Rapid detection of clinically-confirmed bloodstream pathogens in culture-negative specimens

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Background

Delays in the diagnosis of sepsis are common among hospitalised patients and are associated with increased odds of mortality. Rapid detection and identification of a pathogen would allow for more targeted therapy, potentially avoiding unnecessary administration of broad-spectrum antibiotics. We are presenting data generated with the technologies underlying a novel diagnostic test currently under development, LiDia® Bloodstream Infection Test (LiDia® BSI). LiDia® BSI identifies pathogens and key resistance markers, directly from whole blood.

Methods

LiDia® BSI comprises extraction of pathogen DNA from intact bacteria and fungi, removal of background matrix, nested multiplex PCR, and final identification by real-time amplification on a pH-sensing complementary metal–oxide–semiconductor (CMOS) chip (**Figure 1**). The CMOS technology is implemented in an integrated circuit (IC) that incorporates ion-sensitive field-effect transistors (ISFET) to detect the changes in pH associated with the release of hydrogen ions (H^+) during PCR.

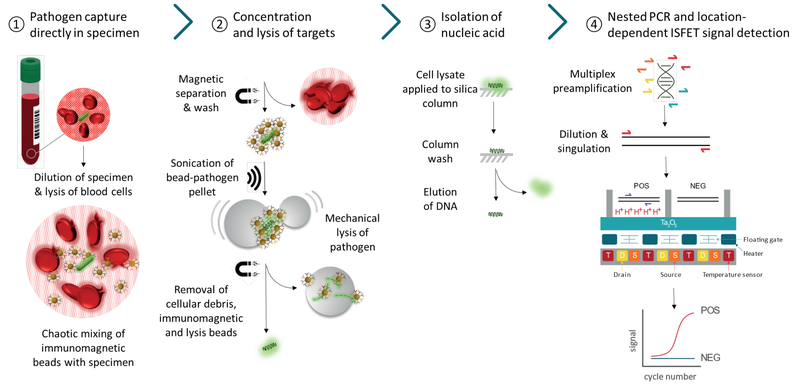


Figure 1: LiDia® BSI process. **1.** Targeted pathogen cells are captured directly in the specimen on magnetic beads coated with polyclonal antibodies. **2.** Bead-cell complexes are separated from background sample matrix (host cells, freely circulating nucleic acid, and other PCR inhibitors) with a magnetic wash step. Sonication is then used to emulsify the concentrated bead-cell complex, followed by the addition of ceramic beads for mechanical lysis. Ceramic lysis beads are separated from the cell lysate via filter, and immunomagnetic beads (and associated cellular debris) are removed via magnetic separation. **3.** The cell lysate is applied to a silica matrix under chaotropic conditions for immobilization of the nucleic acid. Contaminant proteins and salts are removed by a wash buffer and purified DNA is eluted. **4.** Purified pathogen nucleic acid is amplified in a multiplexed PCR reaction containing primers specific for multiple pathogen targets, followed by splitting of the multiplex mixture into separate reaction vessels, each with a specific nested PCR target assay. Detection of changes in pH associated with the release of hydrogen ions (H^+) during PCR provide a real-time readout of detection events.

Two sites, the Mayo Clinic (Rochester, MN, USA) and the University of New Mexico Health Sciences Center (Albuquerque, NM, USA), collected samples from consenting hospitalized adult patients with suspected or previously documented bloodstream infections. This study was IRB approved for both sites.

All patients enrolled in the study had received antimicrobial therapy within the 48 hours immediately prior to the DNAe study draw, and 35 of the 40 patients had a positive blood culture result 1-2 days prior to the DNAe study draw. Microbiology data for these prior blood cultures are summarized in **Figure 2.** Demographic and clinical data were collected in case report forms, summarized in **Table 1.**

At the time of the DNAe study draw, standard blood cultures (paired blood cultures) were collected first, followed by the DNAe study sample of whole blood in a sodium heparin Vacutainer® (1-10 ml each). Blood cultures were processed onsite, with positives identified by MALDI-TOF or biochemical methods. DNAe samples were shipped on cold packs to DNAe overnight and were processed with the semi-automated LiDia® BSI method to final ID PCR on the IC.

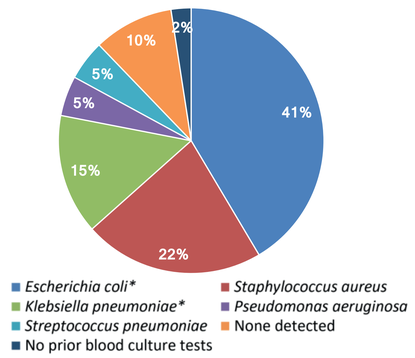
Results

Specimens from 40 patients were tested with the LiDia® BSI method and blood culture with an overall concordance of 78% (**Figure 3**). Three specimens were positive by both LiDia® BSI and paired blood culture for methicillin-resistant *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* (**Table 2**). Results of blood cultures drawn prior to the study samples are noted in the tables, along with a list of all antimicrobials administered to the patient within the 48 hours immediately before the study draw.

The LiDia® BSI method detected pathogens in an additional 9 samples (**Table 3**): *E. coli* (6), *K. pneumoniae*, *Pseudomonas aeruginosa*, and methicillin-resistant *S. aureus* (MRSA). All positive LiDia® BSI results were confirmed by comparison to blood culture results 1-2 days prior to the paired LiDia® BSI/blood culture draw. The 28 remaining samples were negative by paired blood culture and LiDia® BSI method (**data not shown**). Representative ISFET traces from positive LiDia® BSI results are shown in **Figure 4**. Time to result for LiDia® BSI was <5 hours and blood culture results were available 2-5 days after specimen collection.

Table 1: Demographic, clinical data

Demographic, Clinical Summary	Patients n	%
Median age, years	60	
Men	27	67.5
Women	13	32.5
Blood draw method		
Venipuncture	26	65
Central line	10	25
Arterial line	3	7.5
Not recorded	1	2.5
Hospital location		
MICU	10	25
Surgical	8	20
General medical	7	17.5
ED	5	12.5
Oncology	3	7.5
Cardiology	2	5
Neurology	1	2.5
Gastroenterology	1	2.5
Nephrology	1	2.5
Geriatrics/hospice	1	2.5
Trauma/Surgical ICU	1	2.5



**One patient has a polymicrobial infection with E.coli and K. pneumoniae*

Figure 2: Distribution of prior blood culture results for study patients (all drawn 1-2 days before paired BC/DNAe study samples)

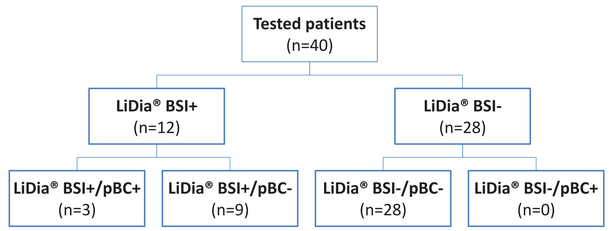


Figure 3: Performance of LiDia® BSI method compared to paired blood culture (pBC)

Table 2: LiDia® BSI method positive, paired blood culture positive samples

Sample ID	Prior blood culture result	Antimicrobials given prior to DNAe study draw	Paired blood culture result	LiDia® BSI method result
MAYO-072	*Escherichia coli*	Piperacillin/tazobactam	*Escherichia coli*	*Escherichia coli*
MAYO-085	*Klebsiella pneumoniae* complex	Piperacillin/tazobactam Vancomycin Ciprofloxacin	*Klebsiella pneumoniae* complex	*Klebsiella pneumoniae*
NMR091-1710	MRSA	Ceftriaxone Vancomycin	MRSA	*Staphylococcus aureus* (*mecA*+)

Table 3: LiDia® BSI method positive, paired blood culture negative samples

Sample ID	Prior blood culture result	Antimicrobials given prior to DNAe study draw	Paired blood culture result	LiDia® BSI method result
MAYO-068	*Klebsiella pneumoniae* complex	Piperacillin/tazobactam Cefepime Vancomycin Levofloxacin	Negative	*Klebsiella pneumoniae*
MAYO-071	*Escherichia coli*	Aztreonam Levofloxacin	Negative	*Escherichia coli*
MAYO-074	*Escherichia coli*	Piperacillin/tazobactam Cefepime Vancomycin Metronidazole	Negative	*Escherichia coli*
MAYO-084	*Escherichia coli*	Piperacillin/tazobactam Ciprofloxacin Levofloxacin Fluconazole	Negative	*Escherichia coli*
MAYO-086	*Escherichia coli*	Piperacillin/tazobactam	Negative	*Escherichia coli*
MAYO-091	*Escherichia coli*	Ertapenem Gentamicin Meropenem Sulfamethoxazole/ trimethoprim Vancomycin	Negative	*Escherichia coli*
NMR091-1688	*Escherichia coli*	Piperacillin/tazobactam Ciprofloxacin	Negative	*Escherichia coli*
NMR091-1699	MRSA	Vancomycin Bacitracin	Negative	*Staphylococcus aureus* (*mecA+*)
NMR091-1724	*Pseudomonas aeruginosa*	Cefepime Rifaximin	Negative	*Pseudomonas aeruginosa*

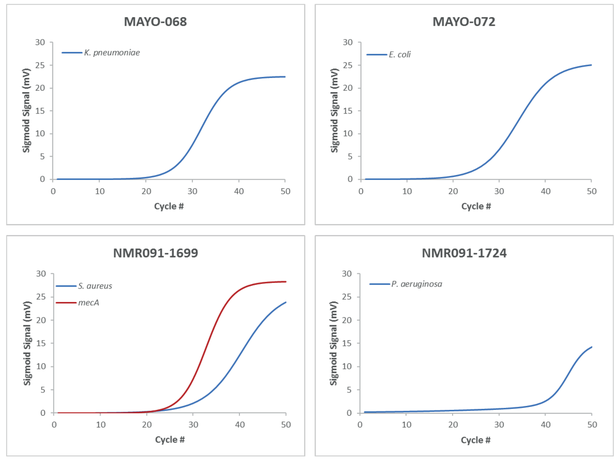


Figure 4: Examples of ISFET traces from positive samples: MAYO-068, MAYO-072, NMR091-1699, NMR091-1724.

Conclusions

This study demonstrated that the LiDia® BSI method can detect pathogens and *mecA* resistance in samples collected from patients receiving antibiotic therapy. Overall concordance with paired blood culture results was 78%. Additionally, given the time to detection by the LiDia® BSI method was significantly shorter compared to standard of care blood culture testing, these results suggest that LiDia® BSI holds promise for the early detection of septic patients, and may add value in cases where prior exposure antimicrobial therapy reduces the sensitivity of blood culture-based diagnosis.

Acknowledgements

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