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Rapid Antimicrobial Susceptibility Testing Directly From Positive Blood Cultures: A Pilot Study on ASTar System (Q-Linea)

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Jian R Bao,[1] Vittal P Ponraj,[1] Robert S Jones,[1] Michelle C Myers,[2] and Kileen L Shier[1]
[1] Quest Diagnostics, Chantilly, VA
[2] Q-Linea, Uppsala, Sweden

Quest Diagnostics
Correspondence: Kileen L Shier
Kileen.L.Shier@QuestDiagnostics.com

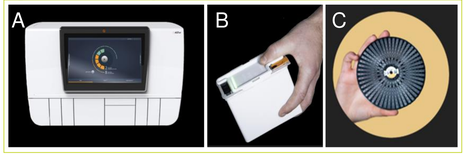
Introduction

- Empirical antibiotic therapy is increasingly risky due to growing antibiotic resistance among infectious bacteria, especially Gram-negative pathogens.
- Rapid antimicrobial susceptibility testing (rAST) enables prompt and precise antimicrobial therapy for deadly infections, such as bacteremia.[1,2] Selection of effective therapy following rAST can save lives and reduce healthcare costs.
- ASTar® (Q-Linea, Uppsala, Sweden) is a rAST system with a simple one-step automated assay that directly uses positive blood cultures with no sample pre-processing required.
- We evaluated the ASTar system's performance on blood cultures that were positive for targeted Gram-negative bacteria.

Methods

- The ASTar system (**Figure 1**), using the investigation use only (IUO) BC G- Kit (Q-Linea), was used to target 14 Gram-negative bacteria (13 non-fastidious).
- The IUO panel disk comprised 23 antimicrobials across 9 drug classes, 18 of which are FDA-cleared (**Table 1**).
- Positive blood samples were from either patient blood culture or spiked blood bottles. Patient samples were evaluated within 16 hours post positive on a blood culture system (BD BACTEC™). Bacterial IDs were initially screened by BioFire® BCID2 panel (bioMerieux) and finalized with MALDI-TOF method (Bruker). Spiked samples were prepared from negative blood culture bottles seeded with bacterial cells ($1.5x10^4$ cells/bottle) and incubated overnight.
- The minimum inhibitory concentration (MIC) was determined by inoculating a cartridge with 0.8 to 1.2 mL unprocessed positive blood culture media and loading the cartridge and drug panel disk into ASTar.
- Comparator MICs were obtained from Vitek 2 (N802 and XN15 cards, bioMerieux). MIC discrepancies from comparison of ASTar with Vitek 2 were re-evaluated on a second Vitek 2 test.

Figure 1. The ASTar System



The ASTar consisted of a single benchtop automation instrument (A) running the IUO BC G− Kit that contained a consumable cartridge (B), frozen insert (single packed reagent), and an IUO drug panel disk (C). Reproduced with permission from Q-Linea.

Methods (continued)

- The MIC data were evaluated for essential agreement (EA), categorical agreement (CA), minor error (MI), major error (ME), and very major error (VME).[3]

Table 1. Antibiotics in the BC G-Kit Panel (*FDA-cleared)

Beta-lactam antibiotics (n=16)		Non-beta-lactam antibiotics (n=7)
Ampicillin*	Cefotaxime	Gentamicin*
Ampicillin-sulbactam*	Ceftriaxone	Tobramycin*
Ceftolozane-tazobactam	Cefoxitin	Amikacin*
Ceftazidime-avibactam*	Cefuroxime*	Tigecycline*
Meropenem-vaborbactam*	Ceftazidime*	Ciprofloxacin*
Piperacillin-tazobactam*	Aztreonam*	Levofloxacin*
Cefazolin*	Ertapenem	Trimethoprim-sulfamethoxazole (TMP-Sulfa)*
Cefepime*	Meropenem*	

Results

- A total of 85 blood samples (patient, n=56; spiked, n=29) were evaluated on the ASTar system for MICs.
- All 14 Gram-negative bacteria evaluated by the ASTar were detected in patient or spiked blood samples (**Table 2**).

Table 2. Number of Samples Positive for Gram-Negative Bacterial Species Evaluated by the IUO BC G− Kit

Bacterial species	Patient samples (n=56)	Spiked samples (n=29)
Acinetobacter baumannii	2	2
Citrobacter freundii	0	2
Citrobacter koseri	1	2
Enterobacter cloacae complex[a]	0	2
Escherichia coli	34	3
Haemophilus influenzae	0	2
Klebsiella aerogenes (*Enterobacter aerogenes*)	1	2
Klebsiella oxytoca	1	2
Klebsiella pneumoniae	9	3
Morganella morganii	1	2
Proteus mirabilis	3	3
Proteus vulgaris	1	1
Pseudomonas aeruginosa	1	1
Serratia marcescens	2	2

[a] *E cloacae* complex refers to *E. cloacae*, *E. hormachei* and *E. asburiae*.

Results (continued)

- Overall, the ASTar achieved an EA of 91.9% and a CA of 92.5%, with errors classified as very major (5.7%), major (1.5%) and minor (5.0%) (**Table 3**).
- In general, ASTar showed better performance on non-beta-lactam antibiotics than for beta-lactam antibiotics, resulting in fewer categorical errors (**Figure 2**).
- The ASTar correctly resulted in the class-A extended spectrum beta-lactamase (ESBL) resistance profiles from 8 bacteria (6 *E coli*, 1 each of *Proteus* sp. and *K pneumoniae* group). The bacteria had the ESBL-encoded CTX-M gene detected on the BCID-2 panel.
- ASTar underperformed on organisms resistant to the 2 carbapenems in spiked samples: ertapenem (58.3% EA with 33.3% VME, a non-FDA-cleared combination) and meropenem (62.5% EA with 35.7% VME, both FDA-cleared & non-cleared).
- The average ASTar run time for rAST from uploading to resulting was 6 hours 15 minutes, representing substantial time savings compared to standard workflows (**Figure 3**).
- Hands-on time for a single sample was 9.5 minutes, including blood bottle handling, labeling, and culture media transferring to uploading.

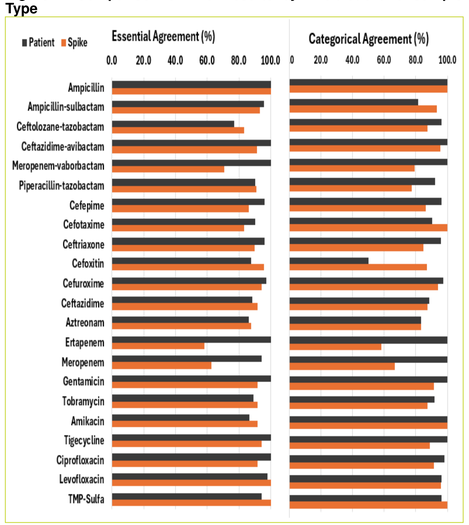
Table 3. Comparison of MIC Results

				Categorical evaluation and target rate (%)			
Sample/antibiotic type[a,b]	Total,[c] n	ED, n	EA,[d] % (≥90)	CA (≥90)	VME (<1.5)	ME (<3)	MI (<10)
Patient blood							
Beta-lactam	639	44	93.1	93.6	5.3	1.3	4.5
Non-beta-lactam	338	15	95.6	97.6	2.0	0.4	1.8
Spiked blood							
Beta-lactam	327	51	84.4	84.4	8.3	5.6	8.9
Non-beta-lactam	162	9	94.4	93.8	1.7	0.0	5.6
Overall							
Beta-lactam	966	95	90.2	90.5	7.3	2.2	6.0
Non-beta-lactam	500	24	95.2	96.4	1.9	0.3	3.0
Total	1466	119	91.9	92.5	5.7	1.5	5.0
Range (%)			58.3-100	50-100			

CA, categorical agreement; EA, essential agreement; ED, essential disagreement; ME, major error; MI, minor error; MIC, minimum inhibitory concentration; rAST, rapid antimicrobial susceptibility testing; VME, very major error.
[a] Antibiotics are 16 beta-lactam drugs and 7 non-beta-lactam drugs (see Table 2 for details).
[b] Cefazolin had no data from patient blood samples.
[c] Calculated from total isolates tested with drug numbers.
[d] ± a 2-fold dilution.

Results (continued)

Figure 2. Comparison of MIC Results by Antibiotic and Sample Type



Essential (left) and categorical (right) agreements from either positive patient samples (blue) or spiked blood samples (orange). Cefazolin was excluded here due to insufficient data. MIC, minimum inhibitory concentration.

Conclusions

- The ASTar system provides automated, acceptable phenotypic MIC results directly from unprocessed positive blood culture medium in about 6 hours, with less than 10 minutes of hands-on procedures required.
- The application of ASTar in clinical laboratories may benefit critical patient care and laboratory operations.

References

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2. Berinson B, Davies E, Torpner J, et al. A multicenter evaluation of a novel microfluidic rapid AST assay for Gram-negative bloodstream infections. *J Clin Microbiol.* 2024;62(10):e0045824. doi:10.1128/ jcm.00458-24
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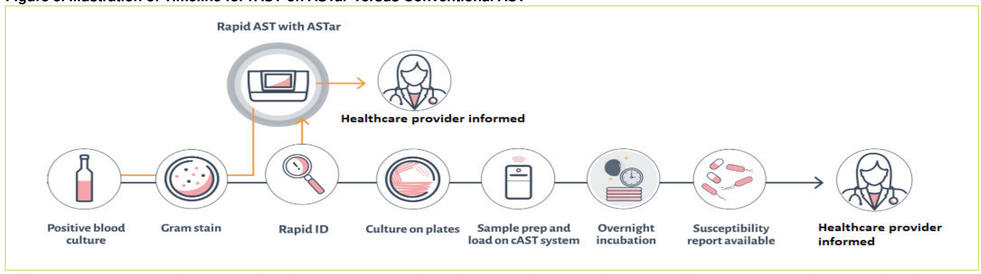
Disclosure

JR Bao, VP Ponraj, RS Jones, and KL Shier are employees of and may own stock in Quest Diagnostics.

Figure 3. Illustration of Timeline for rAST on ASTar Versus Conventional AST



ASTar can be run in parallel with any rapid ID-solution and delivers results directly from positive blood cultures in about 6 hours. According to the manufacturer, turnaround for standard workflows is about 18 hours or longer depending on specific laboratory practices. Modified with permission from Q-Linea.