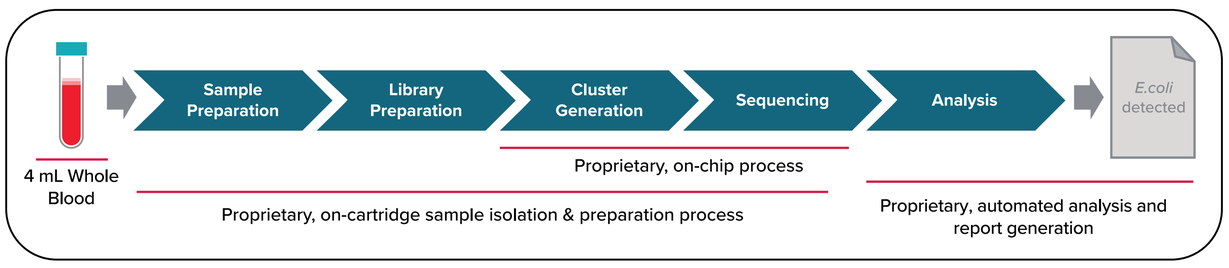


Figure 1. The BSI/AMR assay was executed with a combined manual workflow using DNAe sequencing. The assay is designed for integration into a rapid automated platform.

METHODS AND MATERIALS

Whole-blood samples spiked with known organisms at a range of concentrations 30, 10, 3 CFU/mL were processed through a manual version of the DNAe BSI/AMR workflow (Figure 1) to illustrate and benchmark performance at intermediate steps. Following sample preparation and library preparation, samples were sequenced using the proprietary DNAe sequencing technology. DNAe sequencing outputs were analysed with the DNAe analysis pipeline (Figure 2). All prepared samples were also analysed with Illumina sequencing as an orthogonal confirmation method.

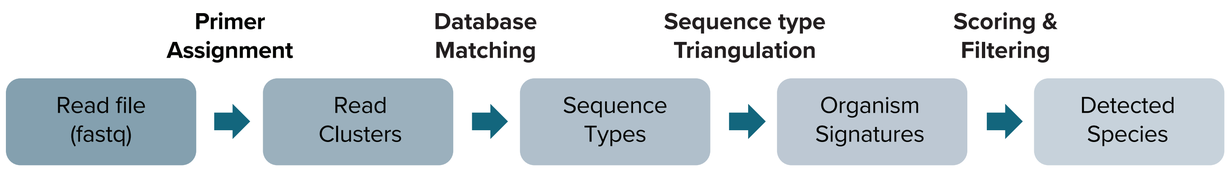
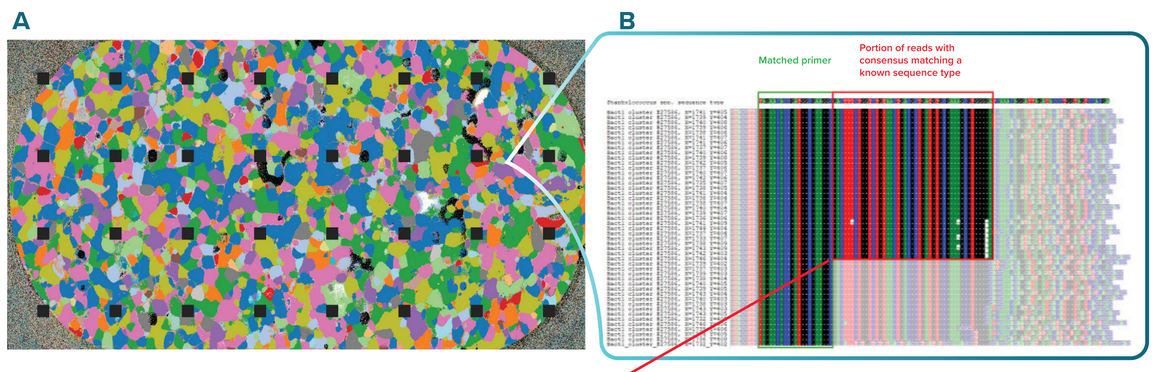


Figure 2. DNAe analysis pipeline workflow.

The DNAe analysis pipeline achieves a quasi-universal breath of coverage by triangulating sequenced reads from a mix of pan-bacterial and pan-fungal targets (Figures 2 and Figure 3).



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						Bacterial reads							AMR reads (3/24 loci)			Fungal reads		
Detection	Score	N_clusters	read_length	read%	N_reads	#1	#2	#3	#4	#5	#6	#7	mecA	gyrB#1	gyrB#2	#2	#3	#4	#5
Extraction control		53	64.7	7.5	8698														
Well controls		18	58.3	4.14	4804														
Staphylococcus aureus	0.977	291	61.9	46.4	53817	30	2291	17603	17472	6396	76	159	6976	2251	372				
Cladosporium spp.	0.294	46	53.1	5.37	6233											4841	443	885	64

Figure 3. Analysis of a blood sample spiked with MRSA at 10 CFU/ml. A: Clustering of reads on the sequencing chip according to their identified target. B: Database matching for a given cluster. C: Condensed detection report, with the intended target shaded in green. Potential species are sorted and filtered using a score reflecting their abundance in the sample and the completeness of their retrieved signature. Gray shading indicates potential species scoring below the reporting threshold.

Altogether, the detected targets and associated genetic sequences provide distinct signatures that typically identify organisms at the species level, and potentially even down to individual strains or groups of strains (Figure 4).

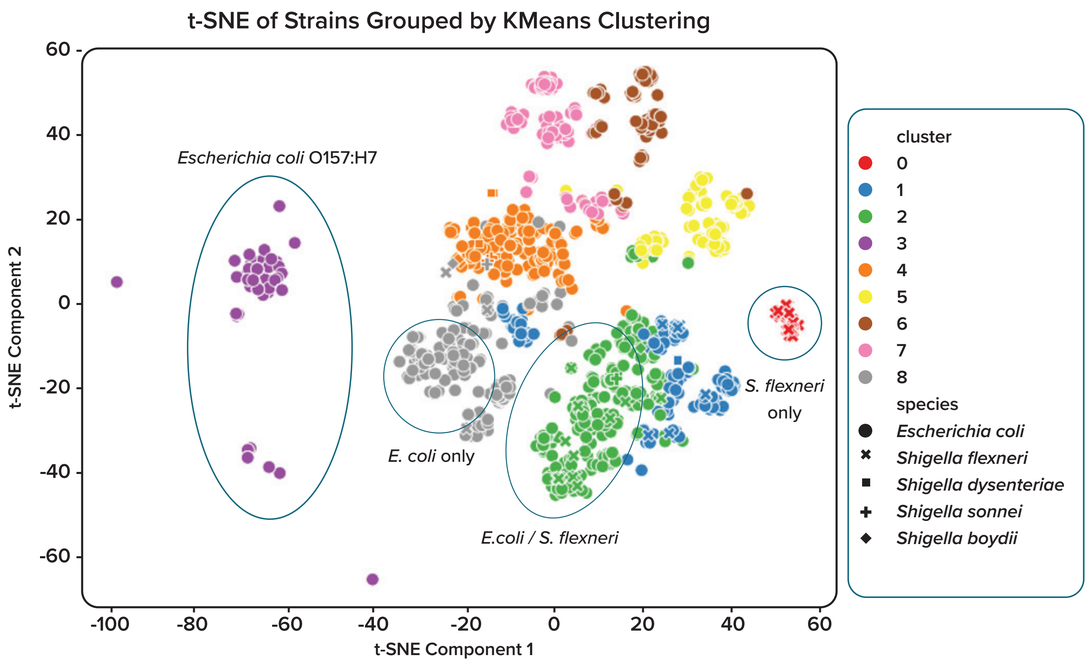

Figure 4. Kmeans analysis of the expected signatures for 1,958 strains of the *E.coli/ Shigella* species complex. For these species, signatures are typically collected across 10 broad-bacterial targets and a variable number of AMR targets. The different colors group together strains with similar signatures that are confidently distinguished from those of other groups, regardless of their species assignment. Whereas most *S. flexneri* strains form a tight, distinct cluster, some strains are undistinguishable from *E. coli* (green) and would be ambiguously identified. Distinct subgroups of *E.coli* (in particular O157:H7) possess unique sequence types that would allow some sub-species differentiation.

RESULTS

The assay was initially tested using a collection of 52 diverse bacterial (Gram-positive and Gram-negative) and fungal species and 25 whole blood clinical samples, demonstrating the correct identification of species spiked at concentrations from 3 to 30 CFU/mL directly from the whole blood sample (full data not shown here, see Tables 1-3 for representative examples).

The analysis pipeline uses key metrics to calculates a "Score" that provides a quantifiable metric for organism identification (Table 1). Column Read% indicates the total percentage of reads assigned to a species. The remaining columns indicate the percentage of reads assigned to each expected sequence type *e.g.* Bac1. Thanks to the built-in redundancy between the coverage of the different targets, the loss of a target *e.g.* Bac1 or Bac5 in some *E. coli* samples does not compromise the species call and only marginally affects the scoring (Table 1).

Sample	Score	Read%	Bac #1	Bac #2	Bac #3	Bac #4	Bac #5	Bac #6	Bac #7	Bac #8	Bac #9	Bac #10	CTX_M	TEM1	TEM2	TEM3	Control
EC_3CFU	0.903	**83.51**		4.06	13.61	2.72		2.27	0.07	2.53	8.12	0.37	4.08	18.92	3.32	23.43	4.10
EC_3CFU	0.93	**84.02**	0.05	2.36	6.70	5.36	0.36	0.81	0.03	4.07	3.23	0.34	8.98	21.69	9.30	20.74	4.97
EC_10CFU	0.921	**92.57**	0.08	3.07	11.62	13.70	0.05	1.40	0.04	5.25	7.11	0.40	8.50	18.23	9.62	13.52	1.11
EC_10CFU	0.907	**91.77**		3.06	15.18	14.3	0.02	2.54	0.05	5.36	4.31	0.21	5.19	17.77	9.60	14.18	1.29
EC_30CFU	0.928	**95.1**	0.93	3.61	5.63	7.48	0.20	3.08	0.02	9.66	10.64	1.26	6.91	18.59	11.8	15.27	1.73
EC_30CFU	0.929	**94.81**	0.58	3.18	9.79	12.09	0.51	2.13	0.09	10.03	8.03	1.65	7.08	12.44	9.10	18.11	1.29

Table 1. Detection of *E. coli* spiked in whole blood at 3, 10 and 30 CFU/ml (2 replicates each).

These expected sequence type signatures provide finer resolutions that enable unique identification of closely related spices. For example, *E. coli* was uniquely detected (to the exclusion of closely related Shigella species) thanks to the detection of a specific sequence type in Bac8 (Table 1); similarly, Bac11 and Bac12 uniquely identify *S. aureus* to the exclusion of other Staphylococcus species (Table 2). This fine resolution tuning can also enable strain level detection for select organisms.

Sample	Score	Read%	Bac #1	Bac #2	Bac #3	Bac #4	Bac #5	Bac #6	Bac #7	Bac #11	Bac #12	mecA	Control
SA_3CFU	0.977	**54**	0.03	2.10	17.68	17.29	6.56	0.07	0.15	2.14	0.34	7.65	5.58
SA_3CFU	0.977	**43.73**	0.18	2.34	16.18	12.75	3.53	0.10	0.10	2.82	0.93	4.79	5.00
SA_10CFU	0.986	**72.02**	0.35	2.54	14.47	20.74	5.66	0.33	0.04	9.66	1.81	16.43	4.46
SA_10CFU	0.977	**47.09**	0.20	4.31	15.47	17.40	4.38	0.39	0.05	0.94	0.16	3.79	2.18
SA_30CFU	0.977	**85.32**	0.95	5.71	15.50	28.57	10.22	0.26	0.17	5.09	1.68	17.17	1.32
SA_30CFU	0.977	**88.75**	1.05	2.77	19.24	25.6	7.00	1.49	0.11	6.74	1.20	23.56	1.70

Table 2. Detection of ***S. aureus*** spiked in whole blood at 3, 10 and 30 CFU/ml (2 replicates each). Next to the first 7 pan-bacterial targets that provide a signature matching several ***Staphylococcus*** species, Bac11 and Bac12 provide finer resolution uniquely identifying *S. aureus.*

In addition to bacterial targets, the BSI/AMR assay can accurately detect a panel of fungal organisms from a single workflow. Table 3 demonstrates the detection of *C. albicans* down to clinically relevant levels of 3 CFU/mL.

Sample	Score	Read%	Fun #1	Fun #2	Fun #3	Fun #4	Fun #5	Control
CA_3CFU	0.924	**17.28**	0.22	11.63	1.60	3.15	0.67	10.45
CA_3CFU	0.918	**19.51**	0.13	10.01	2.09	6.85	0.44	5.94
CA_10CFU	0.908	**15.95**	0.04	12.02	1.67	1.63	0.59	5.35
CA_10CFU	0.892	**14.19**	0.04	13.18	0.52	0.32	0.14	6.27
CA_30CFU	0.924	**36.34**	1.06	25.72	3.35	2.82	3.38	4.87
CA_30CFU	0.936	**37.21**	1.00	26.54	2.68	6.18	0.80	4.08

Table 3. Detection of C. albicans spiked in whole blood at 3, 10 and 30 CFU/ml (2 replicates each).

Testing of the BSI/AMR assay on clinical samples demonstrated a high level of concordance with the gold standard blood culture. The power of the BSI/AMR assay is further shown by the identification of a possible co-infection (Table 4, sample SA984). All samples were run using the DNAe workflow and confirmed on an Illumina MiSeq for discordant resolution. Sample MB984 was discordant with SoC/clinical ID, but the presence of *Staphylococcus warneri* was confirmed with Illumina sequencing. This sample was collected one day after antibiotic treatment, which may have allowed clearance of the infection.

Sample	Clinical ID	Detection	Score	Read%	#1	#2	#3	#4	#5	#6	#7	#8	#10	CTX_M	TEM1	TEM2	TEM3	Control
SD650	*Salmonella sp.*	*Salmonella enterica*	0.936	82.23	1.05	3.93	21.88	18.73	0.06	3.67	0.25	24.72	7.75			0.19		1.29
SA515	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*	0.944	66.15	0.33	8.38	20.89	25.19	0.33	5.77	0.17	3.82		0.16	1.12			0.68
SA773	*Enterococcus faecalis*	*Enterococcus faecalis*	0.991	29.56	0.56	1.85	11.26	14.89	0.73	0.08	0.18							0.54
SA984	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	0.788	13.1		1.04	9.95	1.69	0.16		0.26							1.13
		Staphylococcus warneri	0.725	11.65	0.08	0.13	6.74	4.55	0.16									
MB984	*Fusobacterium nucleatum*	*Staphylococcus warneri*	0.989	19.11	0.13	0.88	10.24	6.5	1.18	0.18	0.02							2.03
SD720	*Morganella morganii*	*Morganella morganii*	0.912	30.42	0.23	1.85	1.59	23.81	-	2.85	0.02					0.07		4.57

Table 4. Organism detection among clinical samples. Unexpected detections of *Staphylococcus warneri* were confirmed via Illumina sequencing.

CONCLUSION & DISCUSSION

We have demonstrated how DNAe's BSI/AMR workflow and sequencing technology detect and identify a comprehensive panel of pathogens and resistance genes at the family, genus, species, and/or down to strain levels directly from whole-blood samples. This underpins the ability of the BSI/AMR assay as a single culture-independent assay offering quasi-universal bacterial and fungal pathogen detection directly from whole blood, making it uniquely positioned to address the limitations of current SoC and sequencing approaches for BSI detection in (suspected) sepsis patients.

Separately, DNAe has combined the proprietary technologies demonstrated here into the fully-automated LiDia-SEQ™ platform (Figure 5). This paradigm shift in the sequencing workflow will enable adoption of sequencing when and where it is needed most to reduce the time to pathogen identification and associated AMR detection.

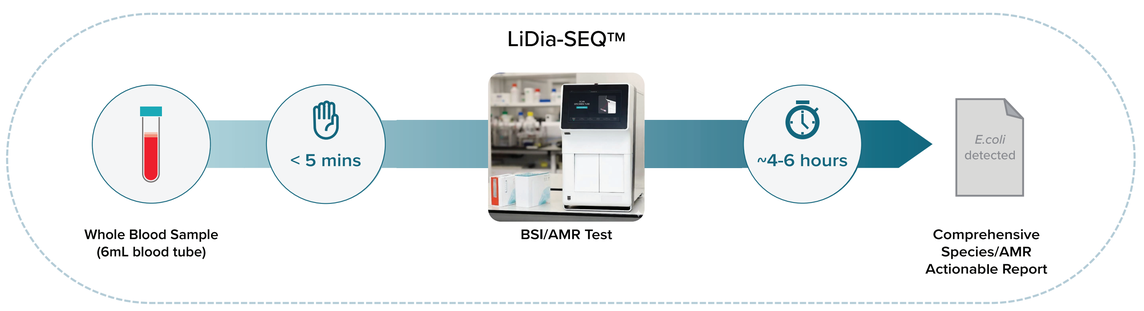

Figure 5. The LiDia-SEQ platform workflow

REFERENCES & ACKNOWLEDGEMENTS

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