P0356

33rd ECCMID Copenhagen, Denmark 15 – 18 April 2023

Implementation of the EUCAST rapid antibiotic susceptibility testing (RAST) method from positive blood cultures by using the BD Kiestra™ TLA system for incubation and reading

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BACKGROUND

Since 2019, EUCAST offers methodology and breakpoints for performing and interpreting antibiotic susceptibility testing directly from positive blood culture bottles (RAST), with first results after 4 to 6 hours incubation.

The BD Kiestra™ TLA system allows the incubation and imaging of the RAST plates according to EUCAST recommendations.

OBJECTIVES

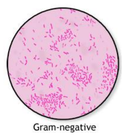
- Validation of **EUCAST RAST from positive blood cultures** in the laboratory
- Automation of incubation and digitalization of the RAST plates in the incubators of the **BD Kiestra™ TLA** system

METHODS

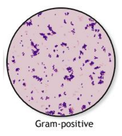
From September to December 2021, prospective inclusion of:

BactALert FA Plus /FN Plus/PF(bioMérieux) blood culture, positive at 8:00 AM

Gram stain



Gram negative bacilli



Gram positive cocci

Suspicion of pneumococci

RAST protocol

125 ± 25 µL

MHE agar

125 ± 25 µL

MHF agar

Disk deposition according to bacterial type

Incubation at 35°C air or +5% CO2

Manual reading after 4-6-8h incubation

Interpretation using EUCAST RAST Breakpoint v3.0

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

Subculture → MALDI-TOF MS identification from 4-6h subculture colonies → AST using Vitek®2 system (bioMérieux) or disk diffusion → Interpretation using EUCAST Clinical breakpoints v11.0

Verification of the method performed and interpreted according to Cumitech 31A : Verification and validation of procedures in the clinical microbiology laboratory

RESULTS

Clinical samples

49 blood cultures out of 119 were included.

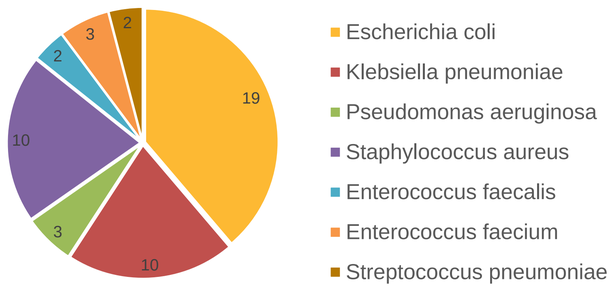


Figure 1: Included blood cultures

70 positive blood cultures were excluded due to absence of RAST breakpoints for the identified species (among which 74% of coagulase negative staphylococci).

RAST results

Readable zones

Readability of RAST was >80% after 4 hours incubation for all species for which 4 hours incubation beakpoints were available, excepted *S. pneumoniae*

	Proportion of readable zones (%)		
Species	4 hours	6 hours	8 hours
Escherichia coli (n=19)	99,52%	100%	100%
Klebsiella pneumoniae (n=10)	100%	100%	100%
Pseudomonas aeruginosa (n=3)	-	93,94%	96,97%
Staphylococcus aureus (n=10)	86,67%	97,78%	100%
Enterococcus faecalis (n=2)	88,89%	100%	100%
Enterococcus faecium (n=3)	85,19%	100%	100%
Streptococcus pneumoniae (n=2)	44,44%	50%	50%

Table1: Proportion of readable inhibition zones after 4, 6 and 8 hours incubation

ATU

Results in the area of technical uncertainty (ATU) were frequent with piperacillin-tazobactam and amikacin on *E. coli.*

ATU rates were <5% for other combination, excepted for *E. faecalis and E. faecium* where vancomycin was in the ATU zone for all samples at each reading time.

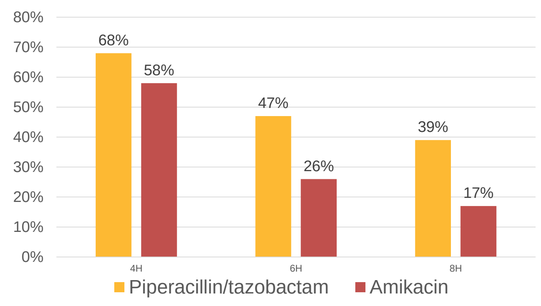


Figure 2: Proportion of results in the ATU with pip-tazo and amikacin on E. coli after 4, 6 and 8 hours incubation

RAST vs Vitek® 2

Performance of RAST compared to Vitek® 2, excluding results in ATU → **Acceptable performance: CA ≥ 90%, MD and VMD < 3%**

No VMD observed

CA=categorical agreement, MD=major discrepancy, VMD=very major discrepancy

Table 2 to 6: Agreement between RAST and Vitek®2 results. Table 7: Agreement between RAST and disk diffusion results

	4 hours		6 hours		8 hours	
	CA (%)	MD (%)	CA (%)	MD (%)	CA (%)	MD (%)
Pip-tazo	4/5 (80%)	1/3 (33%)	8/10 (80%)	2/4 (50%)	9/11 (82%)	2/4 (50%)*
Cefotaxime	19/19 (100%)	0	19/19 (100%)	0	17/17 (100%)	0
Ceftazidime	17/17 (100%)	0	18/18 (100%)	0	17/17 (100%)	0
Meropenem	17/17 (100%)	0	19/19 (100%)	0	18/18 (100%)	0
Ciprofloxacin	18/18 (100%)	0	19/19 (100%)	0	18/18 (100%)	0
Amikacin	8/8 (100%)	0	14/14 (100%)	0	15/15 (100%)	0
Gentamicin	19/19 (100%)	0	19/19 (100%)	0	18/18 (100%)	0
Trimethoprim-sulfa.	18/18 (100%)	0	18/18 (100%)	0	17/17 (100%)	0

Table 2: ***Escherichia coli (n=19)***

* The 2 major discrepancy were solved using disk diffusion method from a fresh agar culture, demonstrating one result in ATU and one concordant with RAST

	4 hours	6 hours
	CA (%)	CA (%)
Pip-tazo	10/10 (100%)	10/10 (100%)
Cefotaxime	10/10 (100%)	10/10 (100%)
Ceftazidime	10/10 (100%)	10/10 (100%)
Meropenem	10/10 (100%)	10/10 (100%)
Ciprofloxacin	10/10 (100%)	10/10 (100%)
Amikacin	8/8 (100%)	10/10 (100%)
Gentamicin	10/10 (100%)	10/10 (100%)
Trimethoprim-sulfa	10/10 (100%)	10/10 (100%)

Table 3: ***Klebsiella pneumoniae (n=10)***

No change observed between 6 and 8h RAST

	6 hours		8 hours	
	CA (%)	MD (%)	CA (%)	MD (%)
Pip-tazo	2/2 (100%)	0	3/3 (100%)	0
Cefepime	1/2 (50%)	1/2 (50%)	2/2 (100%)	0
Ceftazidime	2/3 (67%)	1/3 (33%)	2/2 (100%)	0
Imipenem	2/2 (100%)	0	3/3 (100%)	0
Meropenem	2/2 (100%)	0	2/2 (100%)	0
Ciprofloxacin	3/3 (100%)	0	3/3 (100%)	0
Amikacin	3/3 (100%)	0	3/3 (100%)	0
Tobramycin	3/3 (100%)	0	3/3 (100%)	0

Table 4: ***Pseudomonas aeruginosa (n=3)***

	4 hours		6 hours	
	CA (%)	MD (%)	CA (%)	MD (%)
Cefoxitin	9/9 (100%)	0	10/10 (100%)	0
Gentamicin	5/6 (83%)	1/1 (100%)	10/10 (100%)	0
Clindamycin	3/3 (100%)	0	9/9 (100%)	0

Table 5: ***Staphylococcus aureus (n=10)***

No change observed between 6 and 8h RAST

	CA (%)
Ampicillin	5/5 (100%)
Imipenem	3/3 (100%)

Table 6: ***E. faecalis and faecium (n=5)***

Vancomycin always in ATU

	CA (%)
Oxacillin (screen)	1/1 (100%)
Erythromycin	1/1 (100%)
Clindamycin	1/1 (100%)
Trimethoprim-sulfa	1/1 (100%)

Table 7: ***Streptococcus pneumoniae (n=2)***

IMPLEMENTATION OF RAST ON THE BD KIESTRA™ TLA SYSTEM

From October 2022, considering the greater clinical added value of the RAST on Gram negative bacteria, RAST plates from all morning blood cultures positive for a Gram negative bacillus were, after manual streaking and disks deposition, introduced on the TLA system for incubation and picture.

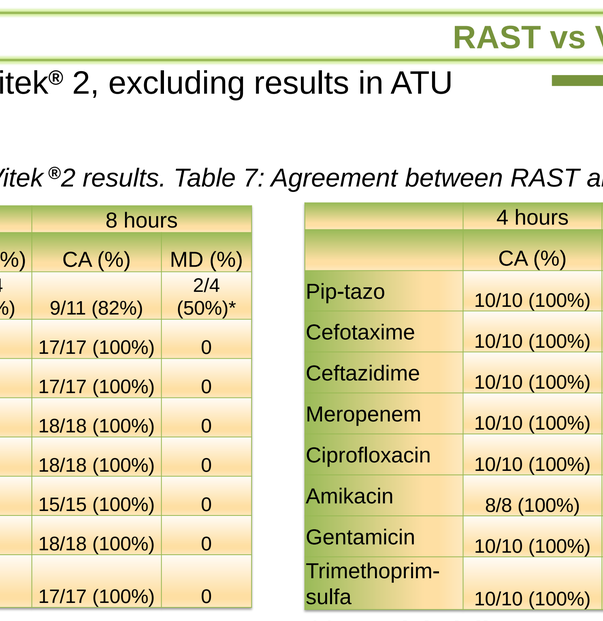


Figure 3: Incubation program in BD Kiestra™ DB Manager software

BD Kiestra

Manual measurement of inhibition zones using BD Kiestra™ ReadA Browser software

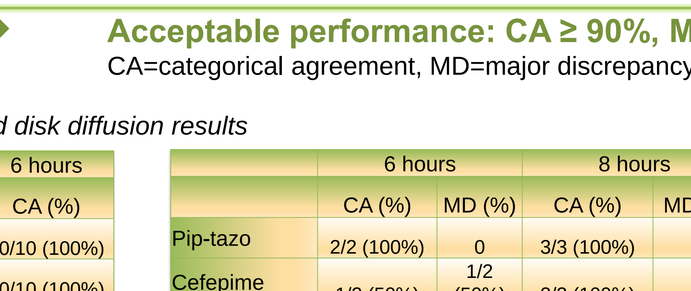


Figure 4 and 5: 4h RAST of a multi-S E. coli

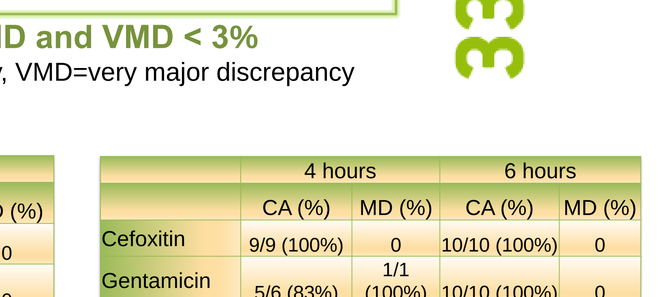


Figure 6 and 7: 4h RAST of a KPC producing K. pneumoniae

CONCLUSIONS

The RAST methodology proposed by EUCAST allows AST results to be reported directly from positive blood cultures after minimum 4 or 6 hours incubation with a great concordance with routine antibiograms performed using a Vitek®2 system. The automation of the process in the BD Kiestra™ TLA system allows the standardization of reading times and easy on-screen interpretation.