REGION KRONOBERG

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

EUCAST Rapid Antimicrobial Susceptibility Testing (RAST) directly from positive blood cultures

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EUCAST development laboratory

Växjö, Sweden

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RAST methodology and performance

Why a standardised rapid method?

- A rapid (and correct) AST result is crucial in the treatment of septicaemia.
- Many microbiology laboratories have developed in-house methods for rapid AST directly from positive blood cultures.
- EUCAST wished to take responsibility for validating **one** RAST method for positive blood cultures to harmonize between laboratories and ensure reliable results.

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
  - Northern Europe 2017: 40 laboratories
  - Southern Europe 2018: 15 laboratories

EUCAST RAST methodology

- EUCAST standard disk diffusion method with modifications of:
  - Inoculum: 100-150 µL, positive blood culture
  - Incubation time: 4, 6 and/or 8 h (depending on species)

- Developed for:
  - *E. coli, K. pneumoniae, S. enterica, P. aeruginosa, A. baumannii, S. aureus, E. faecalis, E. faecium* and *S. pneumoniae*

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Incubation

- 4, 6 and/or 8 hours
  - Read plates within ± 5 minutes of the specified incubation time
  - Re-incubate the plate within 10 minutes
- 16-20 hours intervall
  - Use when it is not possible to read results after 4, 6 and/or 8 hours
  - Breakpoints available for all species

Reading of plates

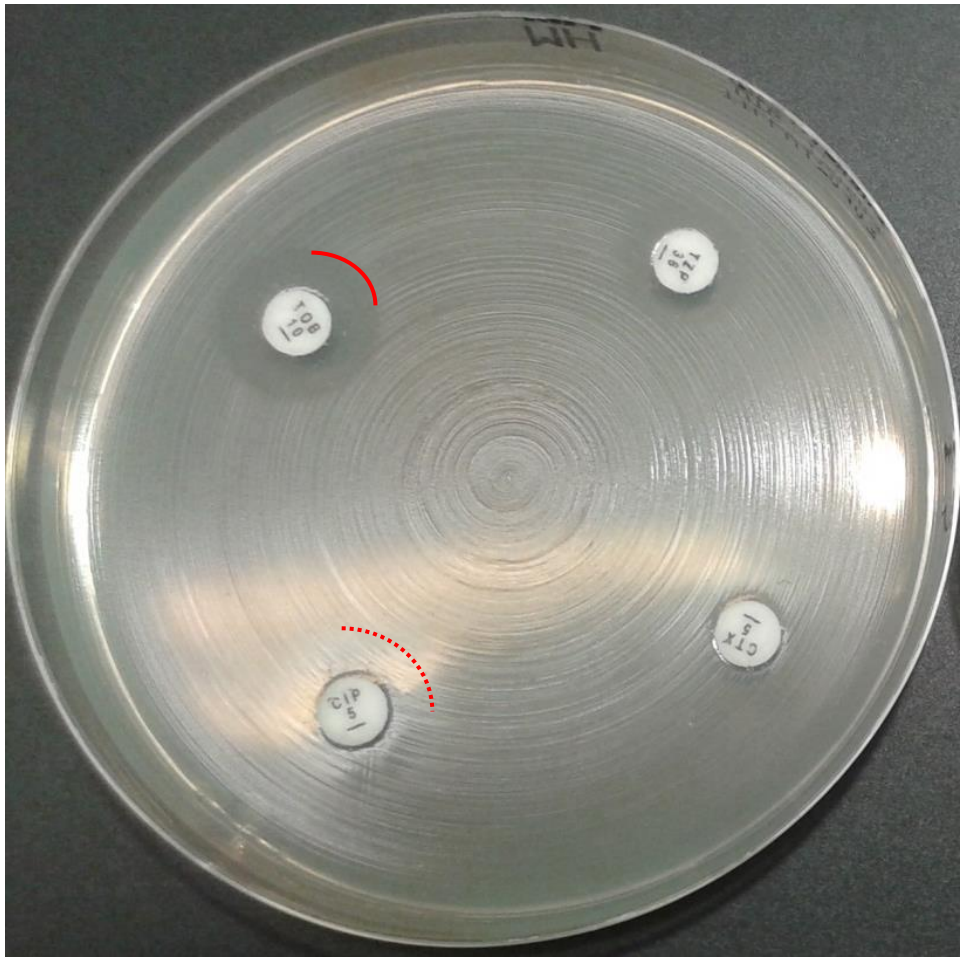
- General reading instructions
  - Hold the plate at 30 cm distance

- 4, 6 and 8 hours
  - Read plates from the front without lid
  - Read zones only when a clear zone edge is visible

- 16-20 hours
  - Read MH plates from the back of the plate
  - Read MH-F plates from the front without lid

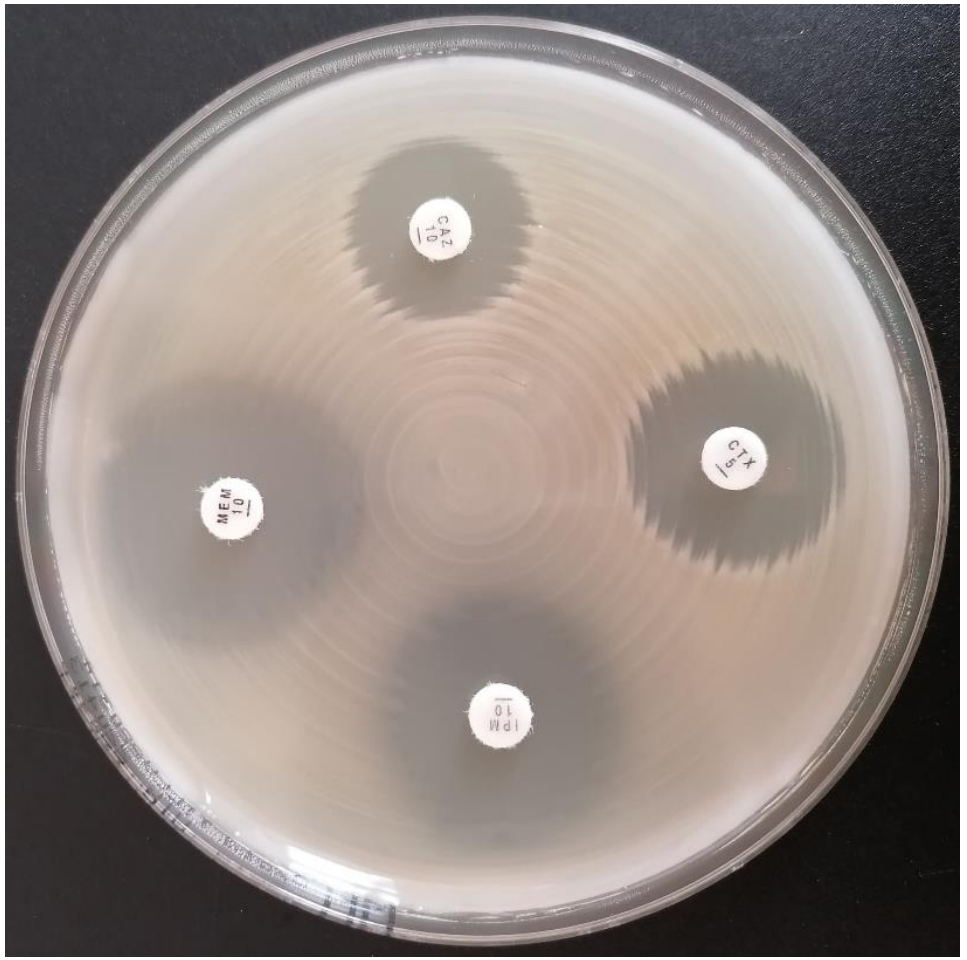
Reading of zones

4, 6 and/or 8 hours



E. coli at 4h incubation

16-20 hours



E. coli at 20h incubation

Readability (%)

The proportion of zone diameters (%) which are possible to read after 4 – 20 h of incubation.

Organism	**4 hours (%)**	**6 hours (%)**	**8 hours (%)**	**16-20 hours**
Escherichia coli	90	99	99	100
Klebsiella pneumoniae	96	98	98	100
Salmonella enterica	93	100	100	100
Pseudomonas aeruginosa	-	88	97	100
Acinetobacter baumannii	99	100	100	ND
Staphylococcus aureus	55	91	95	100
Enterococcus faecalis	93	99	100	ND
Enterococcus faecium	44	93	99	ND
Streptococcus pneumoniae	68	83	95	100

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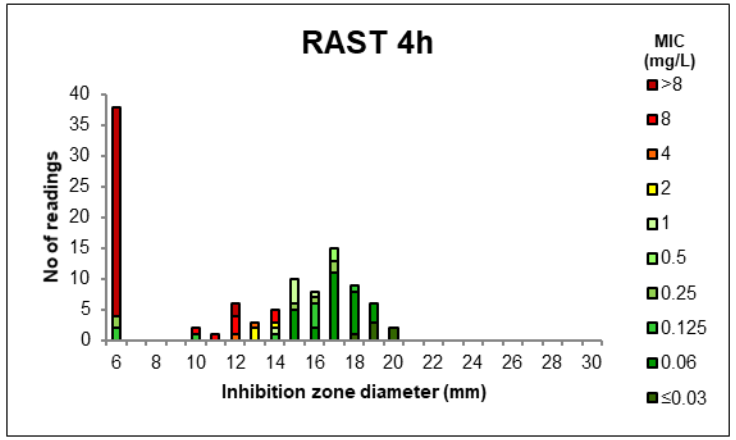
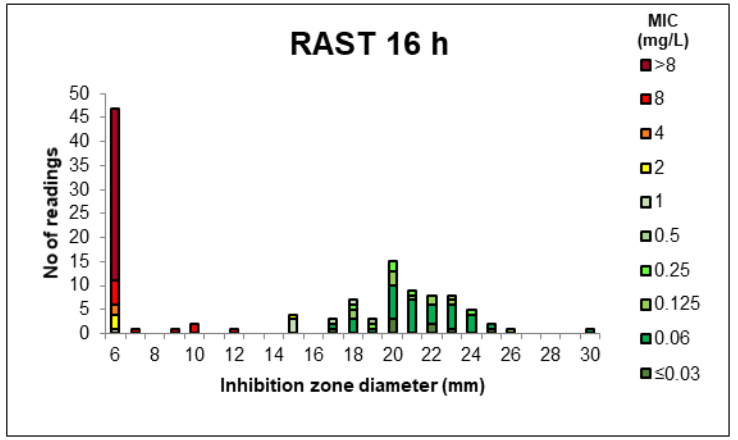
Interpretation of results

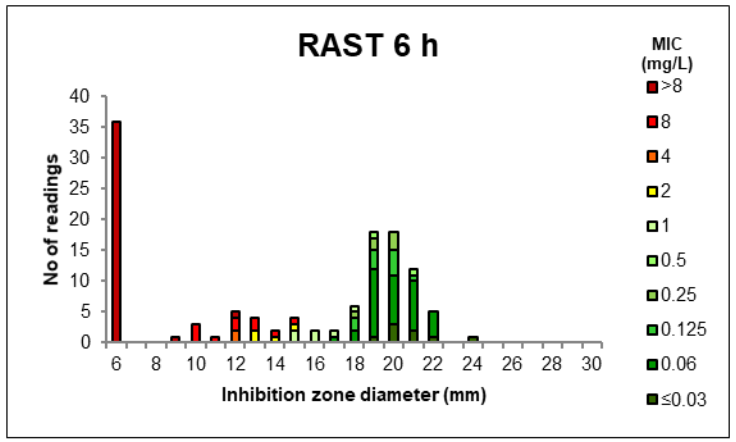
- Interpret inhibition zone diameters according to the latest version of the RAST breakpoint tables.
- Leave the report blank if:
  - Cannot read the zone in a reliable way
  - The zone diameter is in the ATU.

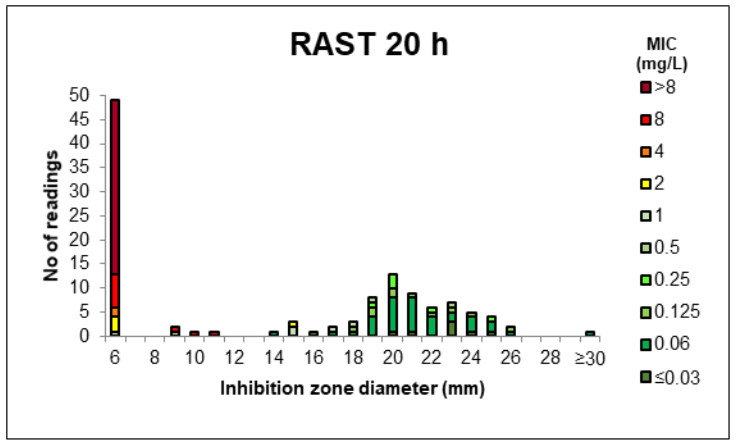
<u>Only interpret results for species with RAST breakpoints!</u>

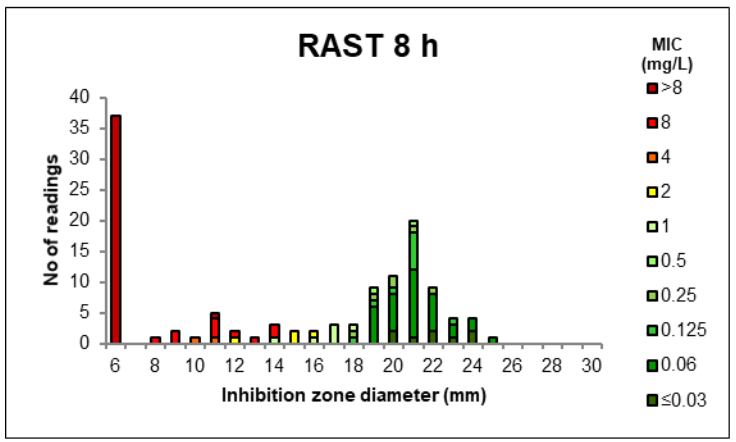
E. coli with cefotaxime 5 µg vs. BMD

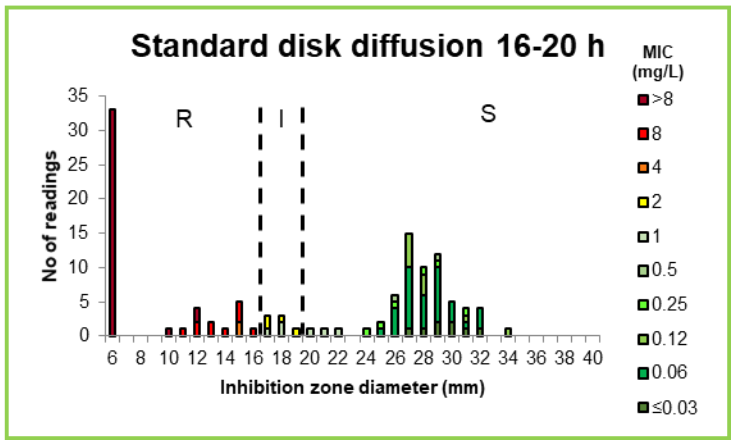












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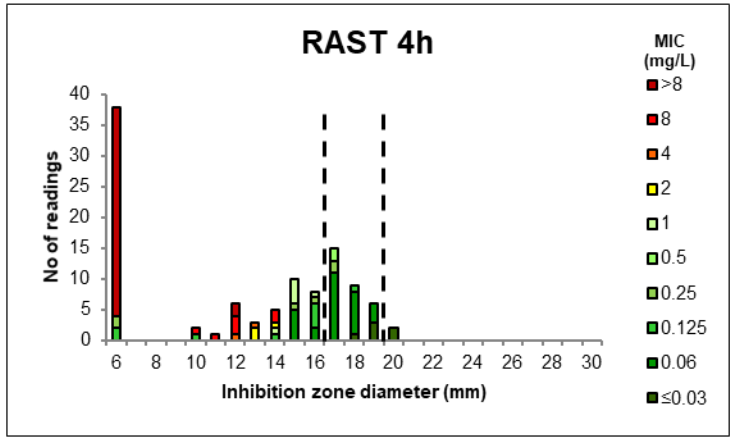

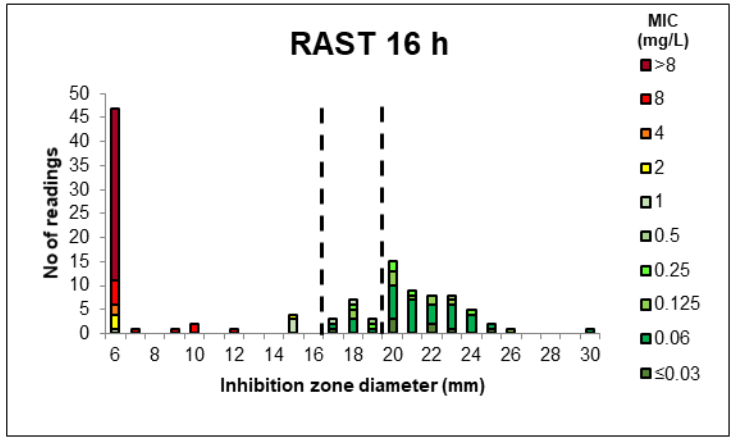

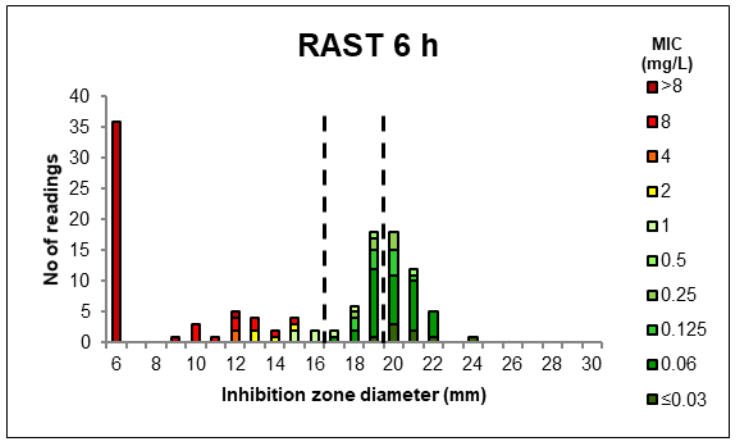

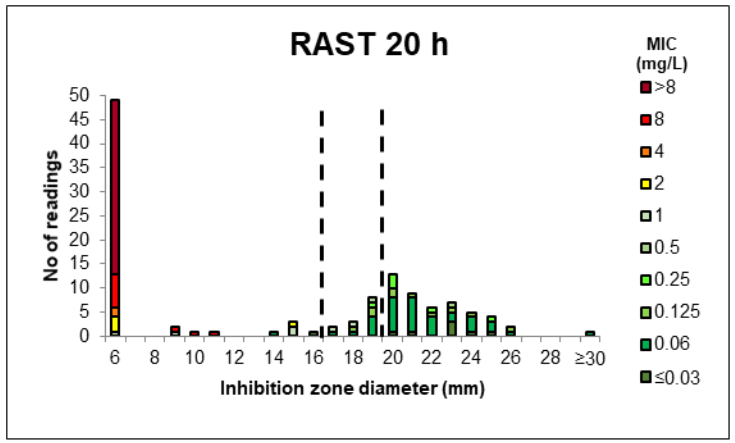

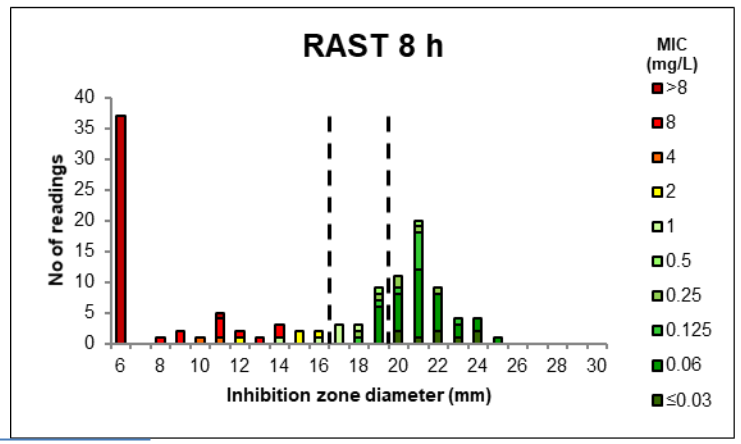

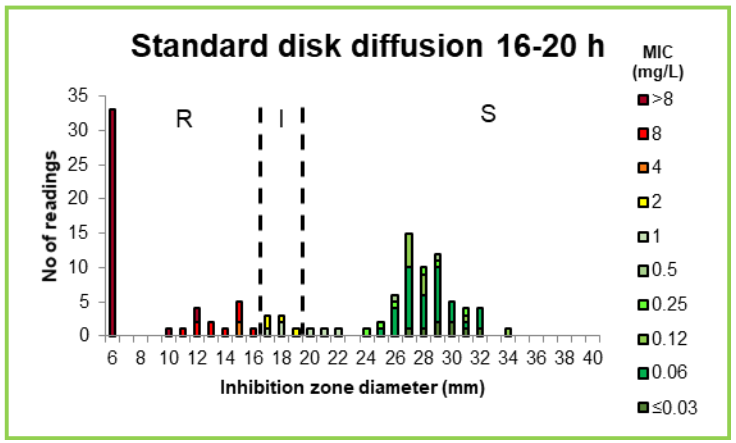

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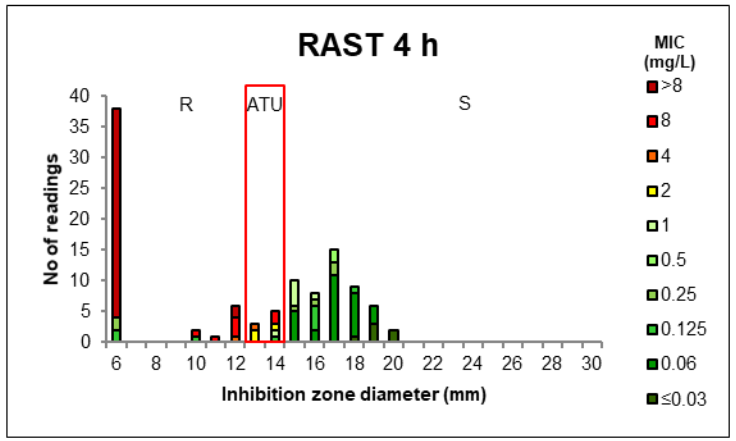
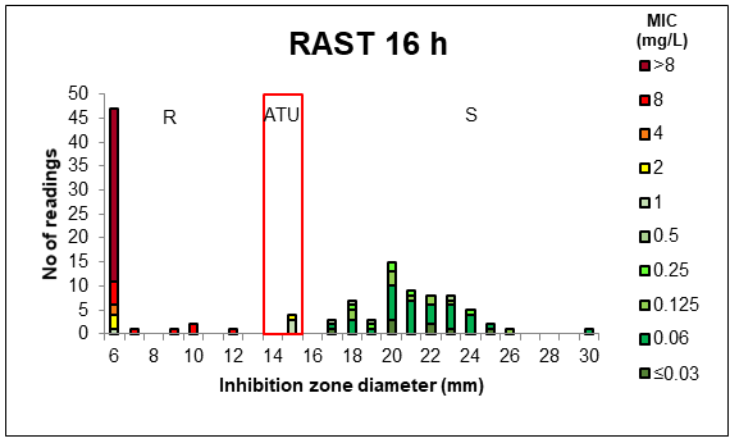
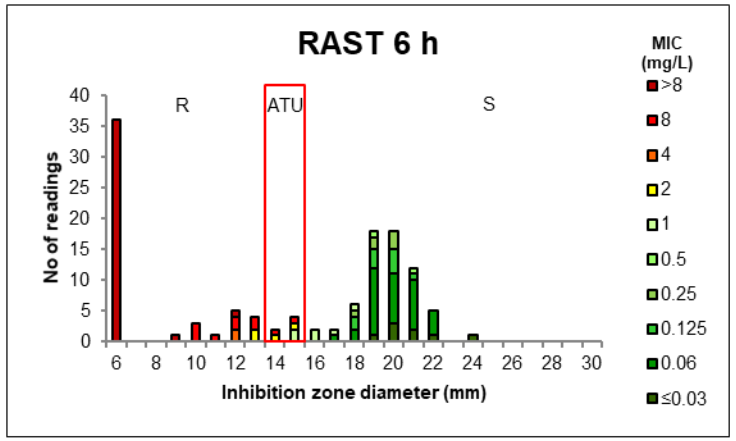
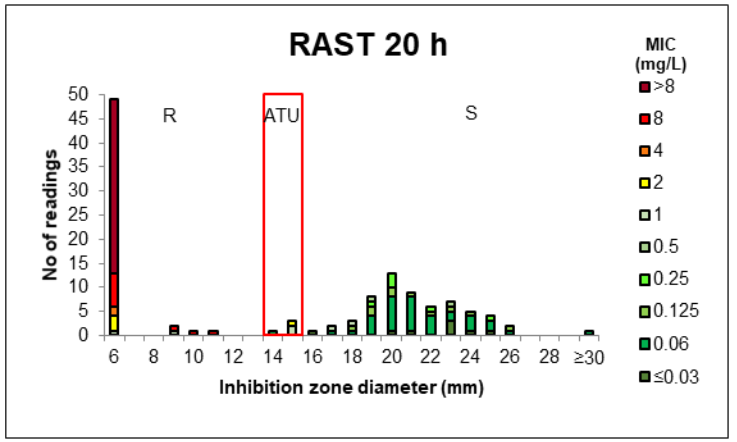
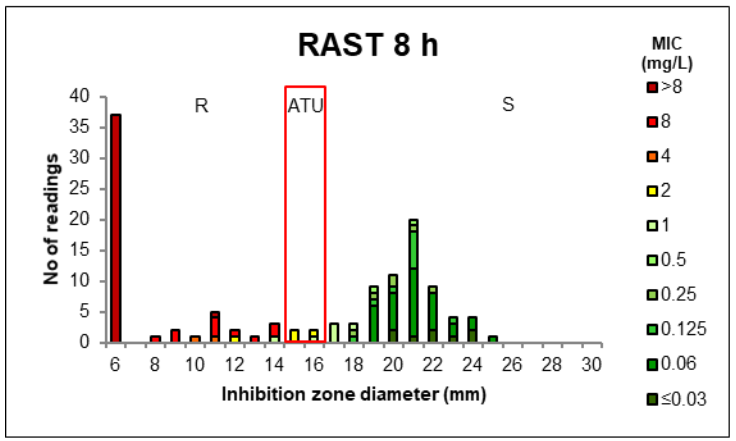
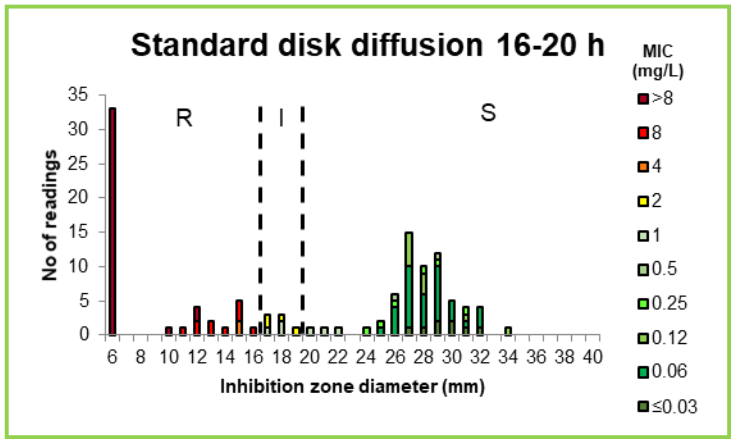




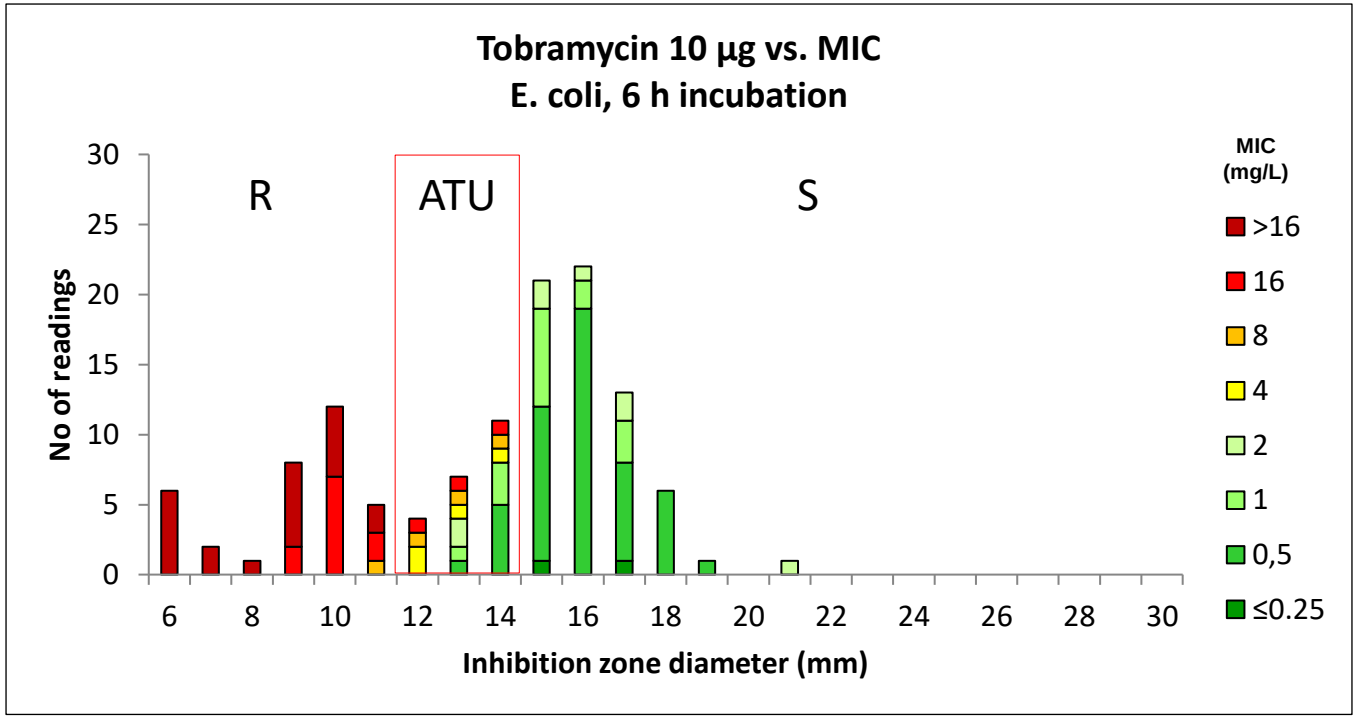








Area of Technical Uncertainty (ATU)



- No AST report was given for results within ATU
  - ATU after 4 or 6 hours = plates are reincubated.
  - ATU also after 8 hours, retest with standard methodology.
  - ATU after 16-20 hours, retest with standard methodology.

Categorical agreement

Northen and Sothern European study, clinical samples and all species.
Number of readable zones 4h: 5811, 6h: 6921, 8h: 6561
Breakpoint version 1.

Incubation time		4h	6h	8h
Results calculated on readable zones (%)				
ATU		16	7.5	5.7
Interpreted o S or R		84	93	94
Errors calculated on zones interpreted to S or R (%)				
Errors	mE	0.6	0.6	0.8
	ME	2.1	1.1	0.9
	VME	0.2	0.4	0.5
		3.0	2.1	2.2

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, 8 and 16-20 hours reading
    3. Practice reading

RAST in a clinical laboratory

Implementation

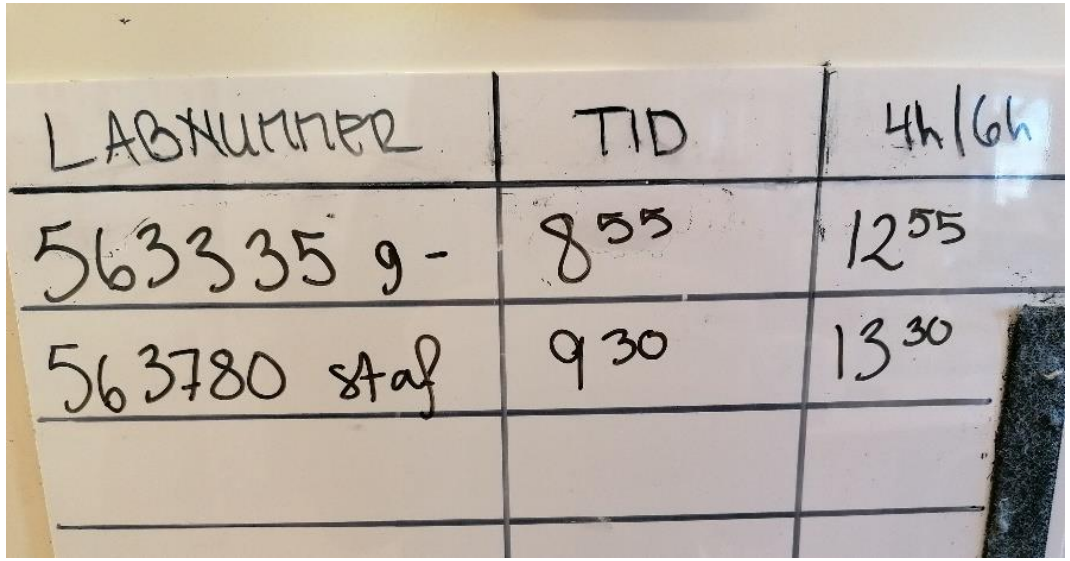
- Should RAST be perform on all bottles?
- Which incubation time?
- How is the results interpreted?
  - Avoid manual interpretation
  - Incorporate in LIS system

- Staff training
  - Theoretical
  - Practical
- Information to clinician

Incubation start	4h reading	6h reading
8.00	12.00	14.00
9.00	13.00	15.00

Daily routine

- Priorities - allocate staff resources
  - Much work in parallel (Gram stain, MALDI-TOF, plating, phone, RAST)



- Read at correct time
  - Timer
  - Whiteboard

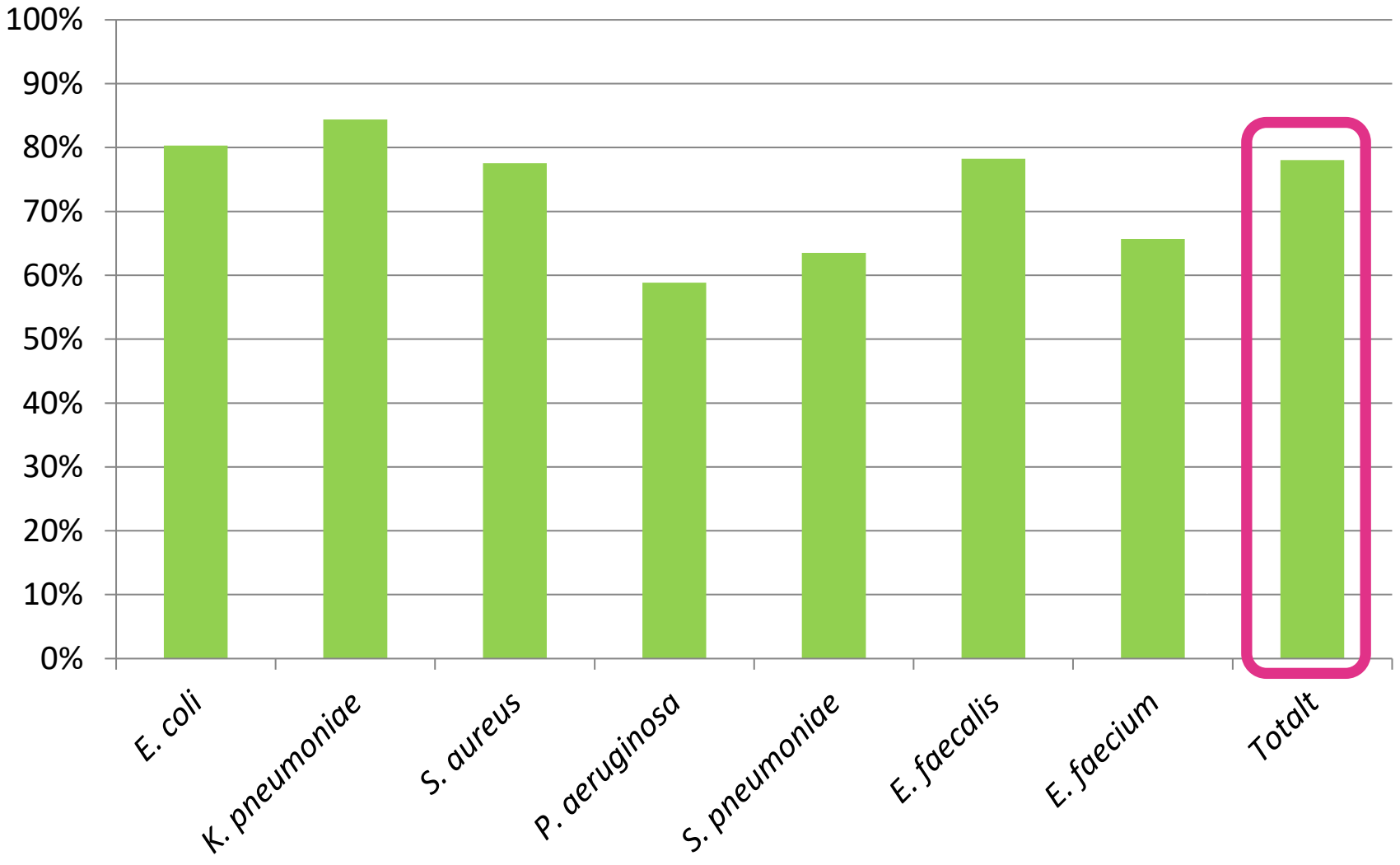
Results from our clinical lab

- Primary reading time 4h
  - First AST results in 5.5 hours from removal of bottle
- Fraction of isolates with completed RAST, in average 78 %

Fraction of isolates with completed RAST

Time period 14 months, RAST 4, 6 and 8 hours

N=1300 isolates

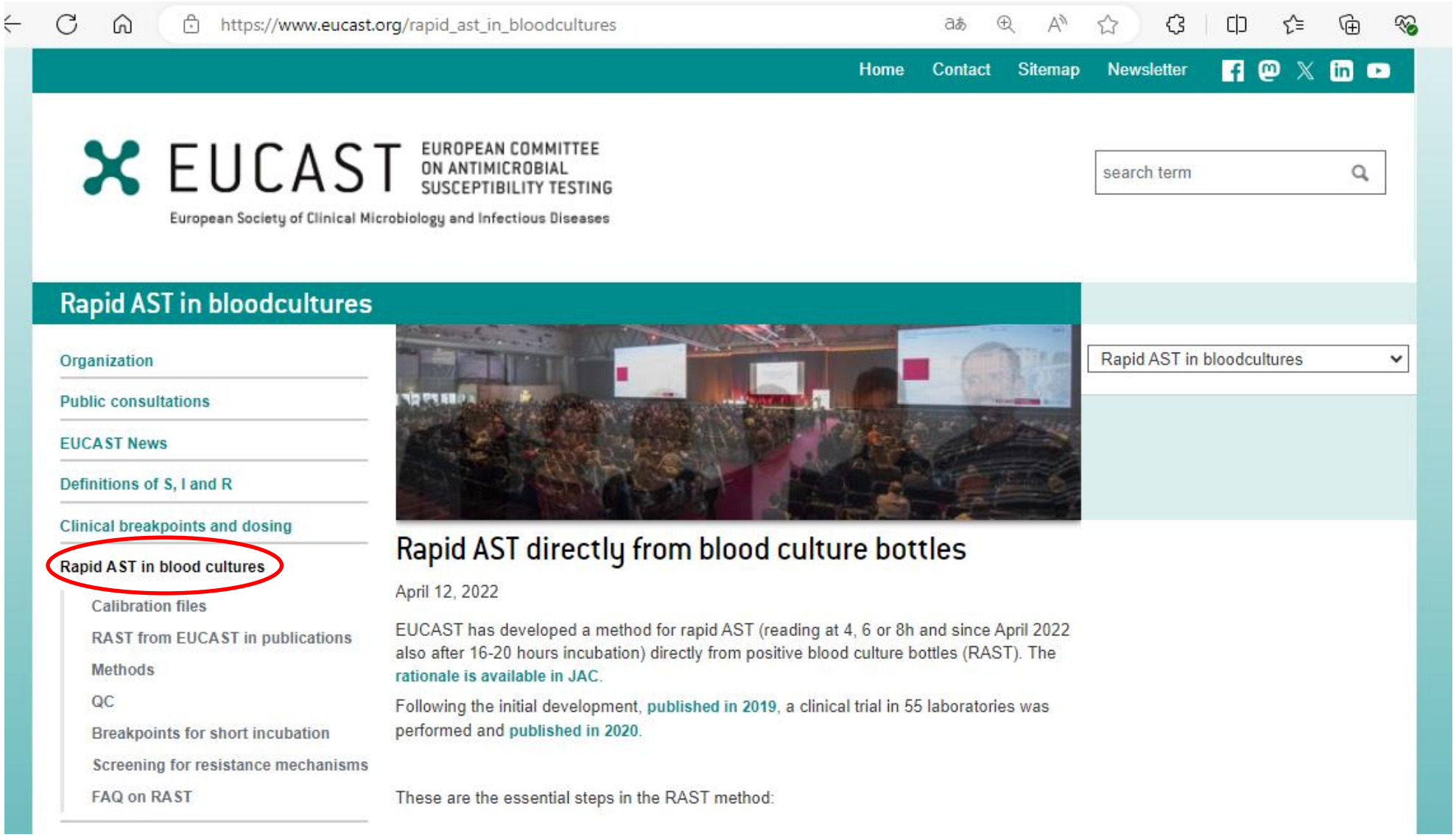


Categorical agreement

RAST 4, 6 and 8 hours during 1 year

Species		***E. coli***			***S. aureus***		
Incubation time		**4h**	**6h**	**8h**	**4h**	**6h**	**8h**
Number of isolates tested		138	223	4	68	139	3
Readable zones (%)		98	100	75	95	98	100
Results calculated on readable zones (%)							
Not interpreted to S or R (ATU)		17	11	10	8	10	0
Interpreted to S		79	83	90	91	87	100
Interpreted to R		4	6	0	1	4	0
Errors calculated on zones interpreted to S or R (%)							
Errors	mE	1.3	0.9	0	0	0	0
	ME	0.1	0.5	0	0	0.8	0
	VME	0	0	0	0	0	0
	Total errors:	1.4	1.4	0	0	0.8	0

EUCAST website



Future

- Breakpoints for new agents and/or breakpoints for additional species when considered relevant by the EUCAST committee

- A similar methodology for urinary tract samples from patients with bloodstream infections – to reduce the time to susceptibility results

Questions?

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