Highly multiplexed molecular pathogen ID followed by phenotypic AST from whole blood using a novel fully automated system

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Background

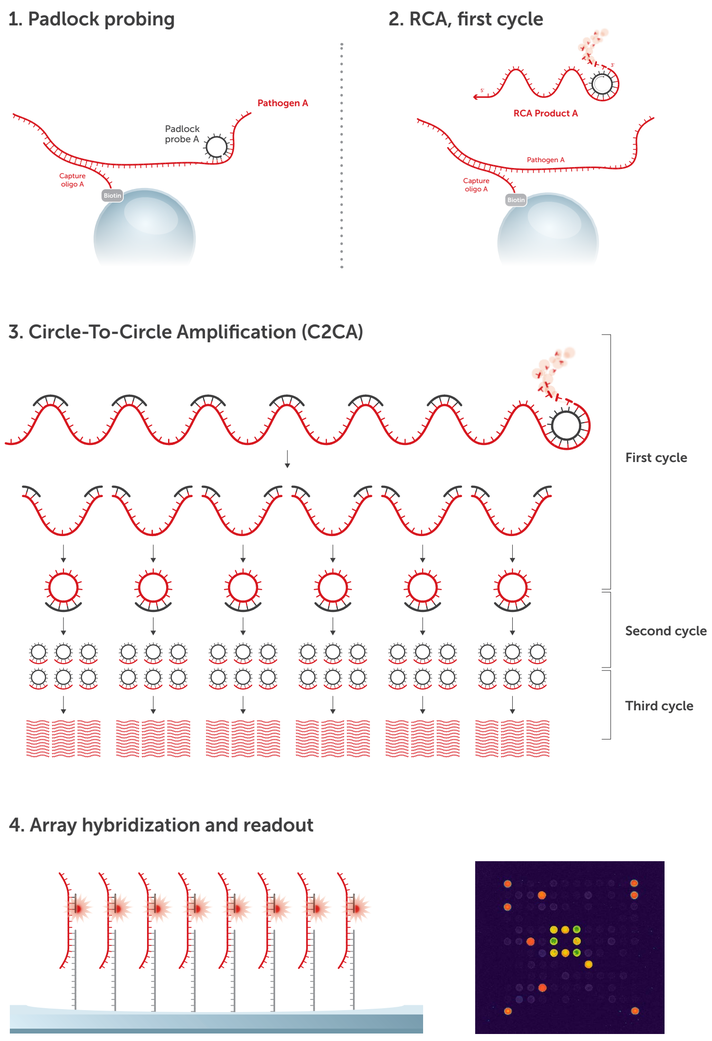
To address the need of more rapid diagnosis of potential pathogens causing sepsis or septic shock in patients, Q-linea is developing a fully automated, high-throughput diagnostic platform, ASTrID®. The future system will perform both molecular pathogen identification (ID) and phenotypic antibiotic susceptibility testing (AST), delivering ID after four hours and AST after an additional six, directly from patient without requirements for positive blood culture. The pathogen panel will cover 95% of relevant pathogens including 33 unique pathogens and 10 groups, as well as 11 resistance markers. The panel of antibiotic substances will contain 30 antibiotics and susceptibility will be reported as Minimum Inhibitory Concentration (MIC) values.

Materials and methods

In an ongoing study clinical blood samples are collected from patients suspected of having sepsis attending the Infectious Disease ER, University hospital of Örebro, Sweden. In addition to the standard 4 blood culture flasks drawn per patient for routine diagnostics, an extra, 5-10 ml, blood sample were taken for the present study. Pathogen ID and AST analysis was performed in a prototype ASTrID system. In this study, an initial enrichment step was included where the blood samples were incubated for 4 hours before start of analysis. Target identification was performed using a multiplexed nucleic acid amplification reaction. Highly specific and selective padlock probes forming circularized DNA strands (1,2) are amplified via rolling-circle amplification (RCA) (3) and subsequent circle-to-circle amplification (C2CA) (4). The resulting RCA products are labelled with fluorescence and detected on a microarray.

The proprietary growth-based AST analysis was performed on a subset of clinical isolates spiked in blood (inoculum consistent with 10 CFU/ml blood).

The Q-linea Molecular Engine



Results

Of the clinical samples collected, 33 samples identified as positive in normal clinical routine diagnostics were selected and are presented in this study. Pathogen identification direct from patient in ASTrID achieved sensitivity of 97% and specificity of 100%, compared to conventional analysis on positive blood culture flasks. When compared with broth microdilution, the ASTrID AST results showed 99% essential agreement and 99% categorical agreement. Overall, the results were highly correlated with traditional technologies and EUCAST guidelines.

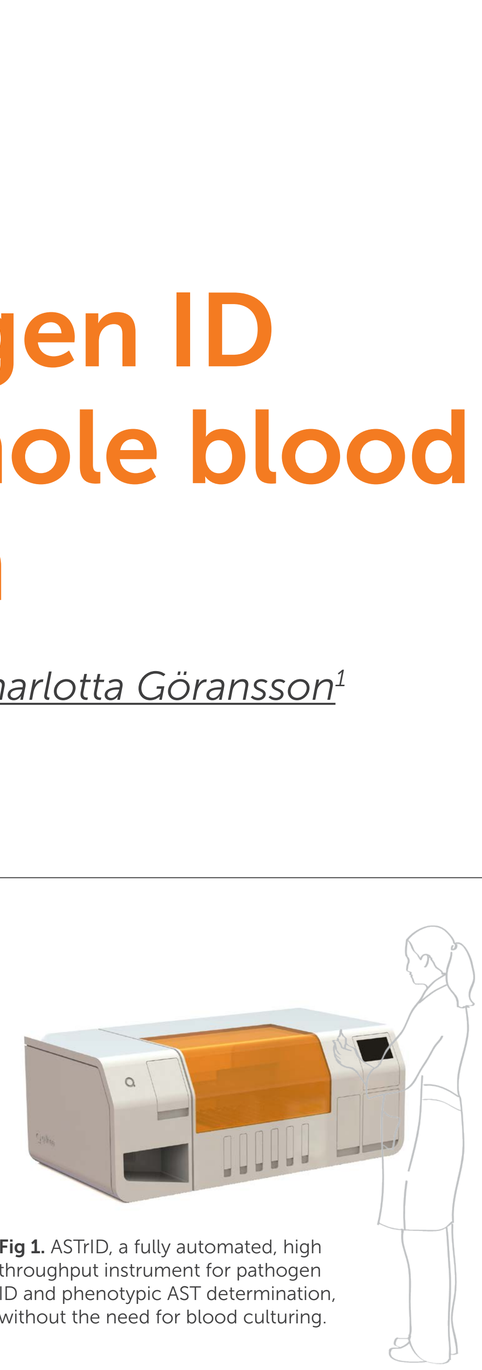


Fig 1. ASTrID, a fully automated, high throughput instrument for pathogen ID and phenotypic AST determination, without the need for blood culturing.

Table 1. Pathogen (*E. coli, S. aureus, S. pneumoniae, C. albicans*) identification direct from patient using ASTrID. ID results were confirmed using traditional methods.

	Tests (n)	Result	%	
True positive events (TP)	33	32	97.0	(Sensitivity)
False negative events (FN)	33	1	3.0	
False positive events (FP)	807	3	0.4	
True negative events (TN)	807	804	99.6	(Specificity)

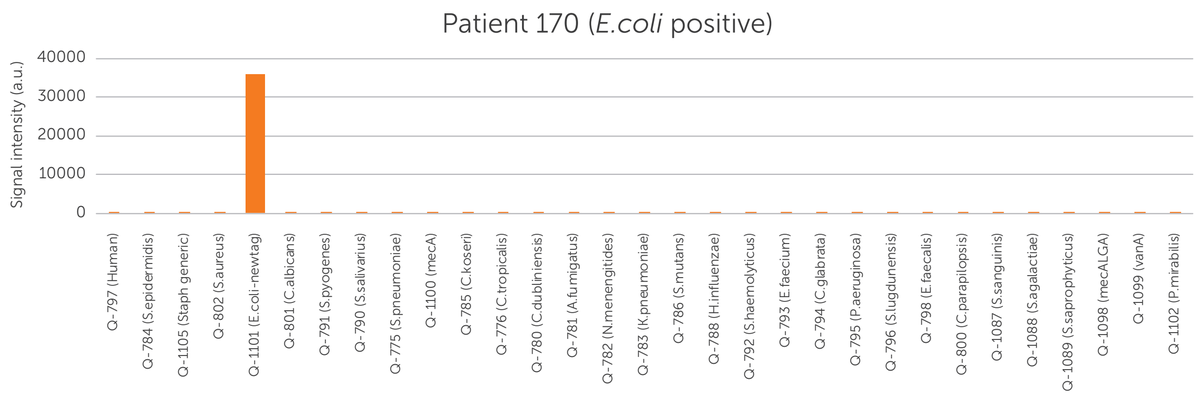


Fig 2. Signal response reported from a multiplexed assay.

Table 2. Phenotypic AST with MIC results in *E. coli* obtained using ASTrID within 12 hours from blood draw compared to MIC determination using broth microdilution according to ISO 20776.

	AST directly from blood, *E. coli*			
	Antimicrobial	Essential agreement (%)	Categorical agreement (%)	Errors
EUCAST	Cefotaxime	17/17 (100)	17/17 (100)	
	Ceftazidime	16/16 (100)	15/16 (94)	1 minor
	Ciprofloxacin	17/17 (100)	17/17 (100)	
	Gentamicin	16/17 (94)	17/17 (100)	
	Meropenem	16/16 (100)	16/16 (100)	

Essential agreement with broth microdilution. Categorical agreement according to EUCAST clinical breakpoints.

References

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Conclusion

With ASTrID, both pathogen identification and antibiotic susceptibility testing can be done within 10 hours. This dramatically shortens the time to adjusted treatment. The molecular pathogen identification method allows high multiplex, high sensitivity testing. The phenotypic antibiotic susceptibility profiling method developed reports reliable data directly from patient samples, without the need to wait for positive blood cultures. The new ASTrID platform from Q-linea has the potential to become a crucial tool against the global challenge of antibiotics resistance, helping to save lives and reduce healthcare costs.

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