Electric field-mediated isolation of bacteria from positive blood cultures enables rapid AST for hard-to-isolate species and improves test performance overall

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Background

The QuickMIC® system rapidly analyses AST of bacteria directly from positive blood cultures (PBCs). However, coagulase-positive staphylococci clusters with blood cells resulting in low bacterial yield during isolation, creating challenges for downstream rapid antibiotic susceptibility testing (rAST). Other difficult species include *P. aeruginosa*, especially with filter-based PBC preparation. Cellectric has developed a direct, filter-free electric field-mediated sample preparation technology for removing non-bacterial components efficiently while preserving pathogen integrity and viability (Figure 1). In this study, the combination of Cellectric Biosciences' sample preparation technology with the QuickMIC rAST technology was evaluated.

Methods

Horse-blood was spiked with bacteria at concentrations of 1-50 CFU/ml and incubated in BD BACTEC™ Plus Aerobic bottles until positivity. The PBC samples were processed using Cellectric Biosciences' prototype device. 10µl (Gram-) and 1ml (Gram+) of the lysate was collected to run the QuickMIC GN cassette in the QuickMIC system. Performance with lysates in QuickMIC was evaluated by comparing inoculum, unusable imaging area, MICs and time-to-result between the workflows using Cellectric's sample preparation and the QuickMIC standard filtering procedure.

Results

A panel of 11 Gram-positive and 14 Gram-negative isolates were tested (Table 1). The Cellectric pretreatment overcame the limitation of Gram-positive detection in QuickMIC by yielding a viable inoculum above the instrument's threshold (Figure 2A). The removal of blood cells was compared by analysing the unusable area in the QuickMIC chambers where the Cellectric preparation notably had less background and unusable area, which translates to overall higher test yield (Figure 2B and C). The TTR was not affected by the lysing preparation (3h 21 min compared to 3h 16 min for QuickMIC standard preparation). Categorical (CA) and essential agreement (EA) were 91.3% and 82.2%, respectively.

Conclusions

- Gram- bacteria
- Gram+ bacteria
- Inoculum in EUCAST range
- Reduced background area
- TTR not influenced

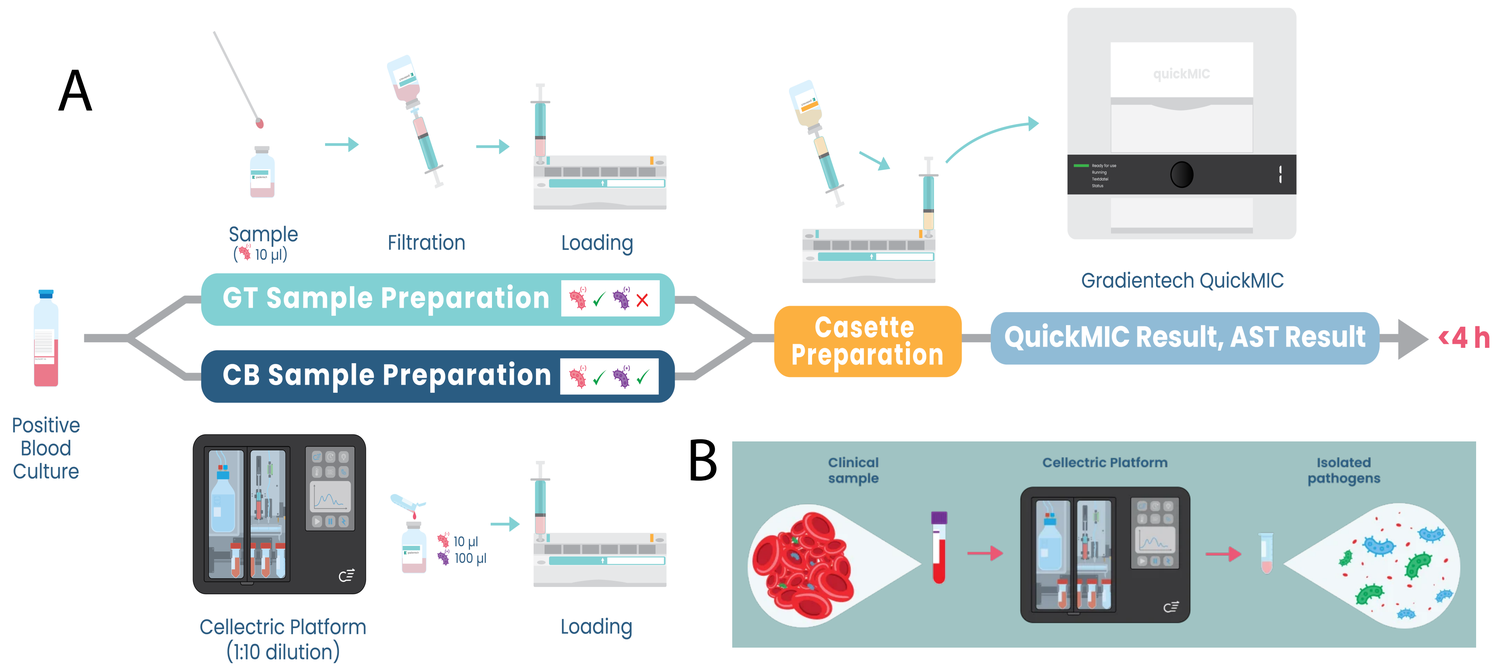

Figure 1 A) *Overview of the Cellectric (CB) and standard QuickMIC (GT) workflows.* ***B)*** *The Cellectric system isolates bacteria from clinical samples using a filter-free electric field-mediated method.*

Species	Strains	Datapoints	S	I	R	EA %	CA %	MiD %	MD %	VMD %
E. cloacae	2	21	16	2	3	81.0	100.0	0.0	0.0	0.0
E. coli	5	96	57	3	36	85.4	89.6	3.1	4.2	3.1
E. faecalis	2	9	5	3	1	88.9	88.9	0.0	11.1	0.0
E. faecium	1	1	1	0	0	100.0	100.0	0.0	0.0	0.0
K. oxytoca	1	11	11	0	0	54.5	100.0	0.0	0.0	0.0
K. pneumoniae	2	21	14	0	7	90.5	95.0	0.0	0.0	4.8
P. aeruginosa	4	25	13	9	3	88.0	85.8	8.0	0.0	4.0
S. aureus	8	35	20	6	9	71.4	89.4	0.0	11.4	0.0
Total	**25**	**219**	**137**	**23**	**59**	**82.2**	**91.3**	**2.3**	**4.1**	**2.3**

Table 1: *Overview of the QuickMIC performance when using the Cellectric Biosciences PBC lysate as starting material. In total, two Gram-positive and six Gram-negative species were tested. The overall essential agreement compared to BMD reference testing was 82.2 %, and the categorical agreement was 91.3 %, for all species included.*

A

Species	Inoculum	SD
E. cloacae	2.95×10^5	2.91×10^5
E. coli	4.17×10^5	2.07×10^5
E. faecalis	1.67×10^6	2.19×10^6
E. faecium	3.60×10^6	-
K. oxytoca	2.61×10^5	-
K. pneumoniae	4.21×10^5	4.48×10^5
P. aeruginosa	1.26×10^5	6.20×10^4
S. aureus	5.54×10^5	4.56×10^5

B

Preparation	Unusable area	
	Mean (%)	SD (%)
Filter	34.6	4.5
Cellectric	12.1	5.8

C

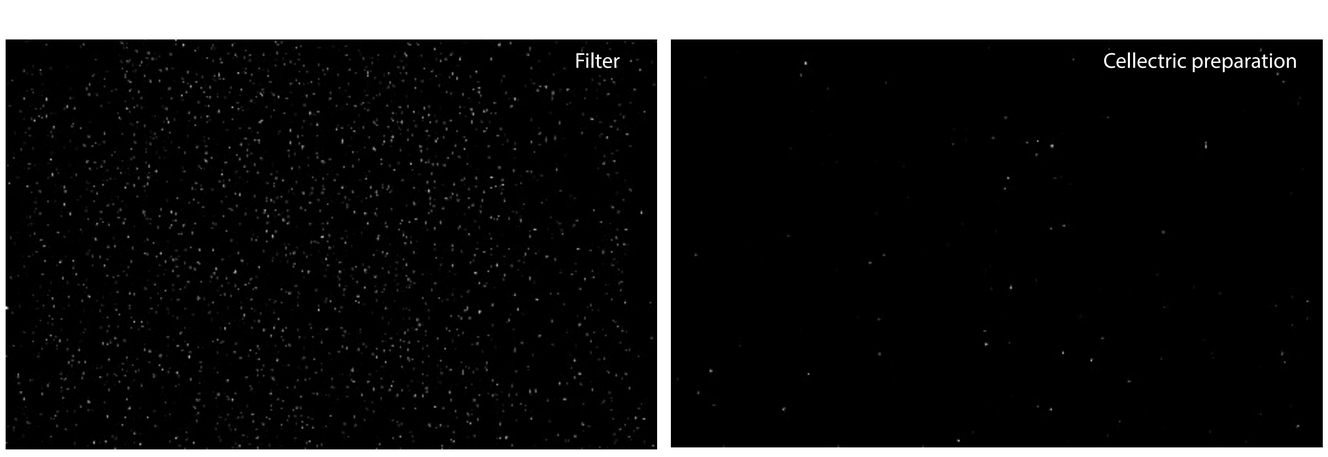

Figure 2: ***A)*** *Inoculum concentration per sample obtained from the QuickMIC system for all tested species. The average inoculum retained from the Cellectric preparation was consistently above the minimum needed in QuickMIC (1 x 105 cfu/mL).* ***B)*** *The reduction in background noise in the QuickMIC system was measured by comparing the unusable area (resulting from debris such as remaining blood cells) in the chambers in the QuickMIC cassette. Preparation with the Cellectric platform reduced the background from 34.6 to 12.1% blocked area, which increases the likelihood of a test chamber generating a result, and thus improves test yield.* ***C)*** *A visual representation of the background in the QuickMIC system when preparing the blood samples using the standard QuickMIC filter-based preparation method and the Cellectric device. The white dots correspond to blood cells and other debris remaining from the PBC after bacteria isolation.*

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