

EUCAST method for Rapid Antimicrobial Susceptibility Testing directly from positive blood cultures

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Why a standardised rapid method?

- A rapid (and correct) AST result is crucial in the treatment of septicemia.
- Many microbiology laboratories have developed in-house methods for rapid AST (RAST) directly from positive blood cultures.
- The antimicrobial resistance levels are increasing making it more important with a RAST result.
- EUCAST wished to take responsibility for validating **one** RAST method for positive blood cultures to harmonize between laboratories and ensure reliable results.

AST= Antimicrobial susceptibility testing

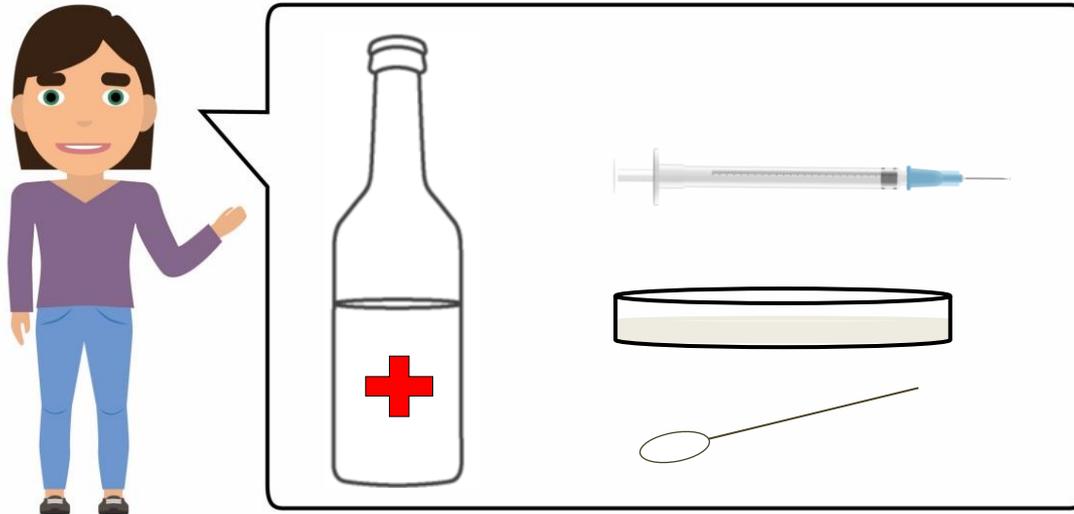
EUCAST Rapid AST (RAST)

- Based on EUCAST standard disk diffusion
 - Directly from positive blood cultures
 - Incubation time 4, 6 and 8 hours
 - Reporting final results
- Developed for:
 - *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*,
S. aureus, *E. faecalis*, *E. faecium* and *S. pneumoniae*
- Available on EUCAST website, eucast.org
 - Methodology and implementation
 - Breakpoint tables
 - Screening for resistance mechanisms

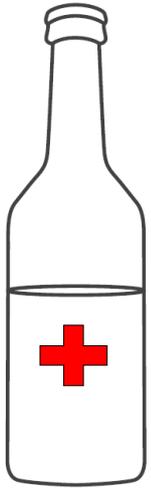
EUCAST RAST validation

- Spiked blood culture bottles
 - Isolates with various levels of susceptibility/resistance
 - Tests performed at EDL
 - MIC with BMD was used as a reference
- Multi-laboratory trial
 - Local clinical isolates
 - Local BC systems, MH media and disks
 - Standard disk diffusion was used as a reference
 - Northern Europe 2017: 40 laboratories
 - Southern Europe 2018: 15 laboratories

RAST methodology



Inoculation of plates

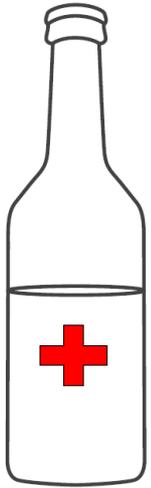


Positive BC
Gram or ID

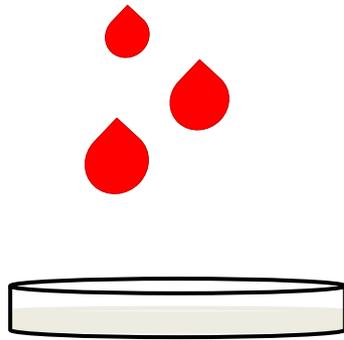
Perform RAST on BC bottles:

- Up to 18 hours after positive signal
- Within 3 hours from removal from the instrument

Inoculation of plates



Positive BC
Gram or ID



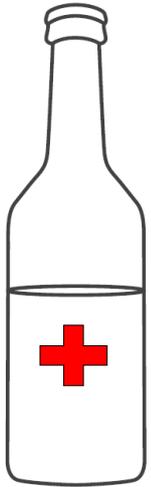
$125 \pm 25\mu\text{l}$



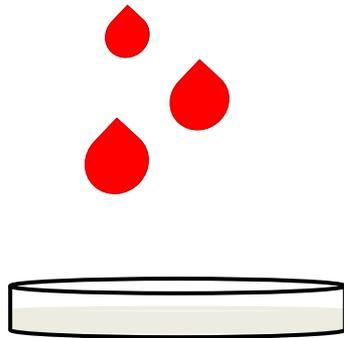
For a 90-mm circular plate

- Drops from a syringe
- Exact amount with a pipette

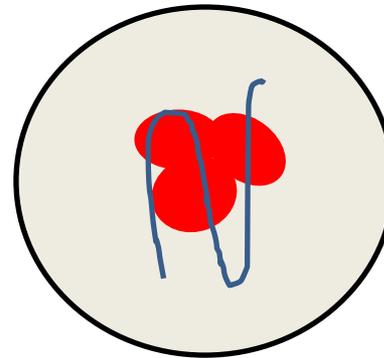
Inoculation of plates



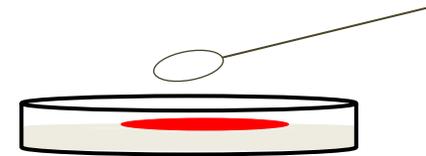
Positive BC
Gram or ID



$125 \pm 25\mu\text{l}$



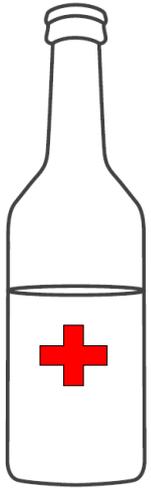
Distribute the
blood



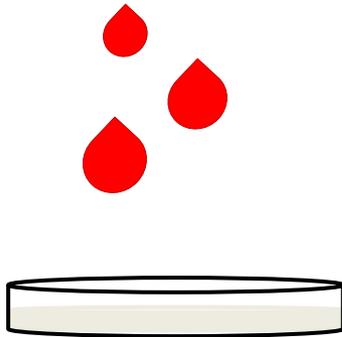
Spread gently
over agar
surface

-Avoid patterns from streaking

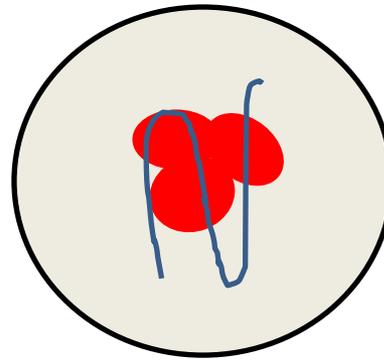
Inoculation of plates



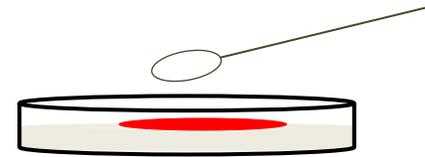
Positive BC
Gram or ID



$125 \pm 25\mu\text{l}$



Distribute the
blood



Spread gently
over agar
surface

Apply antibiotic disks

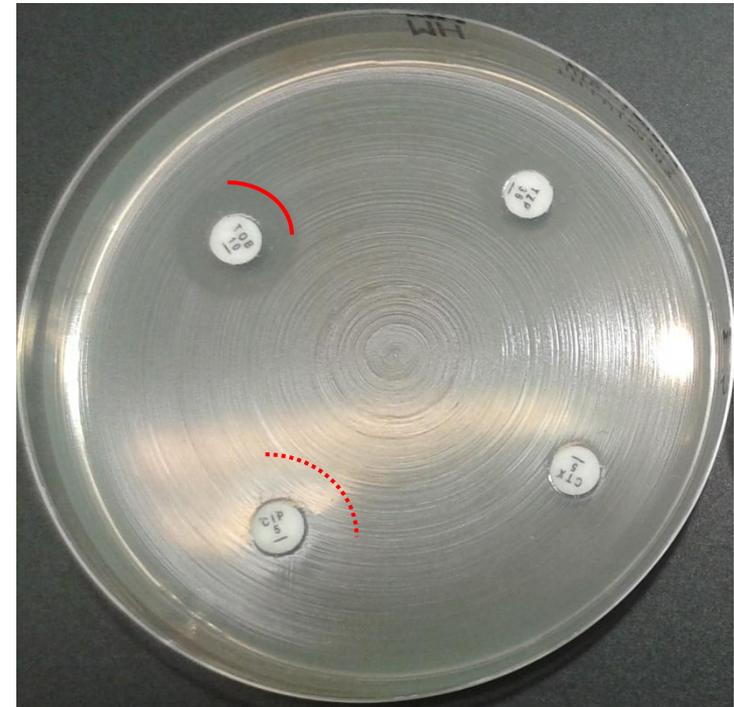
Incubation time: 4, 6 and/or 8 hours

-Read within ± 5 min of the
specified time

-Re-incubate within 10 min

Reading of zones

- Hold the plate at 30 cm distance
- Read inhibition zones from the front without lid
- **Read zones only when a obvious zone edge is visible**
- Disregard thin growth within the zone

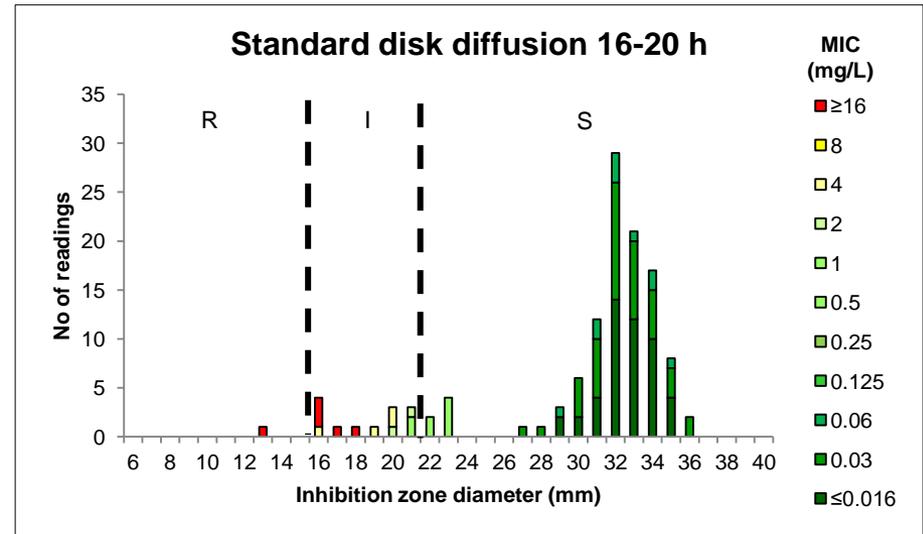
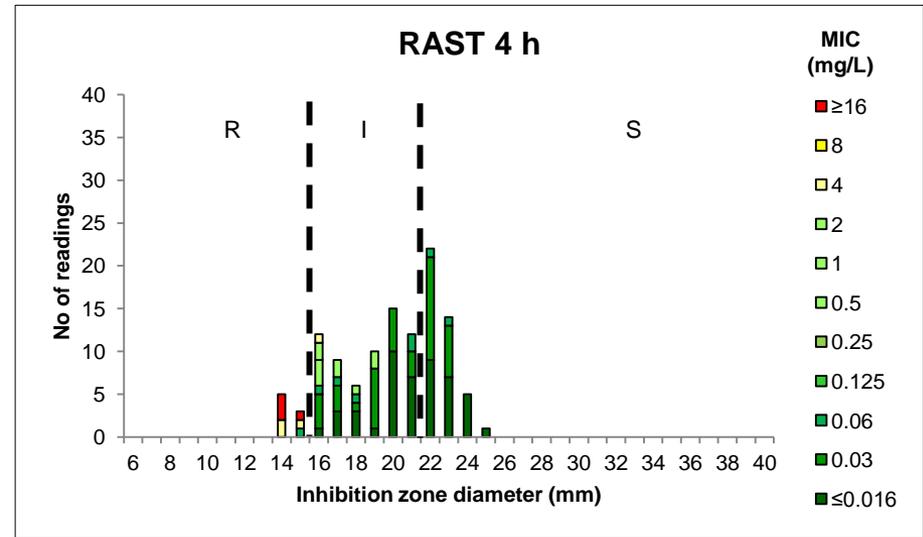


E. coli at 4h incubation

Zones change with incubation time

Poorer separation between susceptible and resistant isolates with short incubation

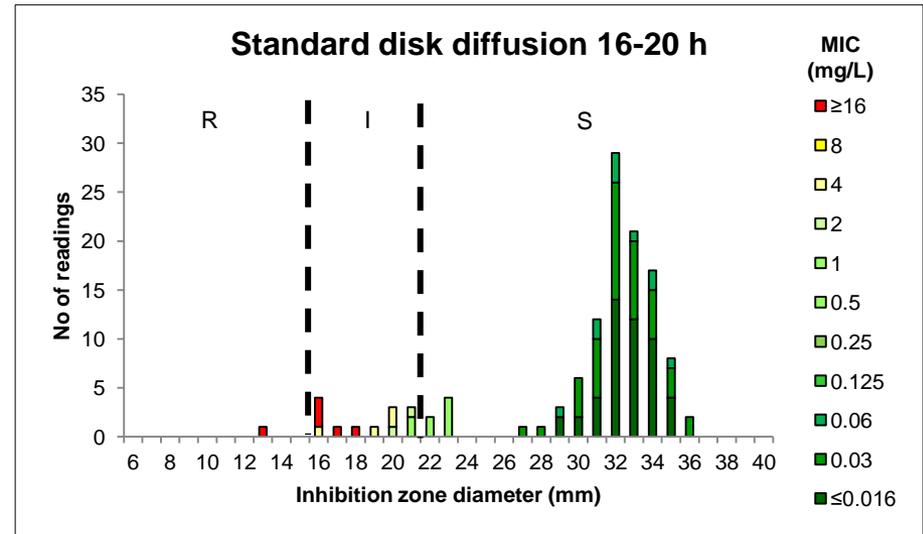
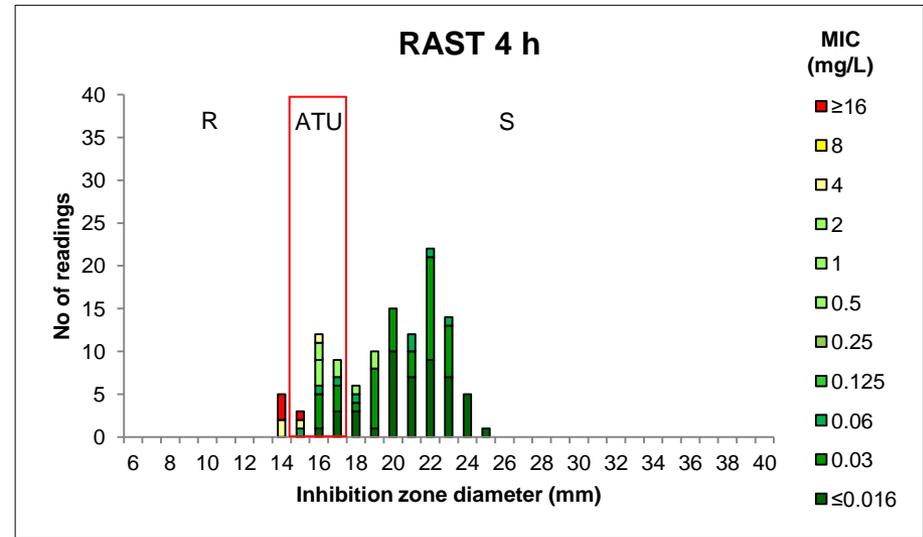
E. coli and meropenem 10 µg vs. MIC



The combination of smaller zones and poorer separation = new breakpoints needed

Specific breakpoints for each organism-agent combination and incubation time.

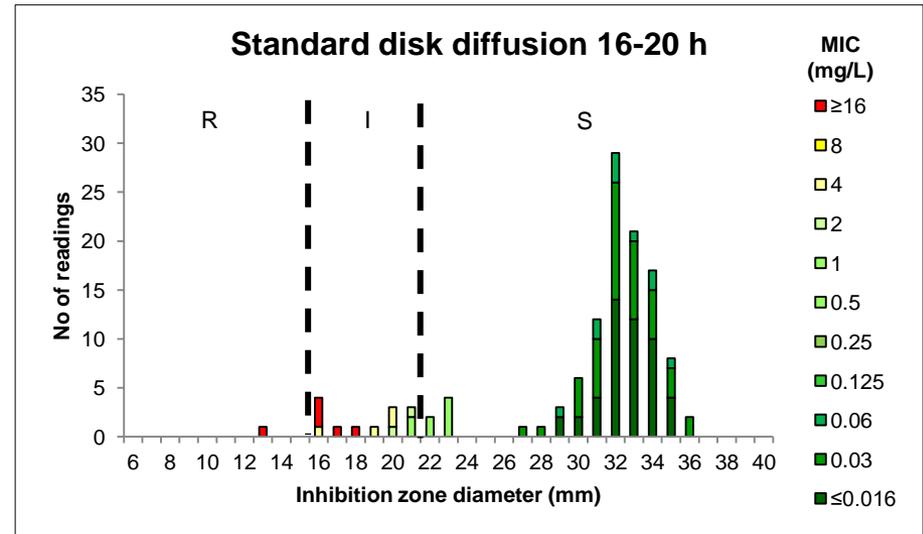
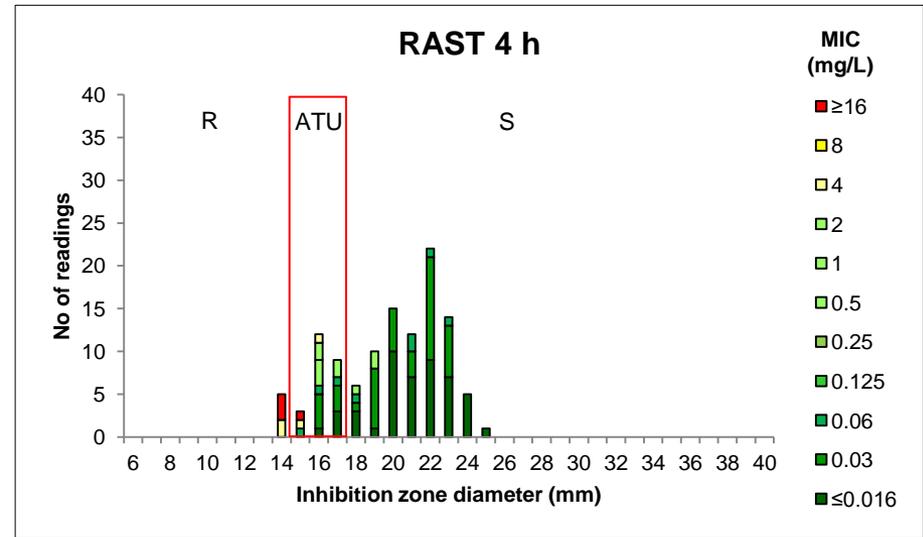
E. coli and meropenem 10 µg vs. MIC



The combination of smaller zones and poorer separation = new breakpoints needed

ATU = Area of Technical Uncertainty Area were interpretations to susceptible and resistant is difficult.

E. coli and meropenem 10 µg vs. MIC



Interpreting results

- Identification is needed
- Results are interpreted into S, R or ATU
- No AST report is given for results within ATU
 - ATU after 4 or 6 hours = plates are reincubated
 - ATU also after 8 hours, retest with standard methodology.
- EUCAST RAST BP table

Only interpret results for species with RAST breakpoints!

Quality Controls (QC)

1. Standard disk diffusion QC – to control AST materials
2. RAST QC - for implementation and changes of the RAST method
 1. From BC bottles with added blood
 2. RAST methodology 4, 6 and 8 hours reading
 3. Practice reading

Performance



Readability (%)

Organism	4 hours	6 hours	8 hours
<i>Escherichia coli</i>	90	99	99
<i>Klebsiella pneumoniae</i>	96	98	98
<i>Pseudomonas aeruginosa</i>	0	88	97
<i>Staphylococcus aureus</i>	55	91	95
<i>Enterococcus faecalis</i>	93	99	100
<i>Enterococcus faecium</i>	44	93	99
<i>Streptococcus pneumoniae</i>	68	83	95

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Organism	4 hours	6 hours	8 hours
<i>Escherichia coli</i>	90	99	99
<i>Klebsiella pneumoniae</i>	96	98	98
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<i>Streptococcus pneumoniae</i>	68	83	95

Min 80 %

Readability (%)

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<i>Streptococcus pneumoniae</i>	68	83	95

Min 95 %

Southern European multi-laboratory trial

- 15 laboratories
 - France (2), Greece (3), Italy (2), Spain (4) and Turkey (4)

Overall resistance (%) and specific resistance mechanism (%)

	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>
Southern Europe	Overall: 11 ESBL:17 Carba: 0	Overall: 43 ESBL: 45 Carba: 38	Overall: 17 MRSA: 29

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Overall resistance (%) and specific resistance mechanism (%)

	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>
Southern Europe	Overall: 11 ESBL: 17 Carba: 0	Overall: 43 ESBL: 45 Carba: 38	Overall: 17 MRSA: 29
Northern Europe	Overall: 4.7 ESBL: 6 Carba: 0	Overall: 3.5 ESBL: 0.6 Carba: 0	Overall: 1.8 MRSA: 1.5

Categorical agreement, *E. coli* (150)

Southern European study

Incubation time		4h	6h	8h
Results calculated on readable zones (%)				
Area of Technical Uncertainty (ATU)		18	8.8	6.9
Interpreted to S or R		82	91	93
Errors calculated on zones interpreted to S or R (%)				
Errors	mE	0.7	1.5	1.6
	ME	2.1	0.9	0.7
	VME	0.3	0.3	0.5
	Total errors:	3.1	2.7	2.8

mE = minor Error, susceptible (S) or resistant (R) with RAST when I-category (I) with standard method.

ME=Major Error False resistance (RAST = R, Standard = S)

VME = Very Major Error, False susceptibility (RAST = S, Standard = R)

Categorical agreement, *E. coli*

Southern European study compared to Northern European

Incubation time		4h	6h	8h			
Results calculated on readable zones (%)							
Area of Technical Uncertainty (ATU)		18	16	8.8	8.8	6.9	6.9
Interpreted to S or R		82	84	91	91	93	93
Errors calculated on zones interpreted to S or R (%)							
Errors	mE	0.7	0.3	1.5	0.4	1.6	0.3
	ME	2.1	1.9	0.9	0.7	0.7	0.6
	VME	0.3	0.1	0.3	0.2	0.5	0.2
	Total errors:	3.1	2.3	2.7	1.2	2.8	1.1
		1.9	2.0	2.3			

Excluding 4 laboratories with 12-17 % errors

Categorical agreement, *S. aureus* (70)

Southern European study

Incubation time	4h	6h	8h	
Results calculated on readable zones (%)				
Area of Technical Uncertainty (ATU)	30	10	7	
Interpreted to S or R	70	90	93	
Errors calculated on zones interpreted to S or R (%)				
Errors	mE	0.0	0.4	0.4
	ME	11	7.7	4.7
	VME	1.1	2.6	2.4
	Total errors:	13	10.6	7.5

Categorical agreement, *S. aureus*

Southern European study compared to Northern European

Incubation time		4h	6h	8h			
Results calculated on readable zones (%)							
Area of Technical Uncertainty (ATU)		30	15	10	8	7	6
Interpreted to S or R		70	85	90	92	93	94
Errors calculated on zones interpreted to S or R (%)							
Errors	mE	0.0	0.3	0.4	0.2	0.4	0.1
	ME	11	2	7.7	1.2	4.7	0.7
	VME	1.1	0.2	2.6	0.1	2.4	0.2
	Total errors:	13	2.5	10.6	1.5	7.5	1.0
		3.8	2.5	0.8			

Excluding 4 laboratories with 12-17 % errors

Future developments

- Breakpoints for *S. epidermidis*
- Breakpoints for more antimicrobial agents for *E. coli*, *K. pneumoniae* and *P. aeruginosa*

<i>E. coli</i> and <i>K. pneumoniae</i>	<i>P. aeruginosa</i>
Imipenem	Cefepim
Levofloxacin	Levofloxacin
Trim-sulfa	Amikacin
Ceftazidime-avibactam	Ceftazidime-avibactam
Ceftolozane-tazobactam	Ceftolozane-tazobactam

Summary

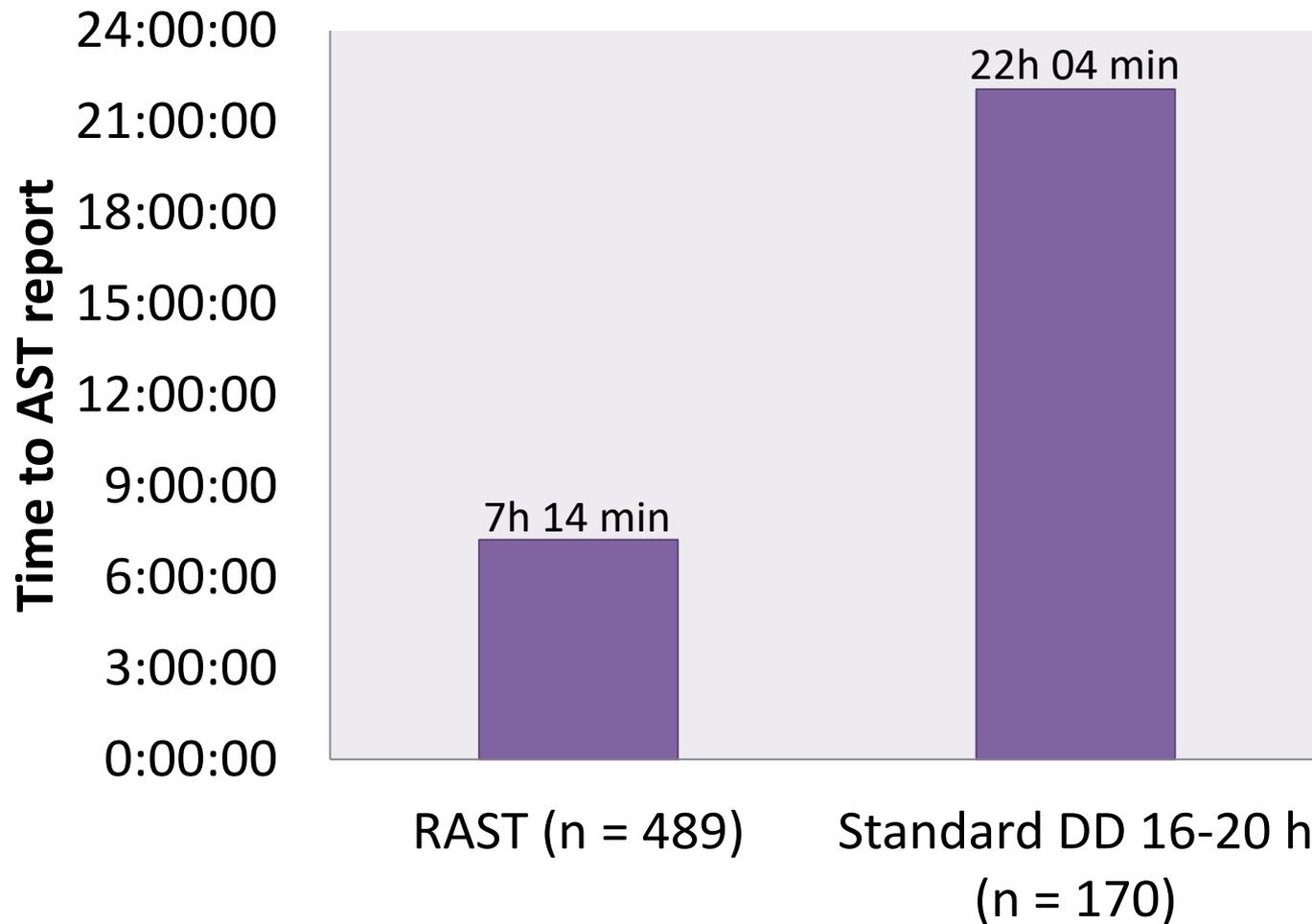
- EUCAST RAST methodology developed and available on www.eucast.org
- Evaluated on 55 clinical laboratories
- Reading after 4-8 h for common sepsis pathogens
- RAST breakpoint table calibrated to EUCAST standard methodology and breakpoints

RAST in clinical laboratory



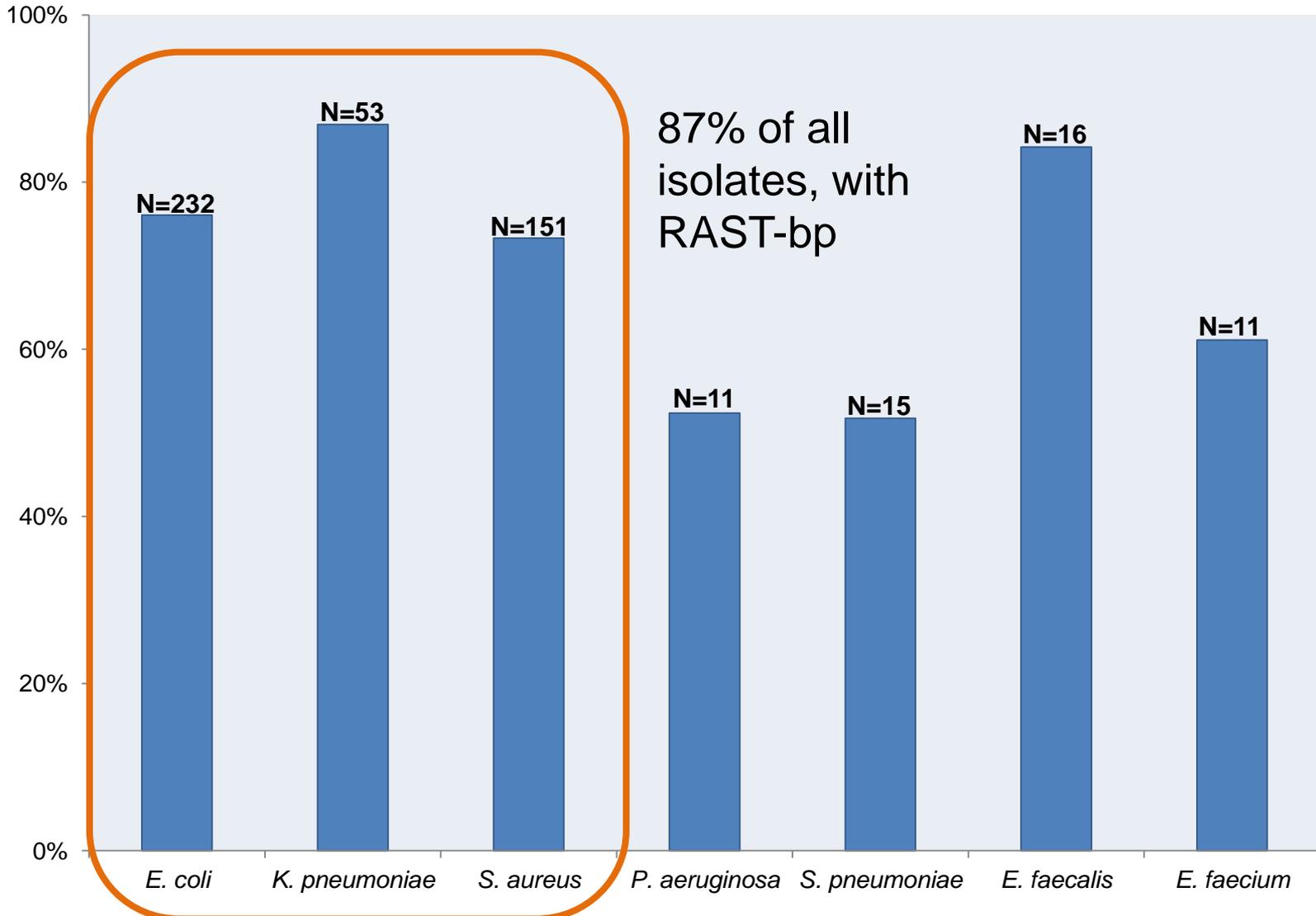
Data from:
May -18 to Feb -19
Around 660 blood
cultures

Time to AST results with RAST and standard disk diffusion (16-20 h)



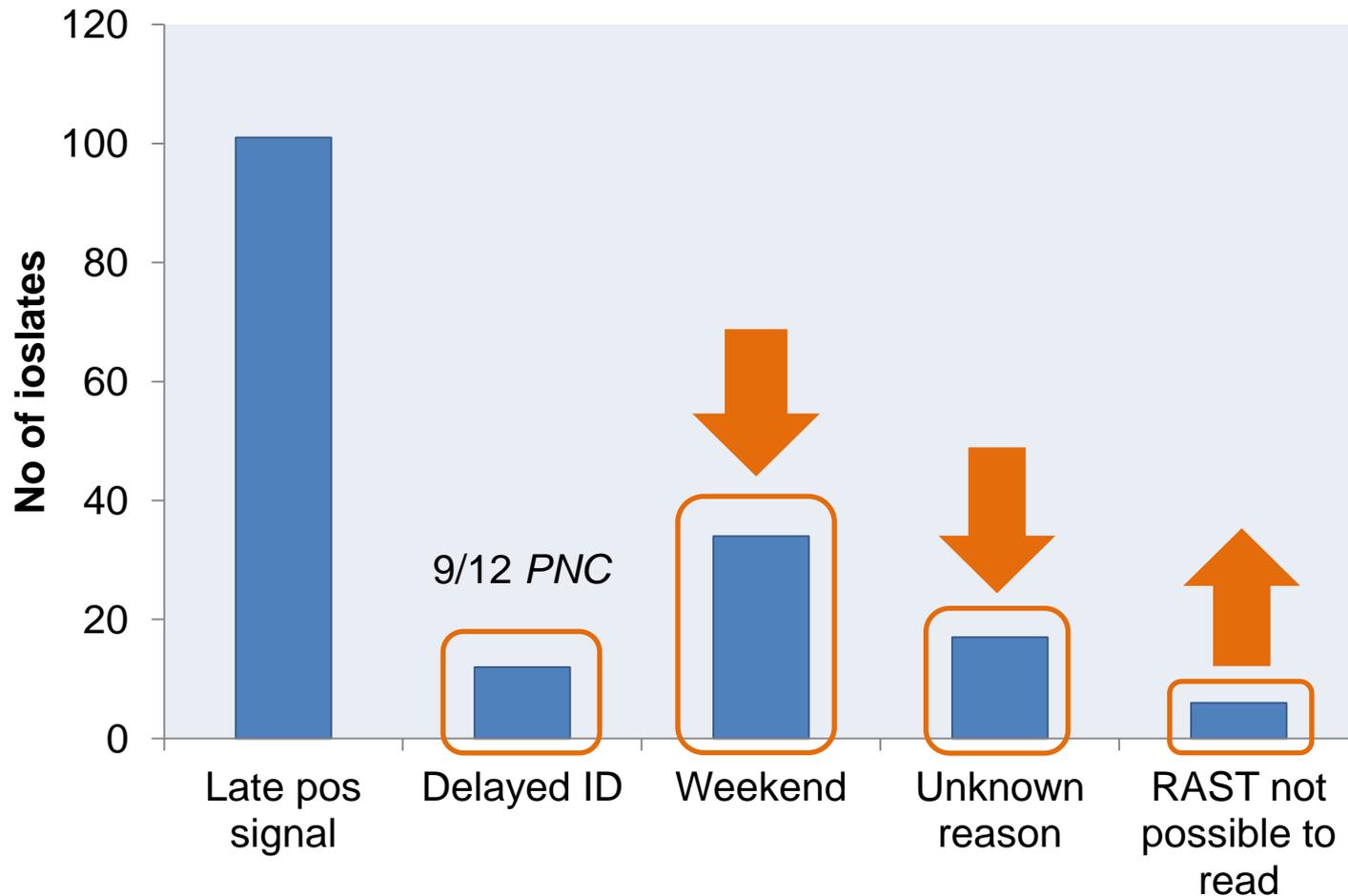
Courtesy of Per Rydström, Clinical Microbiology, Växjö, Sweden

Percent performed RAST, per species



Courtesy of Per Rydström, Clinical Microbiology, Växjö, Sweden

The reason why RAST has not been performed (n=170)

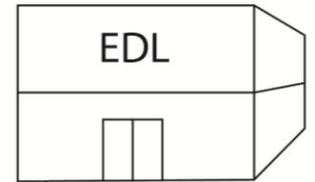


Courtesy of Per Rydström, Clinical Microbiology, Växjö, Sweden

Lessons learned

- RAST decrease the time to first AST result considerably.
- Sometimes difficult with identification
 - *S. pneumoniae*
- Get better at reading zones after 4 hours incubation.
- Very good performance so far.

Contact us!



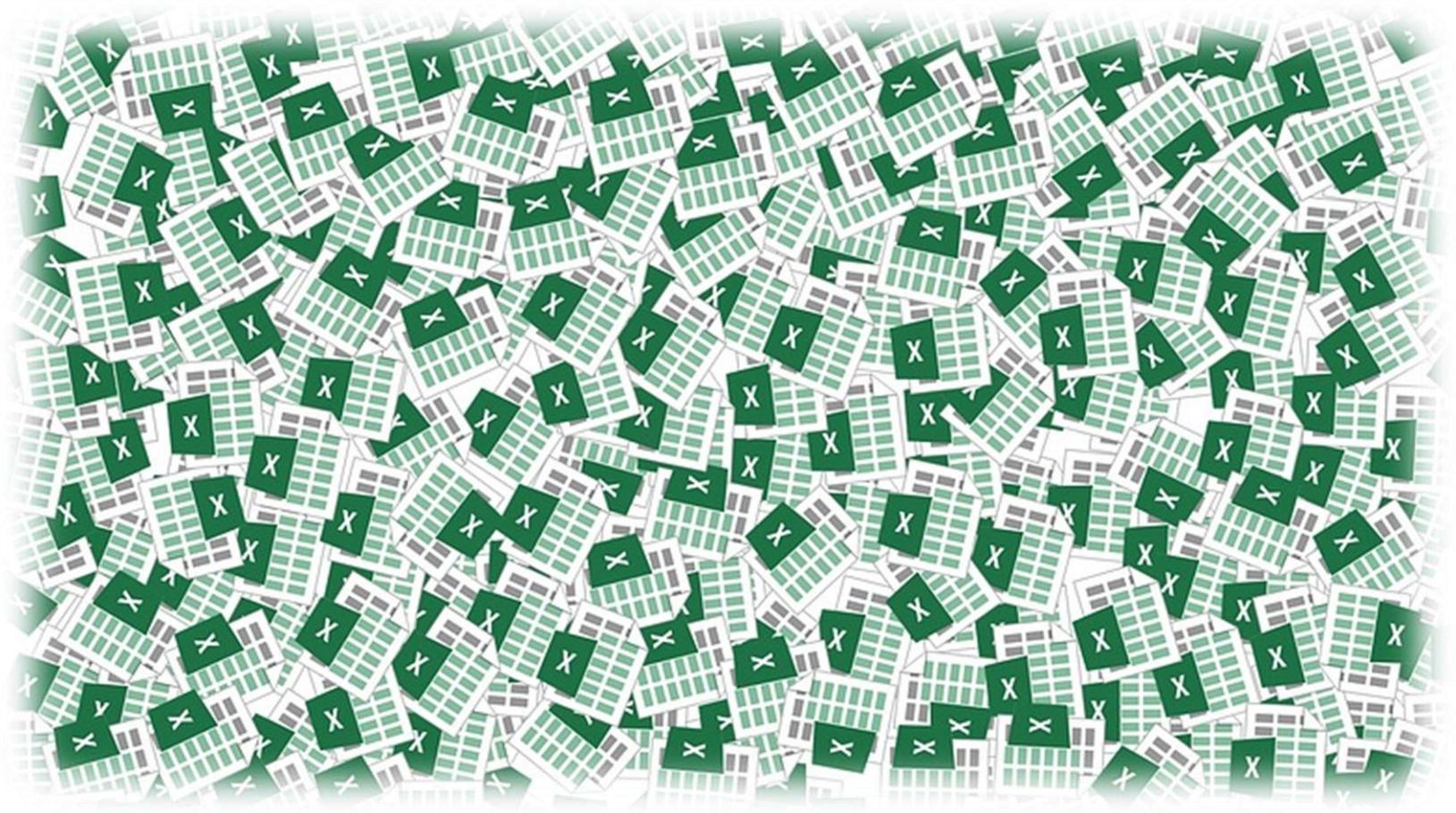
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Acknowledgements

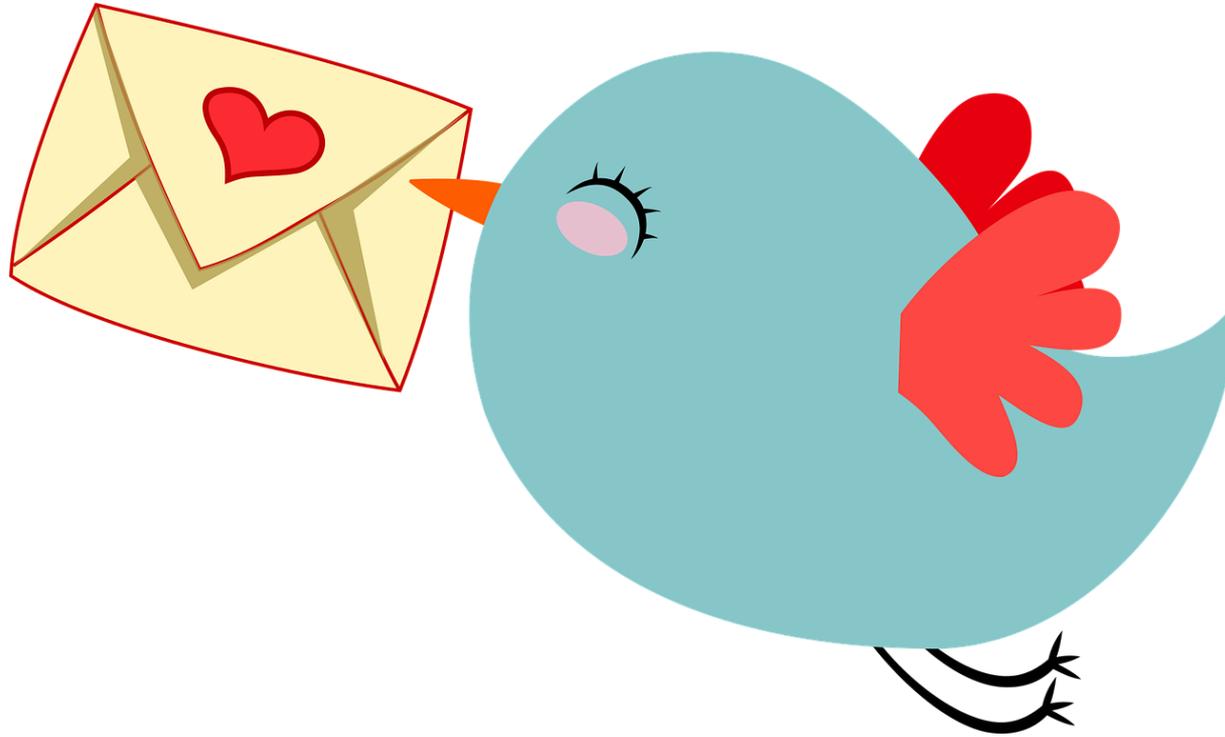
- Erika Matuschek and Gunnar Kahlmeter, EDL
 - Planning and data analysis
- Anna Åkerlund, Clinical Microbiology, Jönköping, Sweden
 - Planning, laboratory work and data analysis
- Martin Sundqvist, Clinical Microbiology, Örebro, Sweden
 - Planning and data analysis
- **All laboratories participating in the multi-laboratory validation studies**

Thank You

Performance



Contact us!



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