Performance of pathogen identification and genotypic expression tests using ASTar® remnant bacterial suspension in Gram-negative contrived positive blood cultures

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Background

ASTar provides phenotypic AST results from Gram negative positive blood cultures in approximately six hours. After approximately one hour of instrument run time, a remnant bacterial suspension may be retrieved from the cartridge for other downstream uses. In this research use only (RUO) evaluation, we tested the performance of the ASTar remnant suspension for lateral flow assays (LFA) and rapid MALDI for identifying genotypic resistance and pathogen identification with well-characterized G- isolates.

Rapid MALDI identification from PBCs combined with targeted LFA may provide a cost-effective alternative to molecular multiplex panels. This evaluated approach has potential to build on existing laboratory infrastructure, reduce per-test and maintenance costs, and limit unnecessary broad testing.

Materials and methods

- Gram-negative strains with available genotypic reference results (CDC[1], ARLG[2], ATCC[3]) were contrived into blood culture bottles. After BC positivity, the samples were loaded onto the ASTar system.
- Remnant bacterial suspensions from the ASTar cartridge were tested (RUO) using NG TEST® CTX M Multi, NG TEST® CARBA 5 (NG Biotech, France), and rapid MALDI-TOF MS identification.
- For each assay, 600 µl of remnant suspension was centrifuged for 3 min at 12,000 g (Figure 1).
- For MALDI-TOF MS, the pellet was spotted onto the MALDI target plate, MALDI matrix was added, and identification was performed using the Bruker Biotyper MALDI system.
- For LFA testing, the pellet was resuspended in 150 µl of kit provided extraction buffer; 100 µl was applied to each test cassette and incubated for 15 min prior to interpretation.

Conclusions

- Remnant suspension from ASTar enabled high performance pathogen ID and genotypic testing.
- Results were delivered approximately 2 hours after start of ASTar runs if run prior to final ASTar MIC/phenotype results.
- This RUO use of the ASTar remnant suspension demonstrated future potential to reduce costs relative to automated multiplex platforms when reflex LFA testing done based on ASTar MIC/phenotype results.

References

1. CDC & FDA Antimicrobial Resistance Isolate Bank: https://wwwn.cdc.gov/arisolatebank/
2. Antibacterial Resistance Leadership Group (ARLG) Biorepository: https://arlg.org/laboratory-center-strain-access/
3. American Type Culture Collection (ATCC): https://www.atcc.org

Results

Workflow

Less than 10 minutes is required to prepare a MALDI run and Lateral flow assay (LFA) run from ASTar cartridge sample remnant.

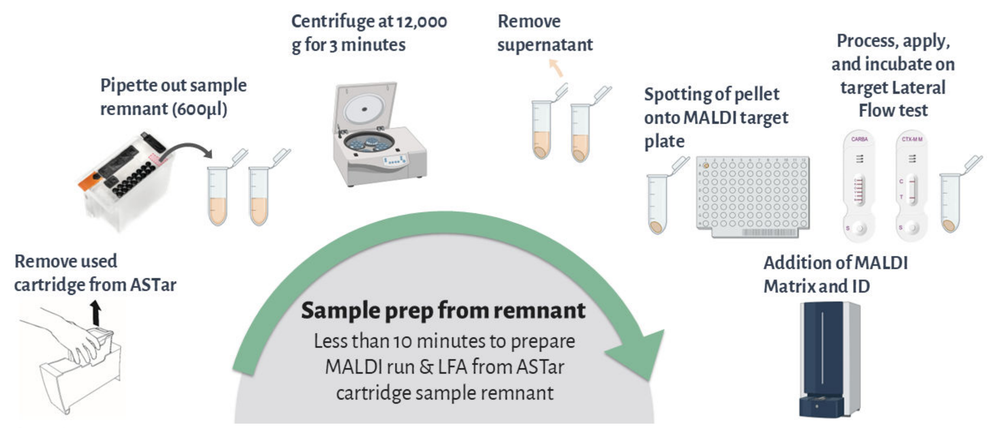

Figure 1: Sample prep workflow from remnant suspension output.

MALDI scoring & LFA reference comparison

Mean (± SD) remnant suspension was 2722 µl (± 300 µl) across 119 Gram-negative samples run on ASTar, and the following Gram-negative species or species groups were identified by rapid MALDI:

- 98 (82%) *Enterobacterales* (ENT)
- 18 (15%) *P. aeruginosa* (PsA)/*A. baumannii*
- 3 (3%) *S. maltophilia*

All 119 samples yielded final high-confidence ID scores >2.0 (Fig. 2), including five samples which initially scored <2.0 and re-scored high-confidence on re-test.

LFA results showed full agreement with reference blaCTX-M positive (30/30) and all carbapenemase positive types except for one reference OXA-48 positive isolate (60/61) (Table 1).

A subset of samples (n=26. 23 *Enterobacterales*, 3 *P. aeruginosa*) were stored refrigerated for 6 h after retrieval of the cartridge before performing the LFA assay. Results were identical to samples processed directly after cartridge retrieval.

Similar results were obtained by the recently available RUO Acineto-5 assay, also on samples stored for 6h after remnant retrieval (9/9) (table 2). This opens the possibility of making rapid reflex LFA testing for resistance markers based on ASTar results.

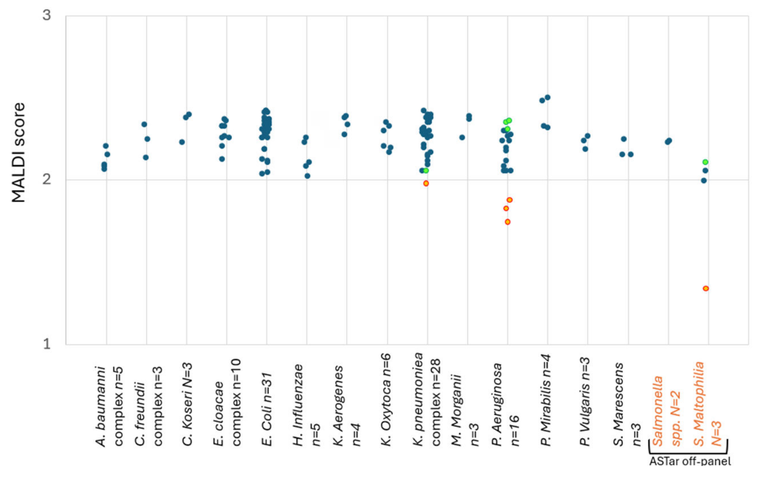

Figure 2. MALDI scoring for 119 samples prepared from remnant ASTar output. Orange dots: initial score <2.0; Green dots score of initially orange dots after re-run, see methods.

Cost savings using ASTar remnant bacterial sample

When syndromic molecular testing is not available, the incremental cost for MALDI and reflex LFA ranges from $1.39 to $4.15 for ENT and from $4.60 to $17.00 for PSA/ACB (Table 4).

When syndromic molecular testing is available, the incremental cost savings for MALDI and reflex LFA ranges from $68.62 to $115.85 for ENT and from $65.40 to $103.00 for PSA/ACB (Table 4).

Table 3. Cost assumptions.

Cost Assumptions		
Item	**Low**	**High**
Syndromic molecular Cost	$70.00	$120.00
MALDI Cost	$1.00	$1.00
LFA Carba 5 Cost	$30.00	$40.00
LFA CTX-M Cost	$25.00	$30.00

Table 1. Comparison of LFA testing using ASTar remnant suspension (research use only) in genotypic confirmed isolates.

Resistance mechanism	LFA results ASTar Sample Prep, n^	Expected resistance mechanisms (Reference, N)	% Concordance (n/N)
CTX-M Multi Enterobacterales	**30**	**30**	**100%**
CTX-M Group 1	13	13	100%
CTX-M Group 2	1	1	100%
CTX-M Group 9	4	4	100%
Negative CTX-M	12	12	100%
CARBA-5 Enterobacterales	**51**	**52**	**98%**
KPC	17	17	100%
NDM	11	11	100%
VIM	3	3	100%
IMP	4	4	100%
OXA-48	4*	5	80%
Negative carba	12	12	100%
CARBA-5 *P. aeruginosa*	**9**	**9**	**100%**
KPC	1	1	100%
VIM	2	2	100%
IMP	3	3	100%
Negative carba	3	3	100%

*1 isolate initially identified as OXA-48 negative was OXA positive on rerun
^ 26 isolates were stored refrigerated and tested 6 hrs after retrieval from cartridge with same results

Table 2. Comparison of LFA testing using ASTar remnant suspension stored defrigerated for 6h (RUO) on genotypic confirmed *A.baumannii* isolates.

Resistance mechanism	LFA results ASTar Sample Prep, n	Expected resistance mechanisms (Reference, N)	% Concordance (n/N)
ACINETO-5 *A.baumannii*	**9**	**9**	**100%**
NDM	2	2	100%
OXA-24	4	4	100%
OXA-58	1	1	100%
Negative ACINETO-5	2	2	100%

Table 4. Cost savings by system and system combination^.

AMR Range (Carb NS/ESBL + ENT)			
	Low	**Med**	**High**
Suggested Carb NS ENT (%)	0.7%	2.0%	4.5%
Suggested ESBL (%)	5.0%	12.0%	20.0%
Molecular Low Cost	$ 70.00	$70.00	$70.00
Molecular High Cost	$120.00	$120.00	$120.00
Cost of Carb NS ENT (Carba 5) + ESBL + (CTX-M)			
MALDI+Reflex LFA w/o Syndromic Molecular (Low) Low Cost [Carba 5 + CTX-M]	$ 1.39	$ 2.10	$ 3.48
ASTar Rapid MALDI + Reflex LFA High Cost [Carba 5 + CTX-M]	$ 1.49	$ 2.40	$ 4.15
Cost Savings Over Syndromic Molecular (Low)	$ 68.62	$ 67.90	$ 66.53
Cost Savings Over Syndromic Molecular (High)	$ 118.51	$ 117.60	$ 115.85
Cost of Carb NS PSA/ACB (Carba 5)			
Suggested Carb NS PSA-ACB (%)	12%	20%	40%
MALDI+Reflex LFA w/o Syndromic Molecular (Low) [Carba 5]	$ 4.60	$ 7.00	$ 13.00
MALDI+Reflex LFA w/o Syndromic Molecular (High) [Carba 5]	$ 5.80	$ 9.00	$ 17.00
Cost Savings Over Syndromic Molecular (Low)	$ 65.40	$ 63.00	$ 57.00
Cost Savings Over Syndromic Molecular (High)	$ 114.20	$ 111.00	$ 103.00

* *ASTar cost is not included since is constant; ^ This experimental workflow is outside the current claims for the ASTar BC G- kit but represents future potenital use.*

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