

# Rapid antimicrobial susceptibility testing from positive blood cultures

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# Why Rapid Antimicrobial Susceptibility Testing (RAST)?

- Increasing number of resistance
- A rapid method for AST in the laboratory also requires:
  - Short transport times
  - Availability of blood culturing cabinets
  - Communication of results with clinicians

# Facts

- Disk diffusion directly from positive blood cultures with a incubation time  $\leq 8$  h
- Reliable S and R categorisation
- Based on EUCAST standardised method
- Available for other laboratories
- EUCAST approved

# Study layout

Phase 1. Inoculated bottles, resistant and susceptible strains

- 2 MH agar manufacturers
- BD BACTEC

-*E. coli*, *K. pneumoniae*,  
*S. aureus*, *S. pneumoniae*

-*Ps. aeruginosa*, *H. influenzae*,  
Enterococci

Phase 2. Routine diagnostics

- BD BACTEC

Phase 3. Additional clinical laboratories

- Different blood culture system

Phase 4. Inoculated different types of blood culture bottles

- BD, BACTEC
- bioMérieux, BactAlert
- Thermo Fisher, VersaTREK

# Factors investigated

Amount of blood  
in bottles (2-10  
mL)

Time from  
positive signal to  
removal from  
cabinet

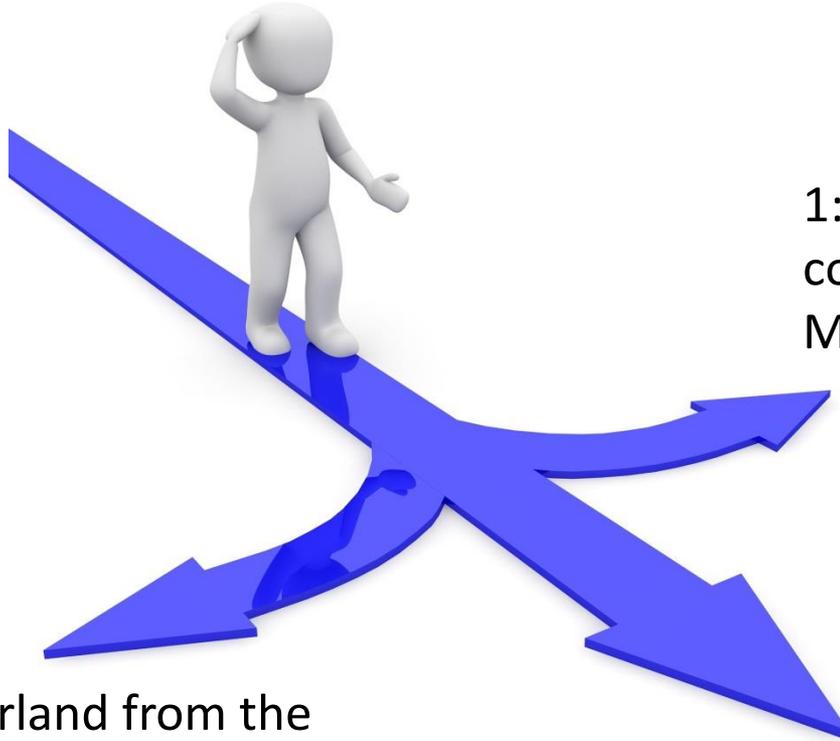


Inoculation  
method

Incubation time,  
test interval 2h-  
8h

Warm (35C) / room  
temperature blood  
culture bottles

# Inoculation method?

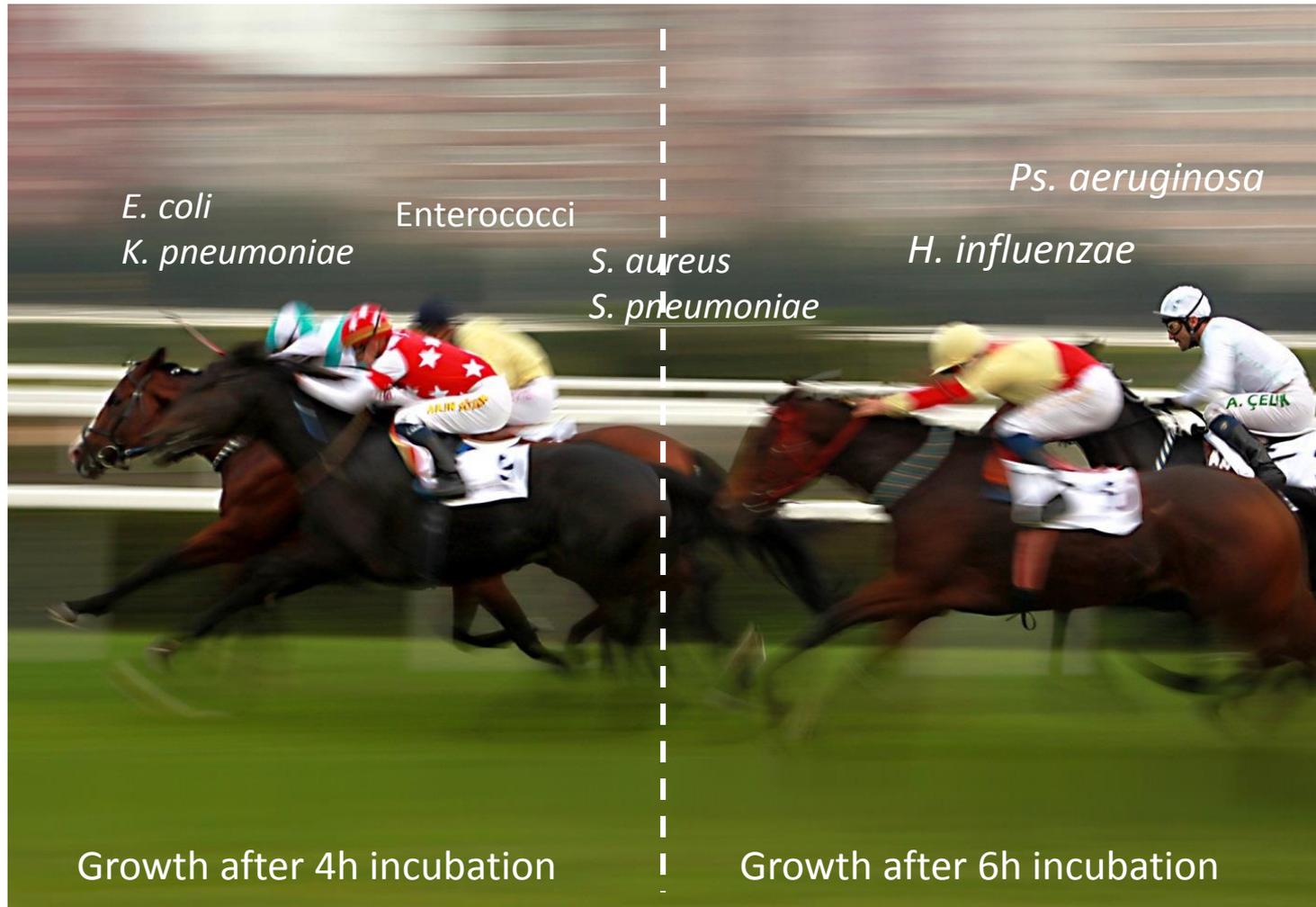


1:10 dilution in saline,  
corresponding app. 0.5  
McFarland

0.5 McFarland from the  
pellet used for direct ID  
with Maldi-tof

Blood directly from bottle  
(100-150  $\mu\text{L}$ )  
Chosen due to shortest  
time to results (1-2h  
earlier)

# How fast do they grow?



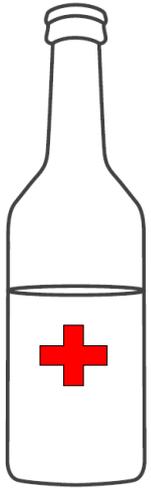
# Suggested methodology

EUCAST standard method for disk diffusion with modified inoculum and incubation time.

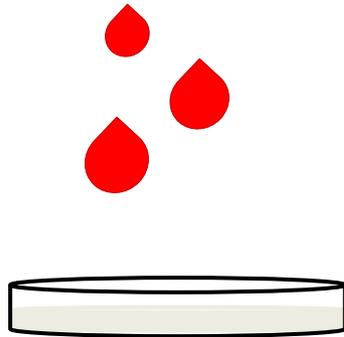
Inoculum: 100-150  $\mu$ l (3 drops from 2mL syringe)

Incubation time: 4, 6 and/or 8 hours

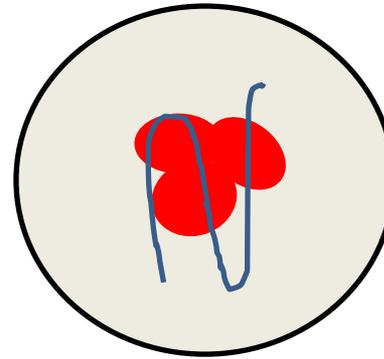
# Inoculation and incubation



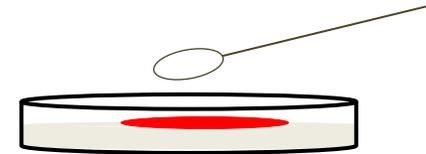
1. Gram or ID



2. 100-150  $\mu\text{l}$  (3 drops)



3. Distribute the  
blood



4. Spread over  
agar surface

5. Apply antibiotic disks

6. Incubation time: 4, 6 and 8 hours

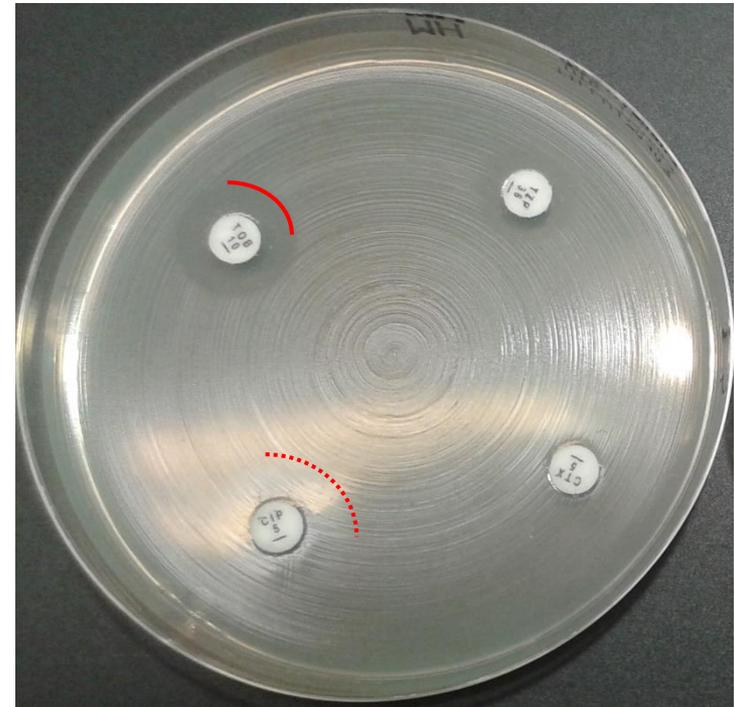
# Reading of zones

## Recommendation

- Hold the plate at 30 cm distance
- Read inhibition zones from the front without lid

## Specific reading instructions

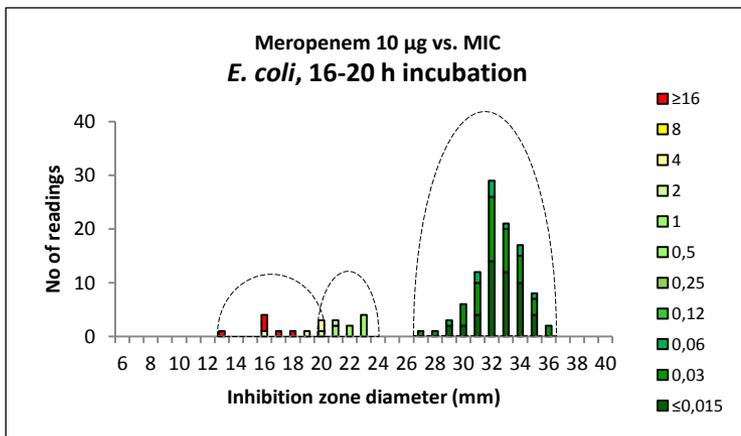
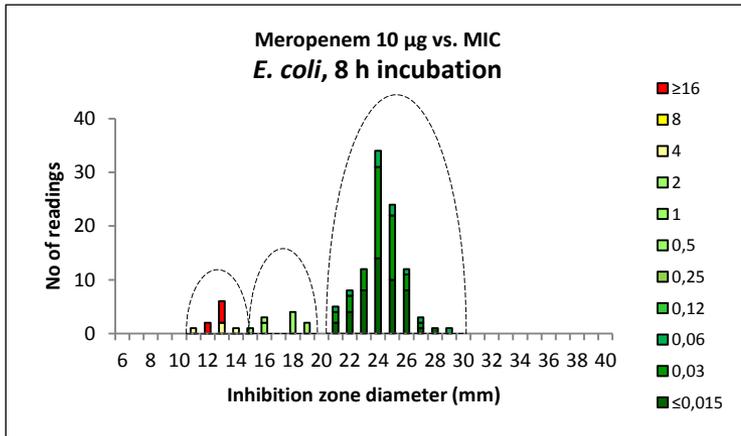
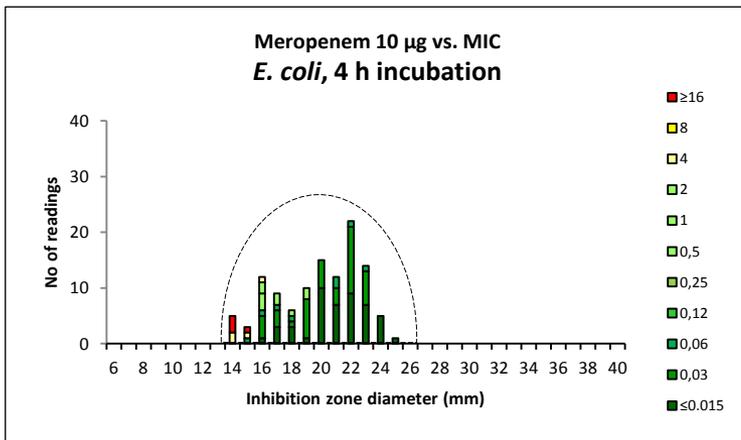
- **Read zones only when a clear zone edge is visible**
- Disregard thin growth within the zone



*E. coli* at 4h incubation

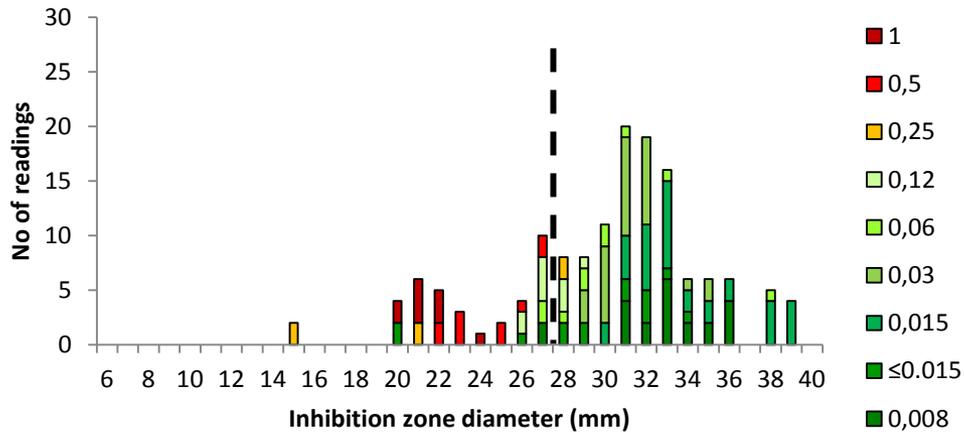
Zone sizes change with incubation time!

Separation improves with increasing incubation time.



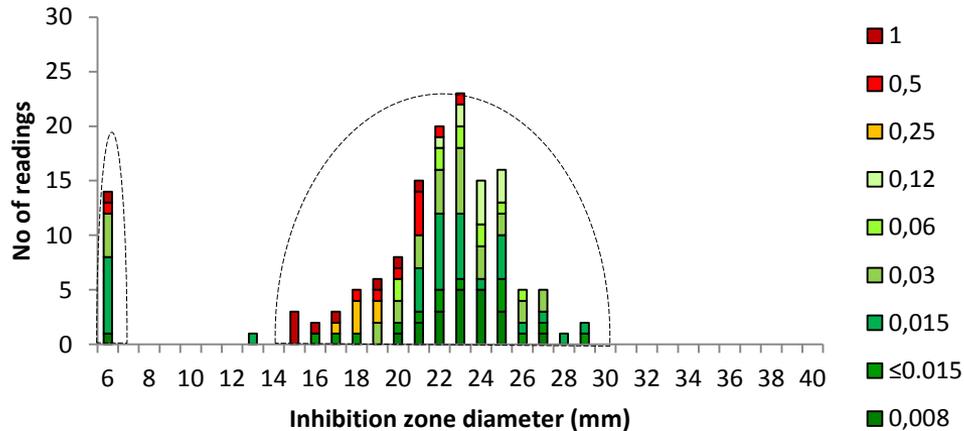
## Zones at short incubation -difficult combinations

**Cefotaxime 5 $\mu$ g vs. MIC  
*H. influenzae*, 16-20h incubation**



Difficult to separate S and R with standard incubation.

**Cefotaxime 5 $\mu$ g vs. MIC  
*H. influenzae*, 8h incubation**

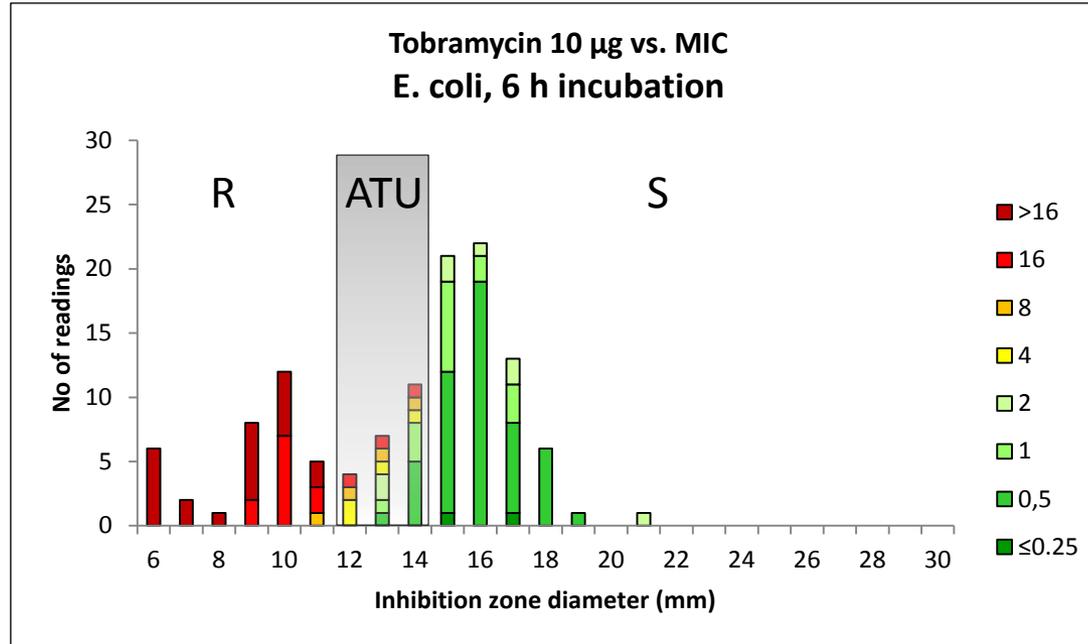


More difficult to separate S and R with short incubation.

# Breakpoints

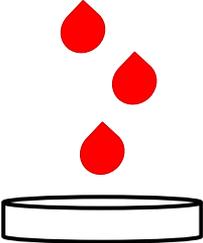
- Set to avoid false susceptibility
- Occasional false resistance accepted
  
- Separate breakpoints per
  - Organism
  - Sometimes per incubation time

# 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.

Gram-staining or  
Identification



ID required

4h

S/R

ATU

Reincub. 2h

6h

S/R

ATU

Reincub. 2h

8h

S/R

ATU

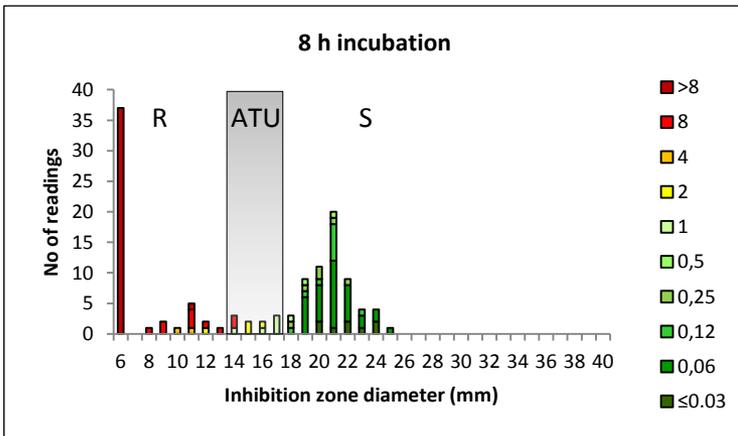
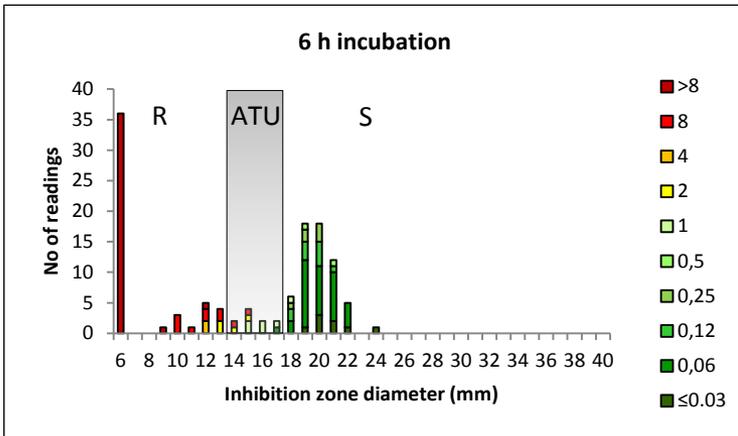
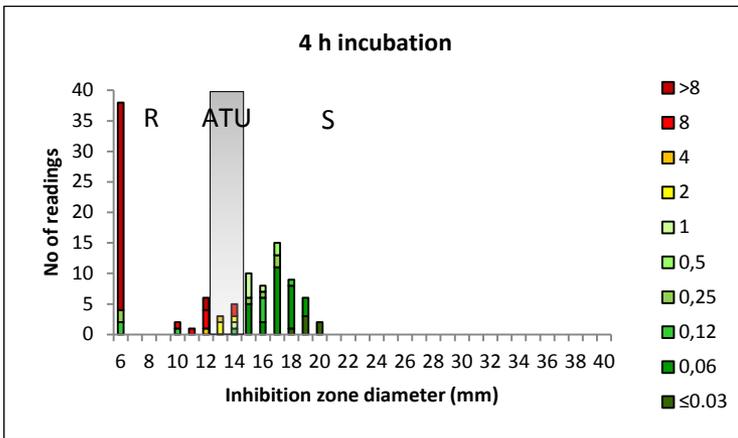
EUCAST standard  
method 16-20h

# Suggested Breakpoint Table

<b><i>E. coli</i></b> Antimicrobial agent and disk content	Proposed breakpoints (mm)								
	4h			6h			8h		
	S≥	ATU	R<	S≥	ATU	R<	S≥	ATU	R<
Piperacillin-tazobactam 30-6 µg	-	≥12	12	-	≥12	12	-	≥12	12
Cefotaxime 5 µg	15	13-14	13	18	14-17	14	18	14-17	14
Ceftazidime 10 µg	15	12-14	12	16	14-15	14	17	14-16	14
Meropenem 10 µg	17	12-16	12	17	14-16	14	17	14-16	14
Ciprofloxacin 5 µg	16	13-15	13	19	15-18	15	20	15-19	15
Gentamicin 10 µg	14	12-13	12	14	12-13	12	14	12-13	12
Tobramycin 10 µg	14	12-13	12	15	12-14	12	15	12-14	12
Amikacin 30 µg	15	13-14	13	15	13-14	13	15	13-14	13

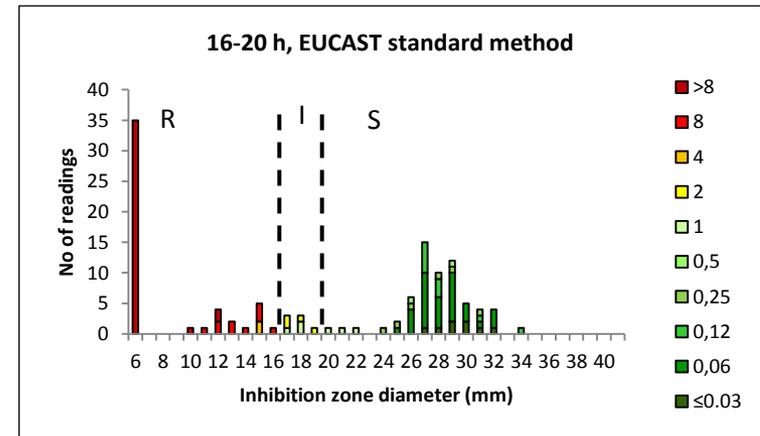
<b><i>K. pneumoniae</i></b> Antimicrobial agent and disk content	Proposed breakpoints (mm)								
	4h			6h			8h		
	S≥	ATU	R<	S≥	ATU	R<	S≥	ATU	R<
Piperacillin-tazobactam 30-6 µg	-	≥12	12	-	≥12	12	-	≥12	12
Cefotaxime 5 µg	15	12-14	12	18	14-17	14	18	14-17	14
Ceftazidime 10 µg	15	11-14	11	16	12-15	12	17	11-16	11
Meropenem 10 µg	15	12-14	12	17	13-16	13	17	13-16	13
Ciprofloxacin 5 µg	18	15-17	15	18	15-17	15	19	16-18	16
Gentamicin 10 µg	14	12-13	12	14	12-13	12	13	12	12
Tobramycin 10 µg	14	12-13	12	13	11-12	11	13	11-12	11
Amikacin 30 µg	15	13-14	13	14	11-13	11	13	10-12	12

# *E. coli* and cefotaxime 5 µg vs. MIC

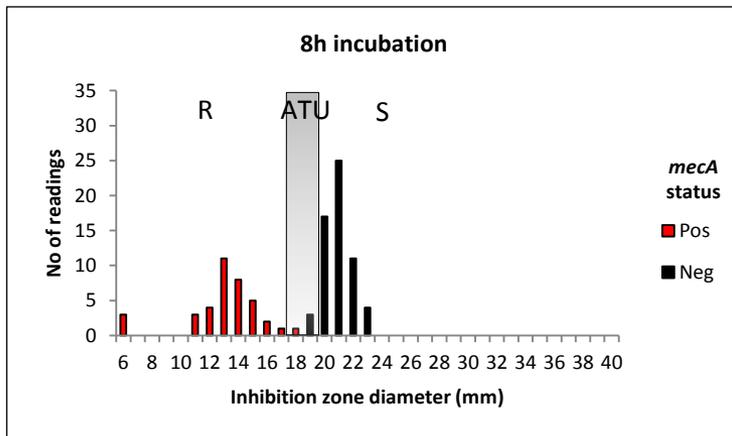
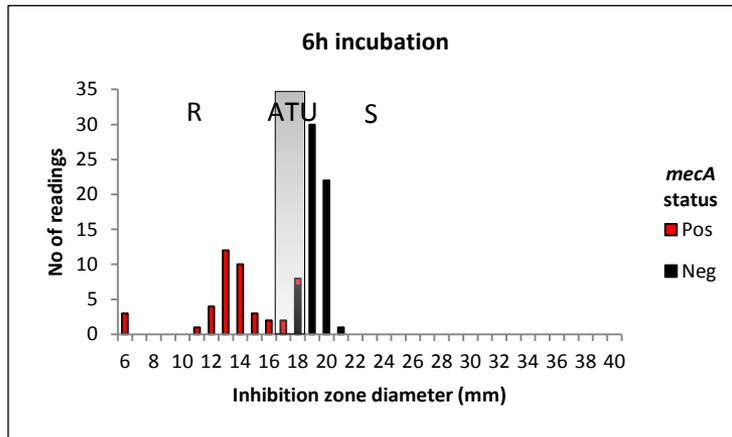
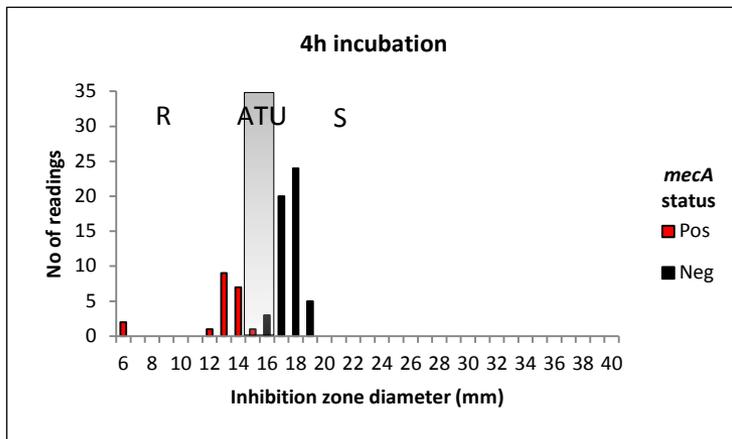


Isolate	MH Agar Manuf.	Incubation time								Reference MIC
		4h SIR	4h zone (mm)	6h SIR	6h zone (mm)	8h SIR	8h zone (mm)	16-20h SIR	16-20h zone (mm)	
1	Oxoid	ATU	13	R	13	R	12	I	17	2
2	Oxoid	ATU	13	R	13	U	15	I	17	2
3	Oxoid	R	6	S	20	S	19	S	27	0,12
3	BD/BBL	R	6	S	19	S	21	S	27	0,12
4	Oxoid	R	6	S	19	S	20	S	29	0,25
4	BD/BBL	R	6	S	20	S	19	S	31	0,25
5	BD/BBL	R	10	S	19	S	21	S	34	0,12

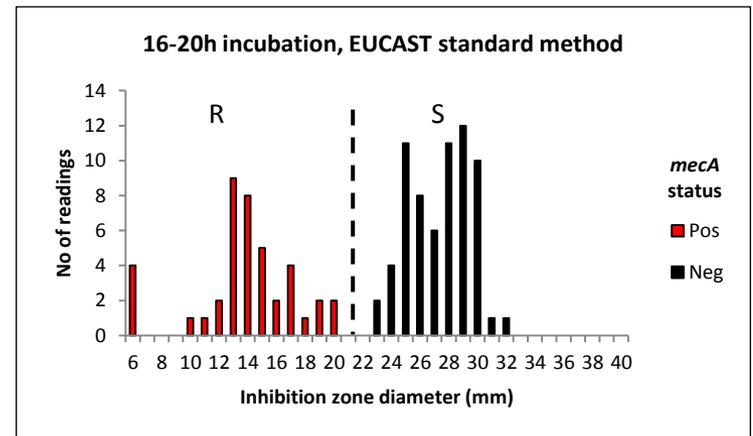
Number of tests per incubation time with incorrect categorisation compared with reference MIC.



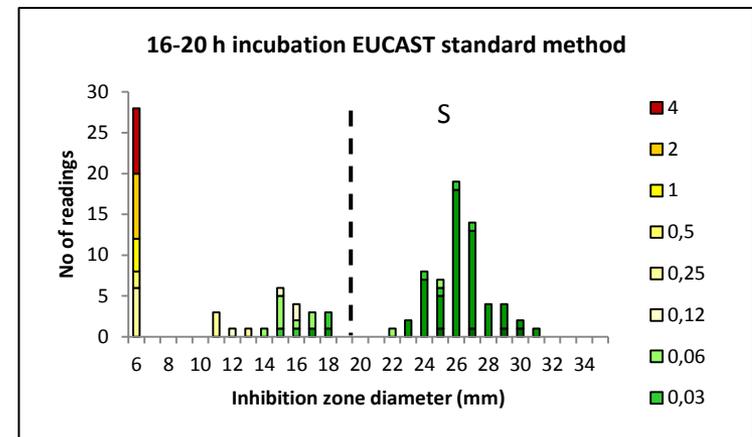
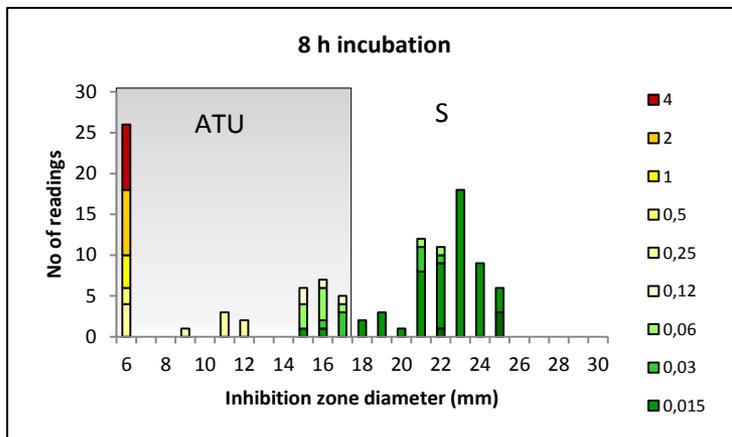
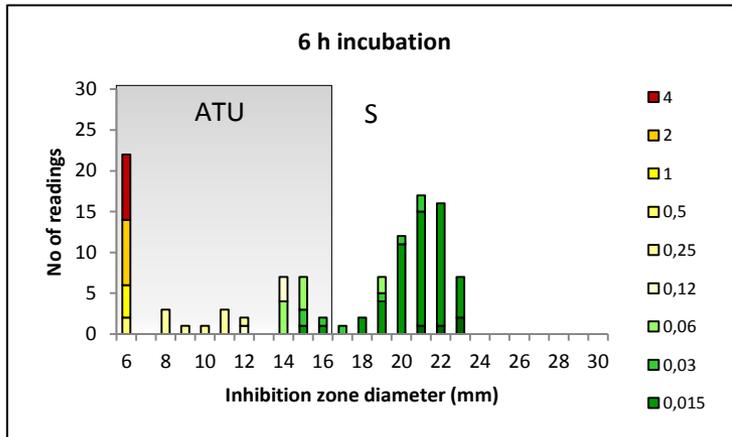
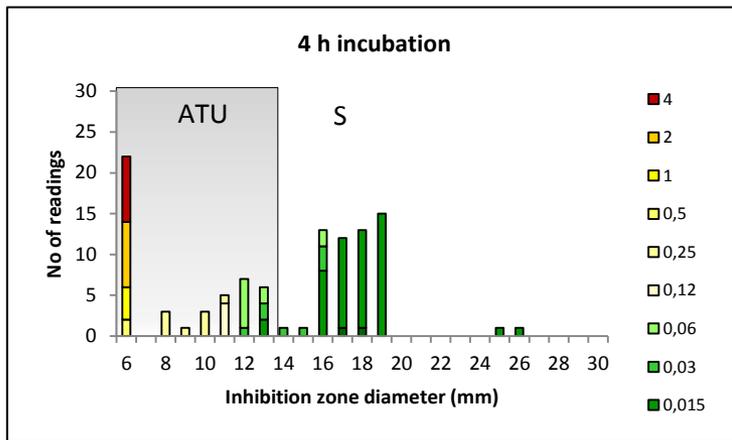
# *S. aureus* and cefoxitin 30 µg vs. *mecA* status



No incorrect categorisation with 4, 6 and 8 hours incubation compared with *mecA* status.



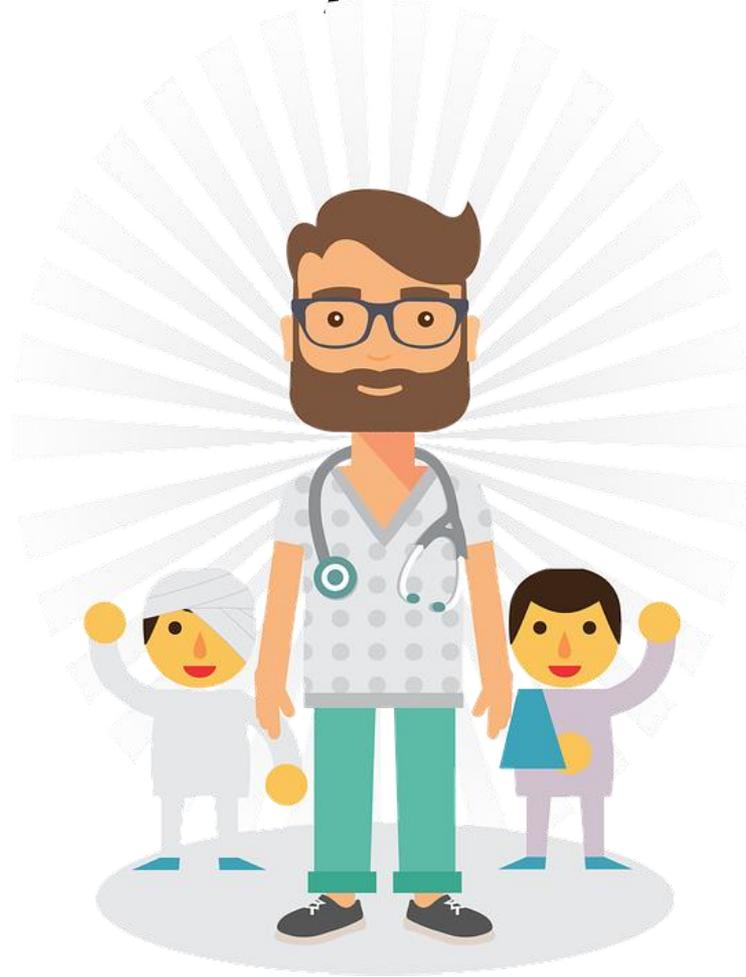
# *S. pneumoniae* and oxacillin 1 $\mu\text{g}$ vs. Benzylpenicillin MIC



# Conclusion

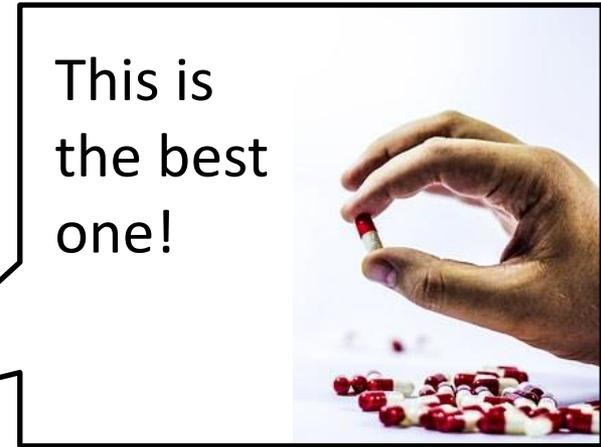
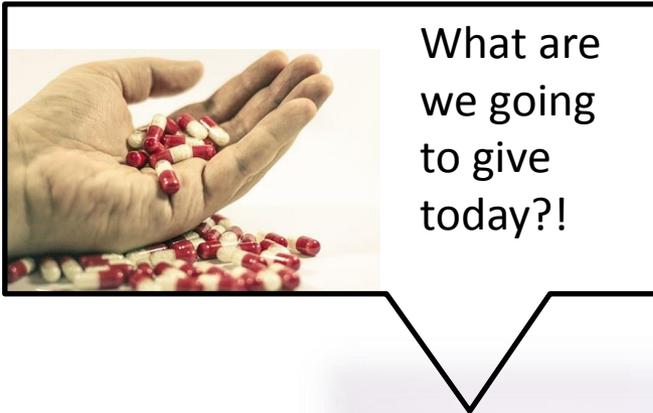
- Rapid antimicrobial susceptibility testing from positive blood cultures
- Results after 4 and 6h
- Area of Technical Uncertainty (ATU)
- Rapid results for sepsis patients

# Thanks for your attention!



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# Thanks for your attention!



Contact: [emma.k.jonasson@kronoberg.se](mailto:emma.k.jonasson@kronoberg.se)



**APPROVED**

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**APPROVED**

**APPROVED**



 **EUCAST** EUROPEAN COMMITTEE  
ON ANTIMICROBIAL  
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

# Aim

- Disk diffusion directly from positive blood cultures with a incubation time  $\leq 8$  h
- Reliable S and R categorisation
- Based on EUCAST standardised method
- Available for other laboratories



EUCAST

