Q-LINEA

A workflow for early and accurate treatment of sepsis

Today's workflow in clinical microbiology laboratories allows targeted treatment of sepsis first after 48–72 hours from blood draw. We present an alternative workflow based on parallel rapid identification using molecular tests or mass spectrometry and ASTar® for antimicrobial susceptibility testing of clinical blood cultures. The workflow was evaluated together with Uppsala University Hospital.

Introduction

To cope with a rapidly growing antimicrobial resistance, rapid diagnostic methods are needed. Currently, antimicrobial susceptibility testing (AST) for sepsis is completed at the earliest 48 hours after blood draw, although workflows may differ between laboratories. Due to this delay, clinicians therefore resort to empiric therapy during the first days of treatment. This blind, and potentially inappropriate, antimicrobial therapy not only drives the development of antimicrobial resistance, but also prolongs patient hospitalization and increases mortality (1, 2).

During recent years, techniques for rapid identification (ID) of bacteria have become available and are being adopted by microbiology laboratories. For bloodstream infections, rapid ID along with antimicrobial stewardship has shortened the time to optimal therapy (3, 4).

Treatment based on pathogen identity is, however, limited by potentially erroneous assumptions on its susceptibility to antimicrobials. For accurate and targeted treatment, not only rapid ID, but also rapid phenotypic AST, is needed. However, techniques for rapid, phenotypic AST to support the timely administration of appropriate antimicrobials are currently very limited.

ASTar – rapid AST results directly from clinical samples

ASTar is a new, automated system for rapid AST (Fig 1). The ASTar proprietary technology is based on broth microdilution (BMD) optimized for high sensitivity and short time-to-result, delivering phenotypic AST with true minimum inhibitory concentration (MIC) results within three to six hours. The first application is sepsis with AST directly from positive blood cultures.

1. Add positive blood culture

The sample preparation cartridge automatically isolates bacterial cells from the sample matrix and adjusts the concentration for a controlled inoculation to the AST disc.

2. Choose AST disc

Q-linea's unique proprietary technology – the AST disc – allows automated time-lapse imaging of bacterial population growth in wells containing different concentrations of antimicrobial agents.

3. Scan and load – tap START RUN

Proprietary algorithms translate visual information into MIC values. Based on EUCAST or CLSI breakpoints, MIC values are interpreted as S, I, or R.

Fig 1. Description of the automated ASTar process.

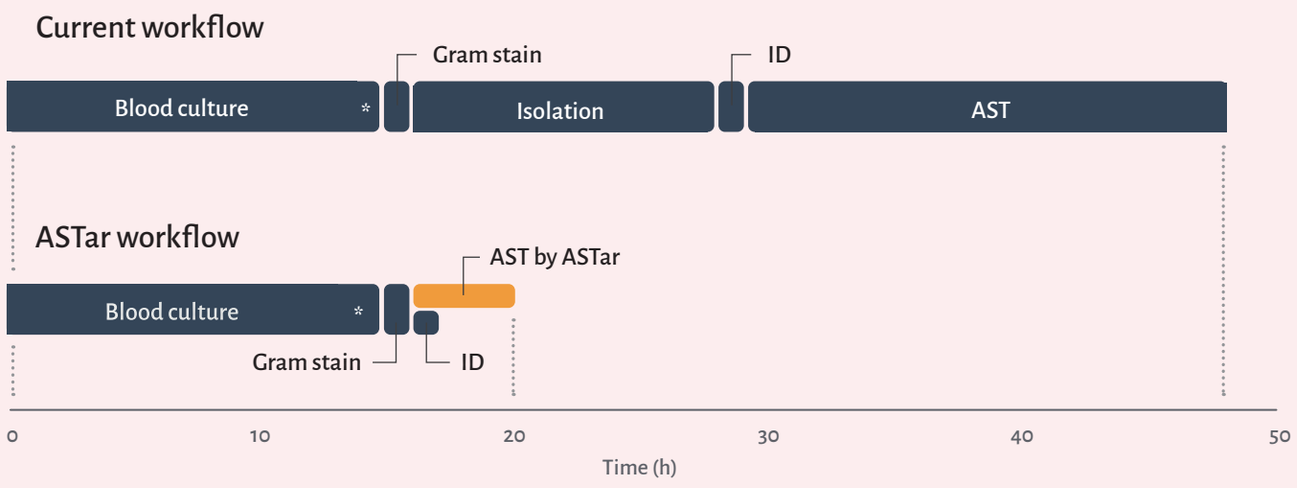

Fig 2. ASTar workflow shortens time to optimized therapy by more than 29 hours compared to current diagnostics. Time for rapid ID may differ depending on chosen method. AST by ASTar can be started independently of ID, which is not needed until creating the final MIC report. Workflows at different clinical microbiology laboratories may differ. A median time to positivity of 14 h was used, based on clinical data from hospitals.
* = positive blood culture.

ASTar workflow

Current workflows for infectious diagnosis of blood cultures in clinical microbiology laboratories are based on sub-culturing microbes from positive blood cultures followed by identification by, for example, semi-automated systems, molecular methods or MALDI-TOF mass spectrometry (MS). Subsequent susceptibility testing is done by overnight disc diffusion or semi-automated systems. In practice, this means that information to support both guided treatment and de-escalation of empirical broad-spectrum antimicrobials is achieved at the earliest by day three. To be able to optimize therapy as soon as possible, both rapid ID and AST are crucial.

We present a workflow (Fig 2) that combines rapid ID with ASTar for rapid AST directly from positive clinical blood cultures. The rapid ID technique can, for instance, be any of the molecular techniques or MS run from prepared blood culture samples (for instance MALDI Sepsityper®, Bruker) or from colonies after short plate culture. The potential of the workflow was evaluated in collaboration with Uppsala University Hospital (UUH).

Evaluation of a prototype ASTar workflow

Clinical samples from patients with suspected bacteremia were collected in aerobic, anaerobic or pediatric BacT/ALERT Plus® (bioMérieux) blood culture bottles and cultured in a BacT/ALERT VIRTUO® system (bioMérieux). Aliquots of positive blood cultures were acquired and directly plated and cultured on agar plates. After incubation for between four and six hours, cultures were identified with Microflex™ LT MALDI-TOF System (Bruker) according to UUH routine procedures. Aliquots from the positive cultures were also taken for AST and assayed directly with ASTar prototype. Reference AST was performed from isolates using BMD.

Rapid MS identification

MS identification scores were above 1.9 for all samples for both gram-positive and gram-negative bacteria and above 2.0 for 23 of 26 samples (Table 1). One sample was excluded from AST, since data from MS of short-term culture indicated sample to be polymicrobial which could not be analyzed in the prototype version of ASTar. Three samples were excluded due to technical failures.

Table 1. Identification of pathogens by MALDI-TOF MS after short-term culture.

	Species	Score		Species	Score
1	*S. epidermidis*	1.98	14	*H. influenzae*	2.19
2	*S. aureus*	2.39	15	*P. mirabilis*	2.31
3	*E. coli*	2.20	16	*S. epidermidis*	1.97
4	*K. pneumoniae*	2.49	17	*S. epidermidis*	1.91
5	*P. aeruginosa*	2.21	18	*S. aureus*	2.38
6	*E. coli*	2.41	19	*E. cloacae* complex	2.15
7	*E. cloacae* complex	2.20	20	*E. coli*	2.23
8	*S. epidermidis*	2.15	21	*S. aureus*	2.49
9	*S. epidermidis*	2.05	22	*S. aureus*	2.43
10	*E. coli*	2.42	23	*S. aureus*†	2.16
11	*S. aureus*	2.36	24	*P. aeruginosa*	2.32
12	*S. aureus*	2.05	25	*C. koseri*	2.47
13	*S. epidermidis**	2.03	26	*P. mirabilis**	2.42

* Multiple species identified in the sample after overnight cultivation.
† This sample contained cultured synovial fluid instead of blood.

Table 2. ASTar susceptibility data from clinically-positive blood cultures, and one synovial fluid sample.

				No. of tests					
Antimicrobial agent	**Total no. of tests**	**EA (%)**	**CA (%)**	**S**	**I**	**R**	**mE**	**ME**	**VME**
Amoxicillin-clavulanic acid	11	11 (100%)	11 (100%)	9		2			
Benzylpenicillin	4	3 (75%)	4 (100%)			4			
Piperacillin-tazobactam	12	12 (100%)	12 (100%)	10		2			
Cefotaxime	11	10 (91%)	11 (100%)	10		1			
Cefoxitin screen	4	-	4 (100%)	4					
Ceftazidime	12	10 (83%)	10 (83%)	11		1	1	1	
Ceftolozane-tazobactam	12	12 (100%)	12 (100%)	11		1			
Meropenem	13	11 (85%)	13 (100%)	12		1			
Ciprofloxacin	13	13 (100%)	12 (92%)	12		1	1		
Levofloxacin	9	8 (89%)	9 (100%)	6		3			
Gentamicin	12	11 (92%)	12 (100%)	11		1			
Tobramycin	12	10 (83%)	12 (100%)	12					
Vancomycin	9	9 (100%)	9 (100%)	9					
Erythromycin	9	9 (100%)	9 (100%)	4		5			
Clindamycin	9	8 (89%)	8 (89%)	5		4	1		
Tetracycline	9	8 (89%)	7 (78%)	6	2	1	1	1	
Tigecycline	9	7 (78%)	9 (100%)	9					
Colistin	11	11 (100%)	11 (100%)	9		2			
Daptomycin	9	9 (100%)	9 (100%)	9					
Trimethoprim-sulfamethoxazole	9	7 (78%)	8 (89%)	7	2		1		
Total	199	91.8%	96.5%						

mE = minor error, ME = major error, VME = very major error

Rapid AST using ASTar

Rapid AST with ASTar was run from clinical positive blood cultures. The different blood cultures contained *H. influenzae*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *E. cloacae* complex, *P. mirabilis*, *S. aureus*, and *S. epidermidis* reported by MS. In total, 199 data points were included yielding an overall Essential Agreement (EA) of 91.8% and a Categorical Agreement (CA) of 96.5% compared to reference BMD MIC (Table 2). In total, 2 major errors and 5 minor errors were observed. No very major errors were identified. Two samples that were interpreted to contain a single pathogen by MS, with scores > 2.0, showed multiple pathogens after overnight incubation on plates. However, ASTar MIC results matched reference BMD for the species reported as monomicrobial by short culture MS.

Conclusion

We evaluated a workflow for rapid ID and AST directly from positive blood cultures using a prototype AST-system for direct testing together with short-term plate incubation and MS analysis as used in routine diagnostics at UUH. The rapid workflow generated AST data that were in good concordance with reference BMD, showing an EA of 91.8% and a CA of 96.5%.

ASTar can be started independently of ID, which is entered before, during or after the AST run to create the final MIC report. AST is initiated directly from a positive blood culture, which means that the total time to targeted treatment is shortened by more than 29 hours compared with current practice. The fully-automated AST solution not only reduces hands-on time to a few minutes, but also improves data quality and, thanks to the comprehensive AST panel, reduces the need for follow-up testing. Faster diagnosis helps clinicians quickly optimize individual antimicrobial treatment. This will help save lives, reduces patient suffering and cut the overuse of broad-spectrum antibiotics.

References

1. Kumar *et al.*, Crit Care Med 34:1589-96, 2006
2. Perez *et al.*, Arch Pathol Lan Med 137:1247-1254, 2012
3. Huang *et al.*, Clin Infect Dis 57:1237–45, 2013
4. Malcolmson *et al.*, J Pediatric Infect Dis Soc 6:178–86, 2017

Save lifetimes

Q-linea is an innovative research, development and manufacturing company that primarily develops instruments and consumables for rapid and reliable infection diagnostics. Q-linea's vision is to help save lives by ensuring antibiotics continue to be an effective treatment for future generations.

Q-linea was founded in Uppsala, Sweden, in 2008 by scientists from the Rudbeck Laboratory at Uppsala University together with Olink AB and the Uppsala University holding company UUAB. We are an interdisciplinary, experienced and highly motivated team with state-of-the-art development and manufacturing facilities in the center of Uppsala Science Park. Q-linea is a publicly-listed company developing inventive systems for in vitro diagnostics for infectious diseases.

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