

EUCAST rapid AST by disk diffusion directly from blood culture bottles.

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On behalf of

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and 40 North European clinical laboratories

and 15 South European clinical laboratories



EUCAST

EUROPEAN COMMITTEE
ON ANTIMICROBIAL
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Improving management of blood stream infections.

- Wide indications for blood culturing (BC).
- Immediate sampling on triaging.
- Delegate to first line nurse to order BC; avoid doctor's delay.
- Mandatory blood culture prior to IV antibiotics.
- Immediate transport of BC bottles to incubator 24/7.
- Incubator available for loading 24/7.
- Immediate species ID (≤ 60 min from positive signal): masspec; genotypic methods; microscopy.
- Immediate AST (tests set up within 60 min)

Solve the boring logistics!

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Rapid AST from positive blood culture (BC)

- **Rapid ID**

- Masspec, various protocols (60 min – 4h)
- Semiautomated devices (Vitek2, Phoenix) (6 – 8h)
- Microscopy (Grampositive, Gramnegative in 10 min)

- **Rapid phenotypic AST (4 – 8h at best)**

- Direct phenotypic AST with reading after 16 – 20 h.
- Short time solid medium subculture followed by
 - regular AST 16 – 20 h (disk, gradient test).
 - shortened AST 6 – 12 h with a regular method (Semiautomated device, disk diffusion, etc)
- A variety of alternative methods (MS, FC, Colorometric/immuno-chromatographic methods, time lapse microscopy) directed at one or two antibiotics only.

- **EUCAST direct phenotypic AST with short incubation and calibrated adjusted breakpoints.**

Why is EUCAST involved?

- **A new system with a different and uncontrolled inoculum and a shorter than normal incubation time will need**
 - recalibration of the whole system
 - alternative breakpoints adapted to short time incubation
 - a system which can tolerate an increased variability
 - and most probably short and standard (16 – 20h) incubation will need different breakpoints.
- Requests for standardisation from many colleagues

EUCAST was tasked with....

- developing a method based on **standardised methodology** and equipment and material ...
 - available to all microbiological laboratories.
 - valid for the most important septicemia pathogens
 - for agents commonly used in septicemia
 - with as few complicated steps as possible
 - which can be quality controlled
 - where breakpoints were validated for each of the short reading times
 - freely available on the EUCAST website.

We systematically controlled the influence of...

Three manufacturers of blood culture bottles

- BD, BACTEC
- bioMérieux, BactAlert (two different bottles were included)
- Thermo Fisher, VersaTREK

The amount of blood in bottles (2-10 mL)

Time from positive signal to removal from cabinet (0.5 – 18h)



Inoculation method

Incubation time, test interval 2 – 8h

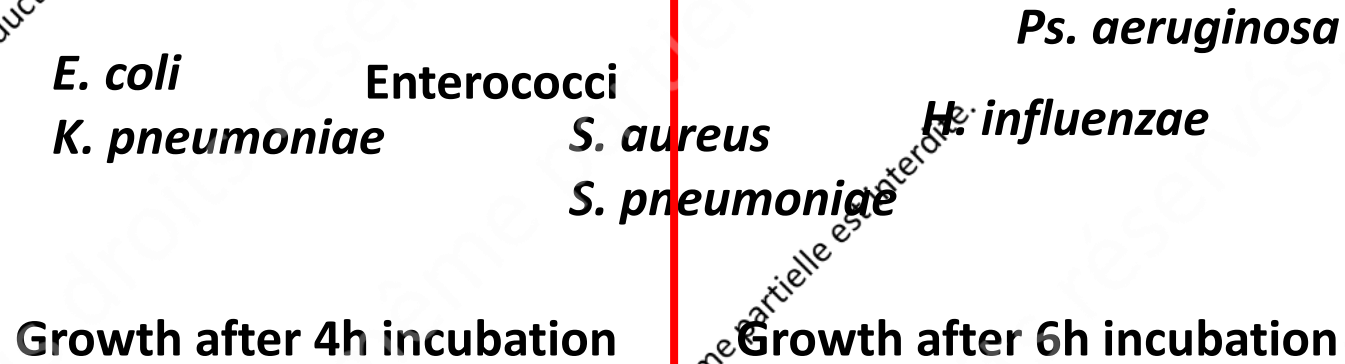
The temperature of blood culture bottles

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What to expect after 4, 6 and 8h?



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EUCAST rapid AST basic methodology

- Directly from BC bottle, no centrifugation – keep the system “warm”
- 100-150 μl (3 drops from 2mL syringe)
- Streak MH and MH-F plates (room temperature)
- Place disks on plates
- Incubate at 35 - 37C
- Read zones after 4, 6 **and** 8h
 - Read zones only when a clear zone edge is visible
 - Disregard thin growth within the zone
- Interpret zone diameters using the specific BP tables on EUCAST website (available from Dec 2018).

1. Spiked bottles

- Clinical isolates + sterile horse blood
- Wild type isolates and organisms with multiple resistance mechanisms (MRSA, ESBLs, KPC, Oxa48, VRE)

Escherichia coli

Klebsiella pneumoniae

Pseudomonas aeruginosa

Haemophilus influenzae

Staphylococcus aureus

Streptococcus pneumoniae

Enterococcus faecalis and *E. faecium*

During 2019:

Acinetobacter spp

Staphylococcus epidermidis

Antimicrobial agents

E. coli* & *K. pneumoniae

- Piperacillin-tazobactam
- Cefotaxime
- Ceftazidime
- Meropenem
- Ciprofloxacin
- Amikacin
- Gentamicin
- Tobramycin

Ps. aeruginosa

- Piperacillin-tazobactam
- Ceftazidime
- Imipenem
- Meropenem
- Ciprofloxacin
- Gentamicin
- Tobramycin

S. aureus

- Cefoxitin
- Norfloxacin (screen for FQ resistance)
- Gentamicin
- Erythromycin
- Clindamycin

S. pneumoniae

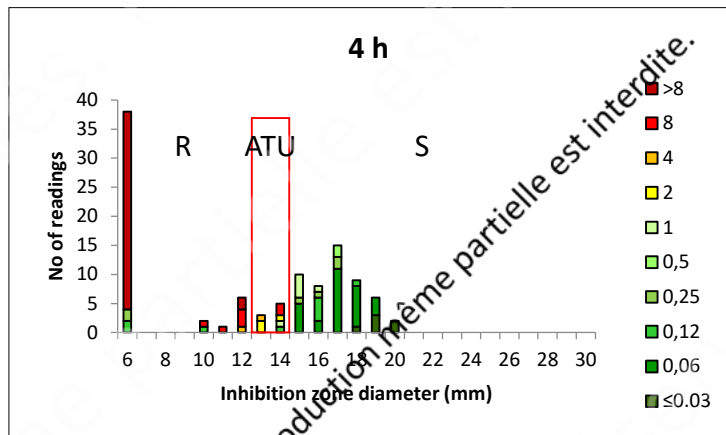
- Oxacillin (PCG)
- Norfloxacin (FQ)
- Erythromycin
- Clindamycin
- Trimethoprim-sulfamethoxazole

E. faecalis* *E. faecium

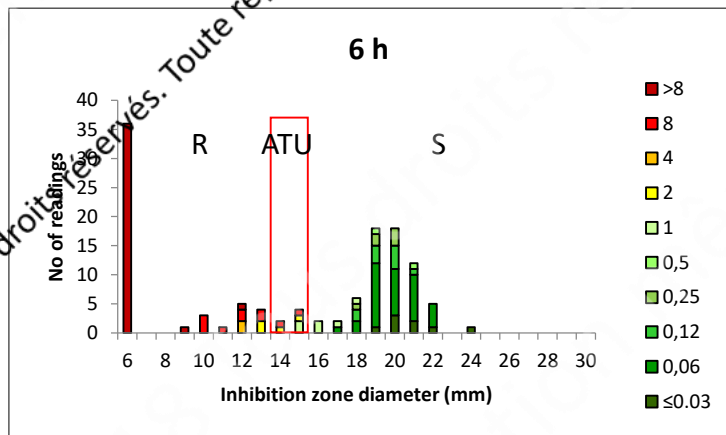
- Ampicillin
- Imipenem
- Gentamicin
- Vancomycin
- Linezolid

Additional agents are currently being validated.

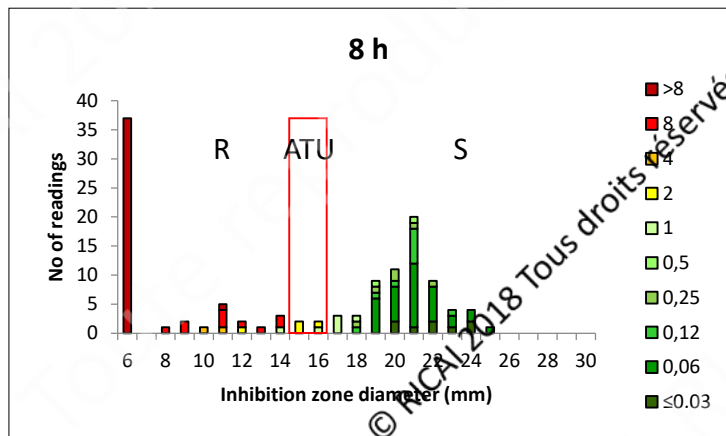
4h



6h



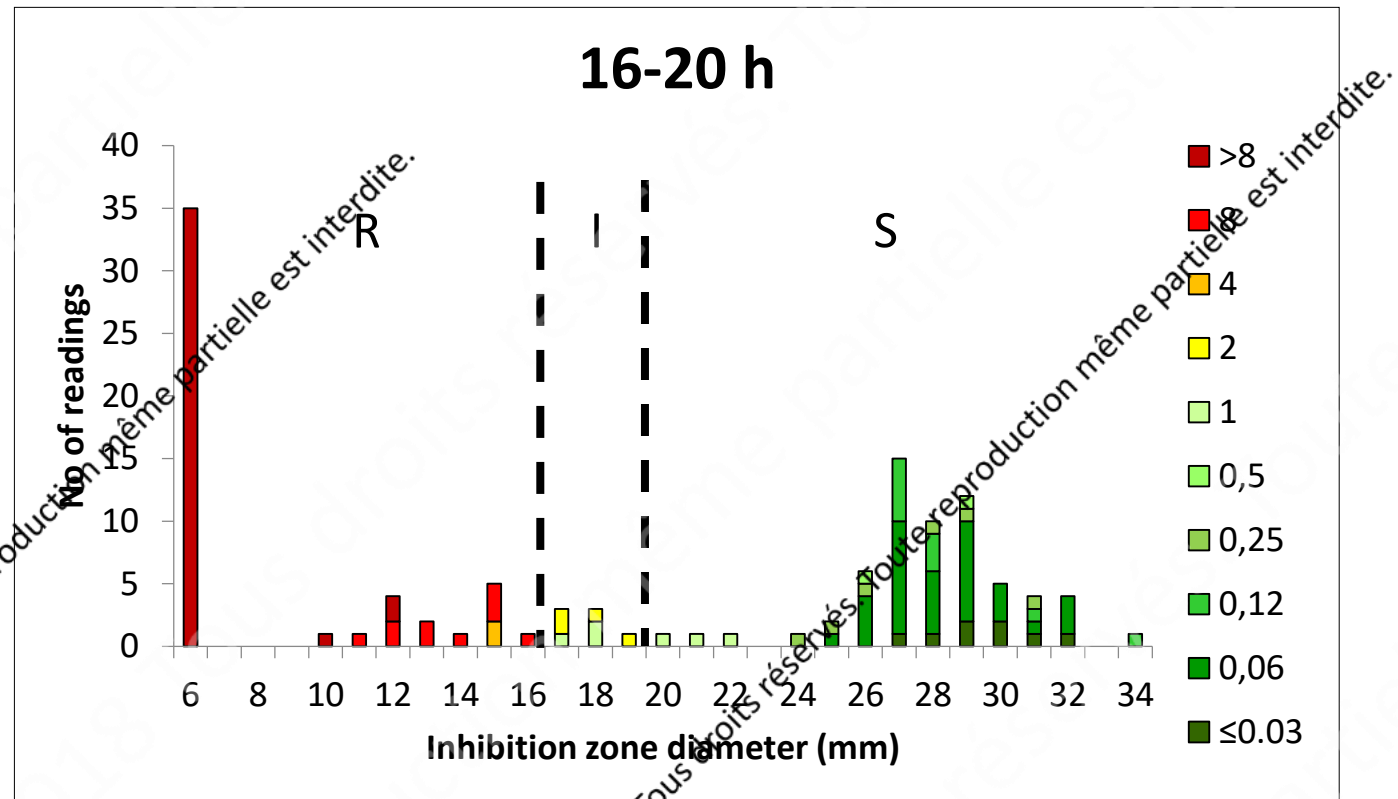
8h



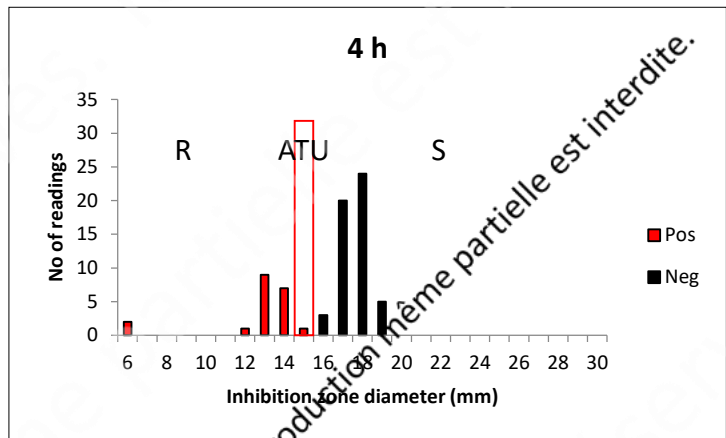
Spiked bottles

E. coli vs cefotaxime 5 µg

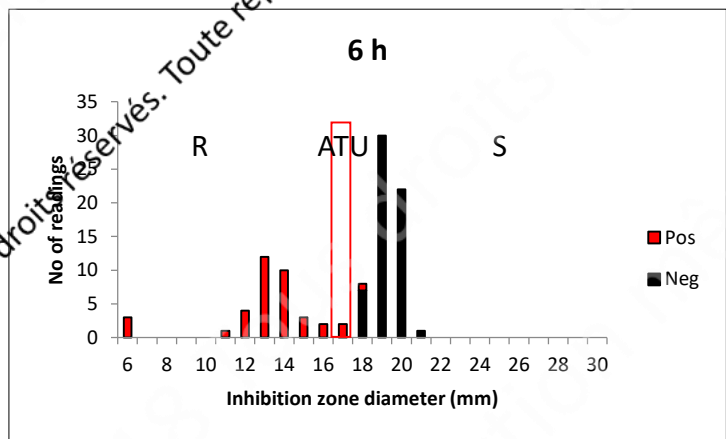
Broth microdilution MIC values



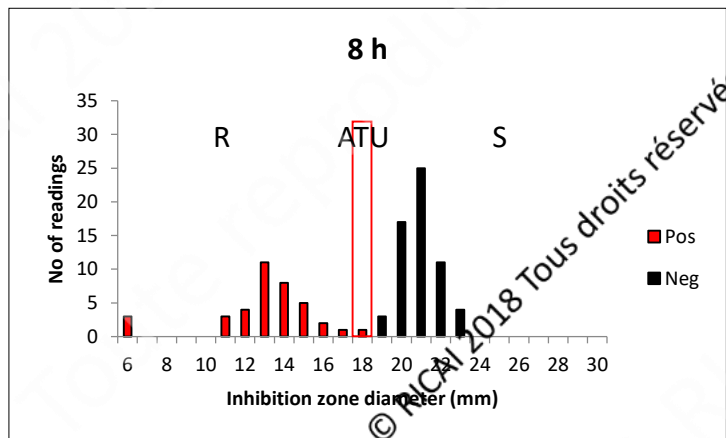
4h



6h

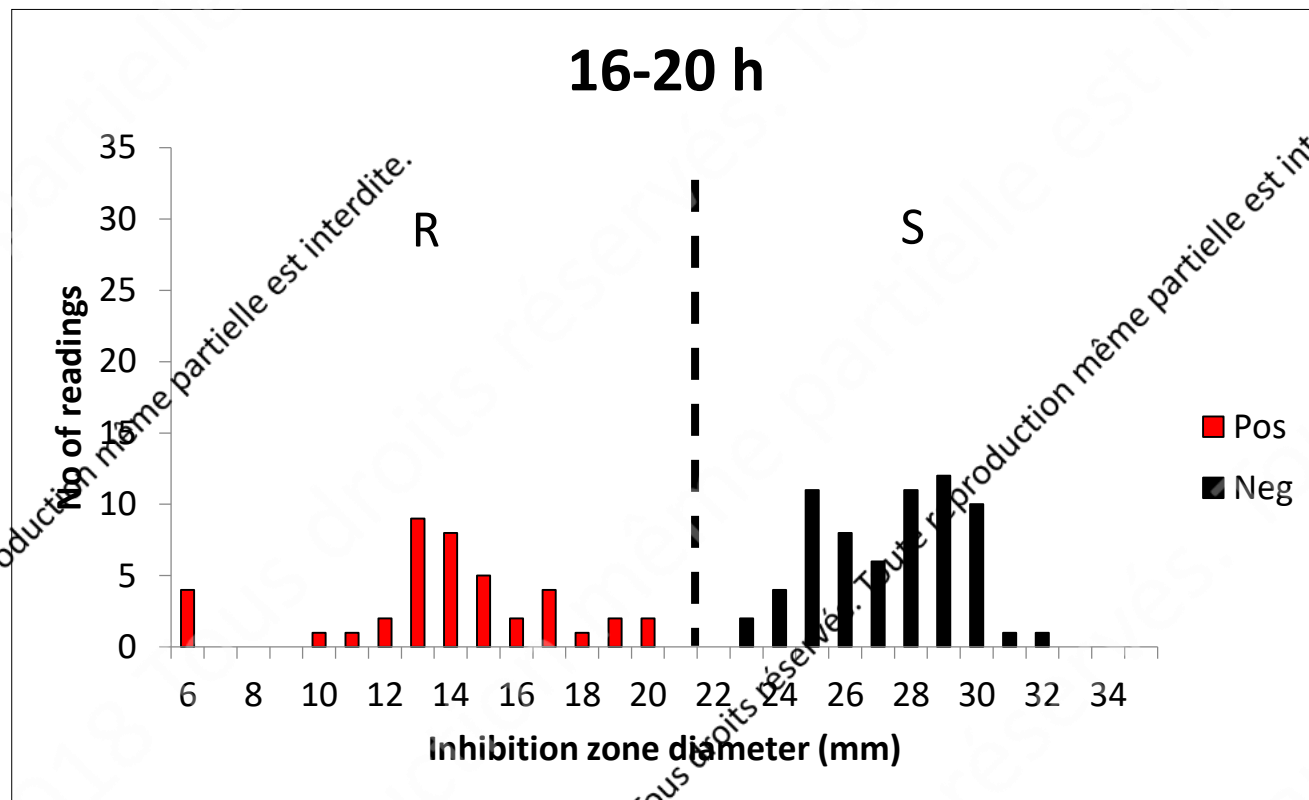


8h



Spiked bottles

S. aureus and cefoxitin 30 µg vs. Vs. mecA/mecC status (PCR)



Rapid AST in blood cultures

Organization

EUCAST News

New definitions of S, I and R

Clinical breakpoints and dosing

Rapid AST in blood cultures

Methods

Breakpoints for short incubation

Expert rules and intrinsic resistance

Resistance mechanisms

Guidance documents

Consultations - New!

MIC and zone distributions and ECOFFs

AST of bacteria

AST of mycobacteria

AST of fungi

AST of veterinary pathogens

Frequently Asked Questions (FAQ)

Meetings

Presentations and statistics

Warnings!

Documents

Videos from EUCAST

Translations

Information for industry



Rapid AST in bloodcultures

Rapid AST directly from blood culture bottles

EUCAST will shortly publish recommendations for short incubation (4, 6 and 8 hours) AST directly from positive blood culture bottles using EUCAST standard disk diffusion. These are the characteristics of the rapid method:

- direct inoculation of disk diffusion plates (MH, MH-F) using 100 - 150 μ L directly from a positive blood culture bottle (BD, bioMérieux and Thermo Fisher).
- no centrifugation or dilution of the inoculum - inoculate plates as for standard EUCAST disk diffusion.
- shortened incubation - 4, 6 and 8 hours with breakpoints adapted to each incubation time.
- breakpoints for each species and each reading time.
- identity of species must be known prior to interpretation of AST results.
- the method is currently validated for the following species.
 - *Escherichia coli*
 - *Klebsiella pneumoniae*
 - *Pseudomonas aeruginosa*
 - *Staphylococcus aureus*
 - *Streptococcus pneumoniae*
 - *Enterococcus faecalis* and *Enterococcus faecium*
- a positive blood culture bottle should be processed 0 - 18 hours after the positive signal.
- zone diameters are read from the front of the plate after removal of the lid.
- not all zone diameters can be read after 4 or 6 hours.
- read zone diameters ONLY when an obvious zone edge can be identified - otherwise reincubate and read after 6 or 8 hours.
- the breakpoint table is specific for EUCAST Rapid AST - do not use the regular breakpoint table. Each species has its own TAB in the table and each reading time (4, 6 and 8 hours) its own section.

Breakpoint table and method to be published before the end of November, 2018.

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AST of bacteria

AST of mycobacteria

AST of fungi

AST of veterinary pathogens

Frequently Asked Questions (FAQ)

Meetings



... Methods 

Method - Rapid AST directly from positive blood culture bottles

EUCAST has validated a method for direct plating of disk diffusion MH and MHF agar plates for reading after 4, 6 and 8 hours of incubation. Incubation can not be prolonged - the method is only validated for the short incubation time.

Currently the method is validated for *E. coli*, *K.pneumoniae*, *Ps. aeruginosa*, *S. aureus*, *E.faecalis*, *E.faecium* and *S.pneumoniae* and for a limited panel of antimicrobials. Work is ongoing to extend the method to more species (amongst which *Acinetobacter baumannii* is high on the list) and more antibiotics.

For methodology, see the document

"EUCAST rapid AST directly from positive blood culture bottles".

For hints on how to implement the rapid AST in the laboratory, see **"Implementation of RAST"**.

EUCAST 26 November, 2018

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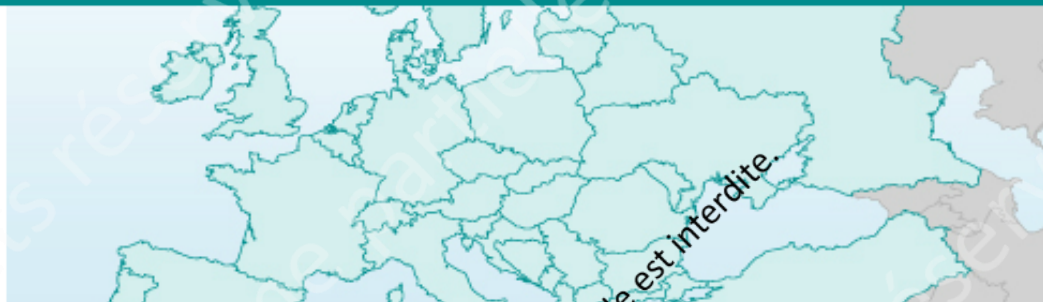
MIC and zone distributions and ECOFFs

AST of bacteria

AST of mycobacteria

AST of fungi

AST of veterinary pathogens



... Breakpoints for short incubation

Breakpoints validated for EUCAST short incubation disk diffusion directly from positive blood culture bottles.

For interpretation of rapid AST directly from blood culture bottles, reading and interpreting inhibition zones after 4 hours, 6 hours or 8 hours, always use the RAST Breakpoint Tables (see below), never use EUCAST standard 16 - 20 h breakpoints (regular EUCAST breakpoint tables).

[Breakpoint tables for Rapid AST directly from blood culture bottles](#) (pdf-file for printing)

[Breakpoint tables for Rapid AST directly from blood culture bottles](#) (Excel-table for screen)

QC recommendations to facilitate the introduction of the methodology in the laboratory are embedded in the breakpoint table (QC tab).

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EUCAST RAST breakpoint tables

- Excel-file for screen and Pdf-file for printing
- Each species has its own table/tab
 - *E. coli*
 - *K. pneumoniae*
 - *Ps. aeruginosa*
 - *S. aureus*
 - *Str. Pneumoniae*
 - *E. faecalis*
 - *E. faecium*
- QC-methodology and QC tables (three ATCC strains) in separate tabs
- ATU explained

EUCAST RAST breakpoint tables

Escherichia coli

EUCAST RAST breakpoint table v. 1.0, valid from 2018-11-28

Zone diameter breakpoints for RAST directly from blood culture bottles

EUCAST rapid disk diffusion method directly from positive blood culture bottles (BCB)
Medium: Mueller-Hinton (MH) agar
Inoculum: 100 - 150 uL directly from a positive BCB
Incubation: Air, 35±1°C
Incubation time: 4, 6 and 8 hours
Reading: Remove lid and read zone edges from the front against a dark background illuminated with reflected light.
[QC for implementation of RAST](#)

Antimicrobial agent	Disk content (µg)	4 hours			6 hours			8 hours		
		S ≥	ATU	R <	S ≥	ATU	R <	S ≥	ATU	R <
Piperacillin-tazobactam	30-6	17	12-16	12	18	14-17	14	18	14-17	14
Cefotaxime ¹	5	15	15-14	13	16	14-15	14	17	15-16	15
Ceftazidime ¹	10	15	12-14	12	16	14-15	14	17	15-16	15
Meropenem ¹	10	18	15-17	15	17	15-16	15	17	15-16	15
Ciprofloxacin	5	17	14-16	14	20	17-19	17	20	17-19	17
Amikacin	30	15	13-14	13	15	13-14	13	15	13-14	13
Gentamicin	10	14	12-13	12	14	12-13	12	14	12-13	12
Tobramycin	10	14	12-13	12	15	13-14	13	15	13-14	13

Notes

1. Screening breakpoints for ESBL or carbapenemase production have not yet been validated. The breakpoints listed are clinical breakpoints. Isolates that are resistant or in the ATU may be suspected of having beta-lactamase mediated resistance.

EUCAST RAST breakpoint tables

Staphylococcus aureus

EUCAST RAST breakpoint table v. 1.0, valid from 2018-11-28

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Reading: Remove lid and read zone edges from the front against a dark background illuminated with reflected light.
[QC for implementation of RAST](#)

Antimicrobial agent	Disk content (µg)	4 hours			6 hours			8 hours		
		S ≥	ATU	R <	S ≥	ATU	R <	S ≥	ATU	R <
Cefoxitin (screen) ¹	30	16	5	15	18	17	17	19	18	18
Norfloxacin (screen) ¹	10	13	≤12	-	14	13	13	15	14	14
Gentamicin	10	14	12-13	12	15	13-14	13	16	15-15	14
Clindamycin ²	2	16	≤15	-	19	16-18	16	19	16-18	16

Notes

1. See comments for the screening test in the current version of the EUCAST Clinical Breakpoint Tables (standard methodology for interpretation).
2. Test for inducible clindamycin resistance: Place clindamycin and erythromycin disks ≤12 mm apart (edge to edge). Look for a D phenomenon after 6 and 8 hours. A positive test can be trusted but a negative test does not guarantee the absence of inducible resistance. Note: For standard clindamycin susceptibility testing, use a separate clindamycin disk (the activity of the erythromycin disk may interfere with the reading of standard clindamycin susceptibility).

RAST QC tables

European Committee on Antimicrobial Susceptibility Testing RAST directly from blood culture bottles

QC criteria for the RAST method where blood culture bottles are inoculated with QC strains.

EUCAST recommends that daily regular QC with standard methodology is performed to control the quality of AST materials and the standardised disk diffusion AST procedure.

The three QC strains in these tables, check the RAST procedure - the inoculation of disk diffusion plates directly from blood culture bottles and the 4, 6 and 8 hour incubation. This QC is relevant when implementing the method in the laboratory, when training new staff or following a change in blood culture system or any other changes in the system.

The QC strains are tested by inoculating blood culture bottles with 1 mL of a 100-200 CFU/mL solution (0.5 McFarland diluted 1:100 000) of the QC strain and with addition of approximately 5 mL sterile blood. The inoculated bottles are incubated in the blood culture instrument and processed according to the RAST methodology following a positive signal.

E. coli ATCC 25922

Antimicrobial agent	Disk content (µg)	4 hours		6 hours		8 hours	
		Range	Target	Range	Target	Range	Target
Piperacillin-tazobactam	30-6	13-18	15-16	15-20	17-18	15-21	18
Cefotaxime	5	14-20	17	17-23	20	17-23	20
Ceftazidime	10	13-19	16	15-21	18	16-21	19
Meropenem	10	14-20	17	18-24	21	16-23	22
Ciprofloxacin	5	19-25	22	22-28	25	23-29	26
Amikacin	30	13-18	15-16	14-20	17	15-21	18
Gentamicin	10	13-18	15-16	14-20	17	15-21	18
Tobramycin	10	13-18	15-16	14-20	17	14-20	17

S. aureus ATCC 29213

Antimicrobial agent	Disk content (µg)	4 hours		6 hours		8 hours	
		Range	Target	Range	Target	Range	Target
Cefoxitin	30	15-19	17	17-22	19-20	19-24	21-22
Norfloxacin	10	13-17	16	14-19	16-17	15-20	17-18
Gentamycin	10	14-19	16-17	15-21	18	15-21	18
Erythromycin	15	15-20	17-18	18-24	21	18-24	21
Clindamycin	2	15-20	17-18	17-23	20	18-24	21

S. pneumoniae ATCC 49619

Antimicrobial agent	Disk content (µg)	4 hours		6 hours		8 hours	
		Range	Target	Range	Target	Range	Target
Oxacillin	1	8-12	10	9-13	11	9-14	11-12
Norfloxacin	10	12-17	14-15	13-18	15-16	13-19	16
Erythromycin	15	16-22	19	18-24	21	19-25	22
Clindamycin	2	15-20	17-18	16-21	18-19	16-22	19
Trimethoprim-sulfamethoxazole	1.25-23.75	13-19	16	14-20	17	14-20	17

Field trials 2017 and 2018

- 40/44 laboratories in northern Europe
- 15/15 laboratories in mediterranean countries (Spain, France, Italy, Greece and Turkey).

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The EUCAST RAST clinical breakpoint are based on data from three studies.

- 1. Spiked bottles with selected difficult isolates**, performed at EDL. Isolates have been tested with the RAST method on MH-agar from Oxoid and BD/BBL. Reference method was BMD.
- 2. Clinical trial northern Europe**, clinical isolates from **40** laboratories. Locally used MH-agars and antimicrobial discs. Reference method is EUCAST disk diffusion 16-20 h.
- 3. Clinical trial southern Europe**, clinical isolates from **15** laboratories. Locally used MH-agar and antimicrobial discs. Reference method is EUCAST disk diffusion 16-20 h.

Blood culture bottles, media and disks used

Blood culture bottles

- Bactec
- BactAlert (old and new)
- VersaTREK

Media

- Oxoid (Thermo Fisher)
- BBL (BD)
- Agricon Ricerche
- bioMérieux
- Bio-Rad
- Liofilchem
- LIP/Annin

Disks

- BD
- Bio-Rad
- I2A
- MAST
- BioMaxima
- Oxoid
- Rosco

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E. coli

Spiked bottles, n=60 (each isolate tested on MH from two manufacturers)

Clinical trial northern Europe, n=430

Clinical trial southern Europe, n=150

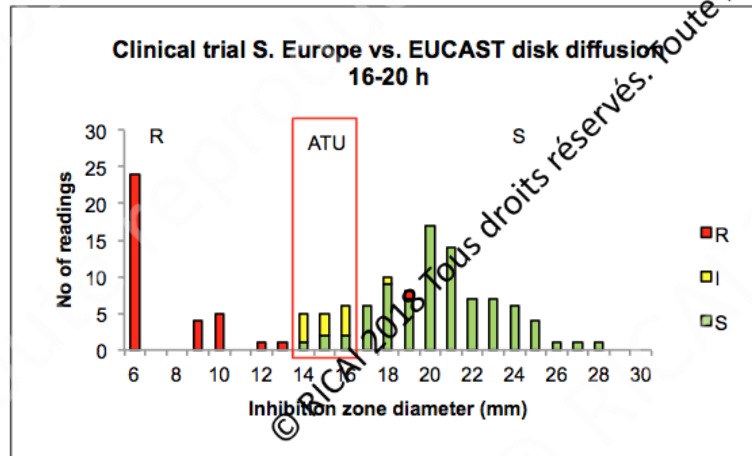
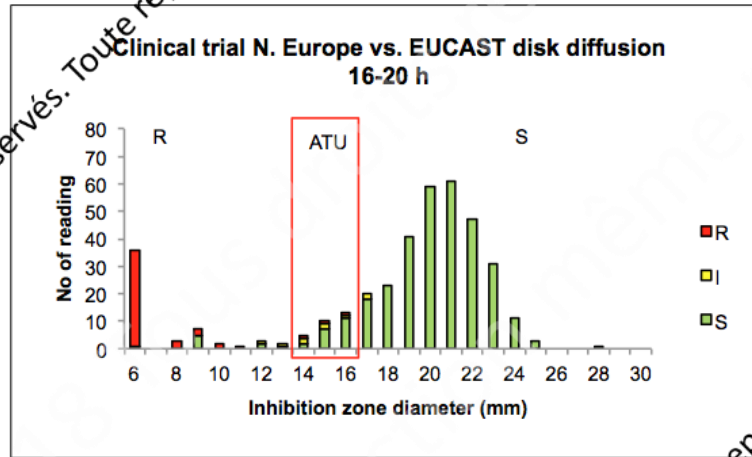
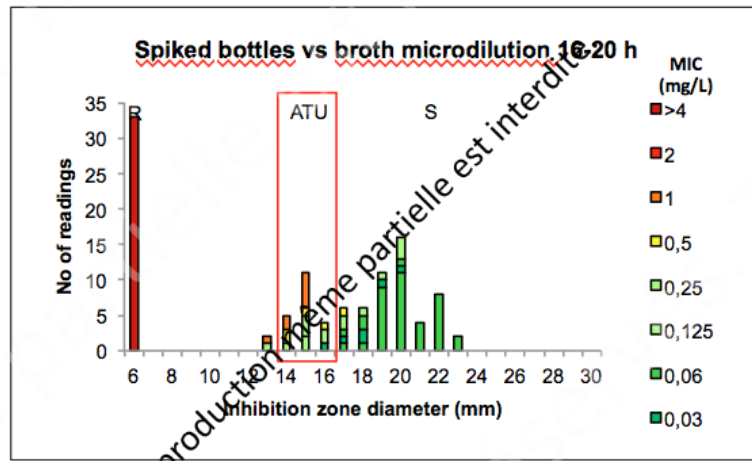
E. coli	4h (%)	6h (%)	8h (%)
Spiked bottles	90	100	100
Clinical trial northern Europe	91	99	99
Clinical trial southern Europe	91	98	99

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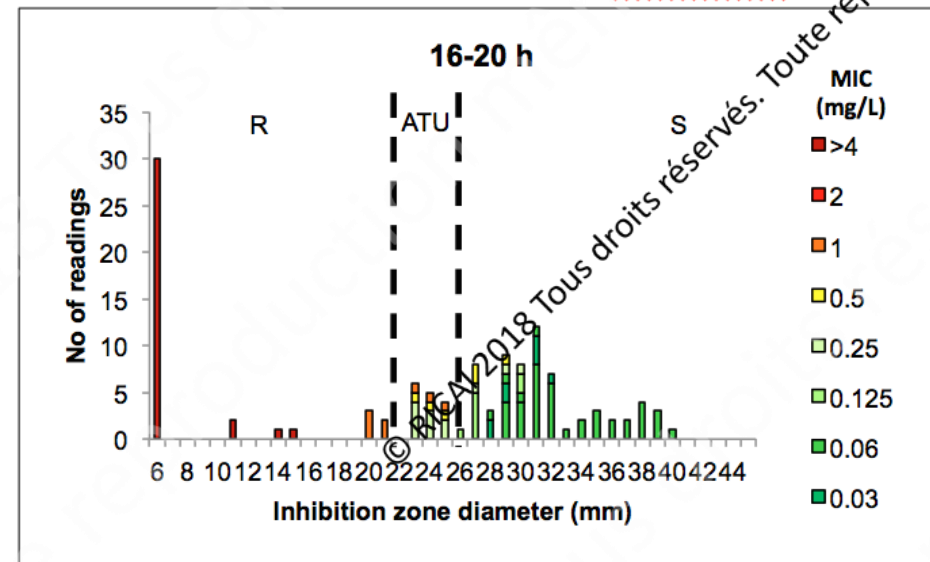
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E. coli and ciprofloxacin 5 µg RAST 4 h

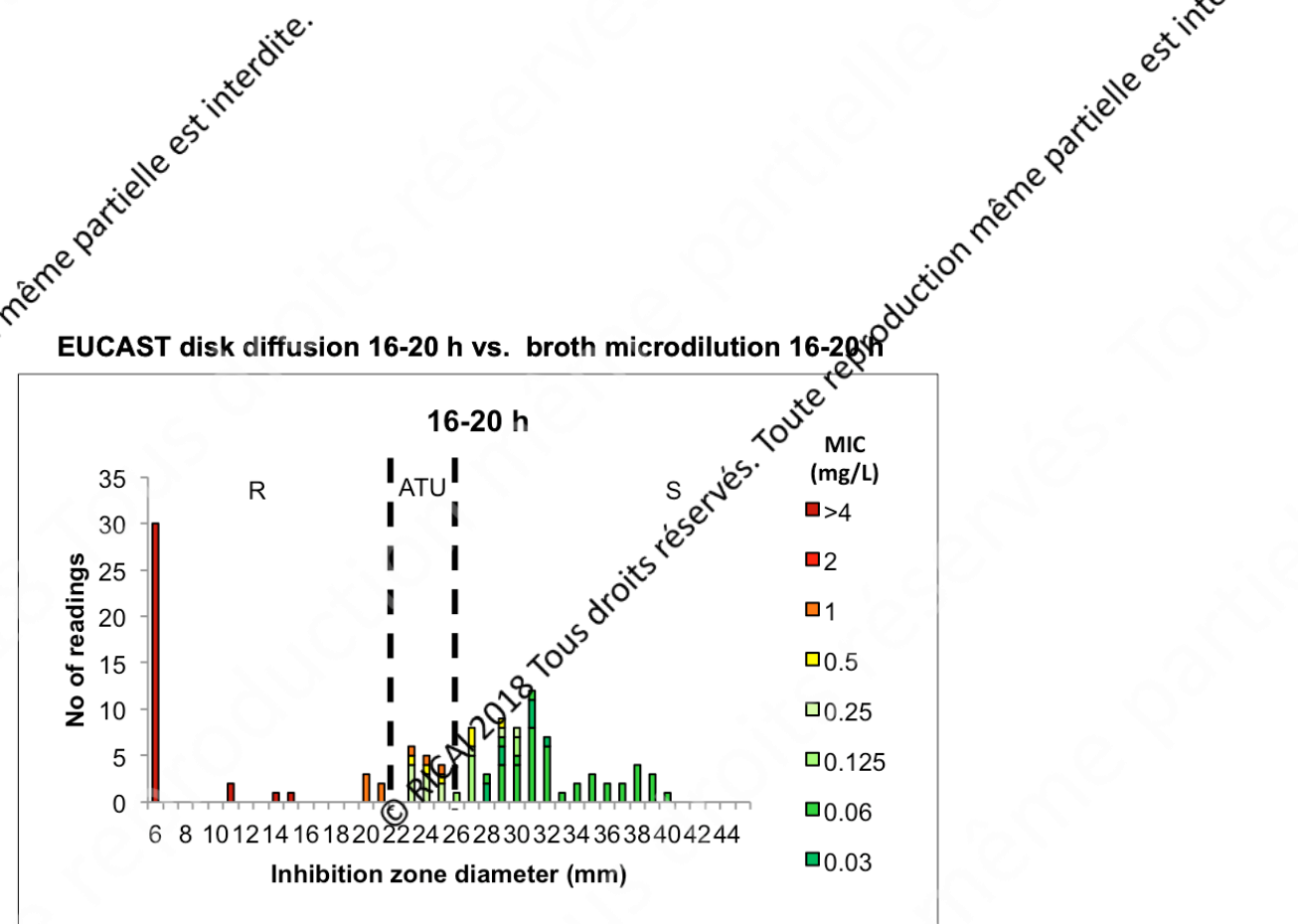
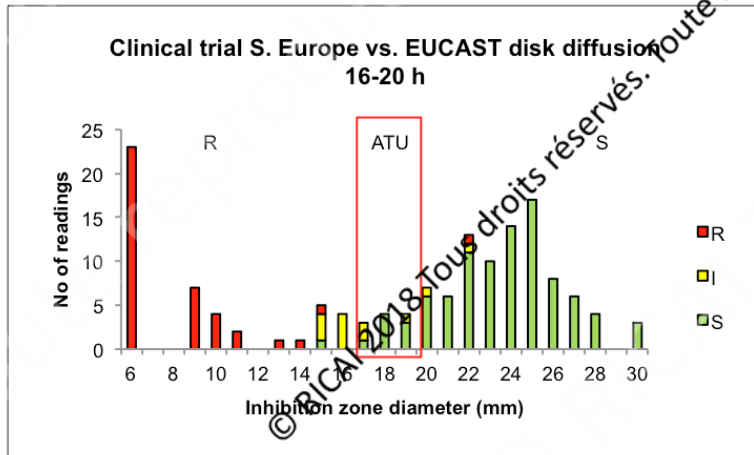
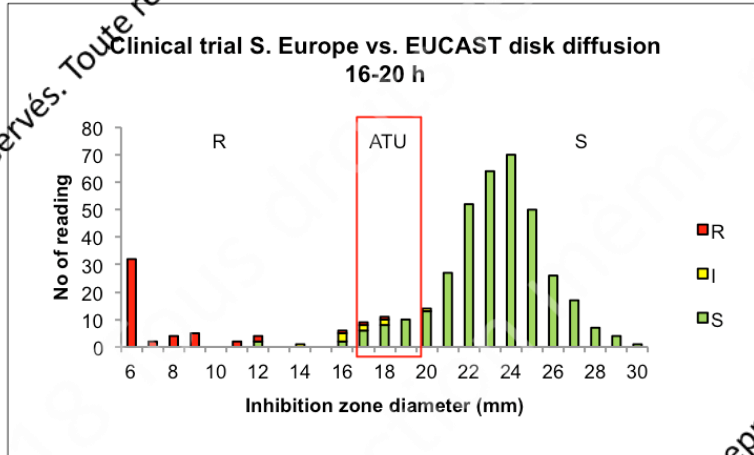
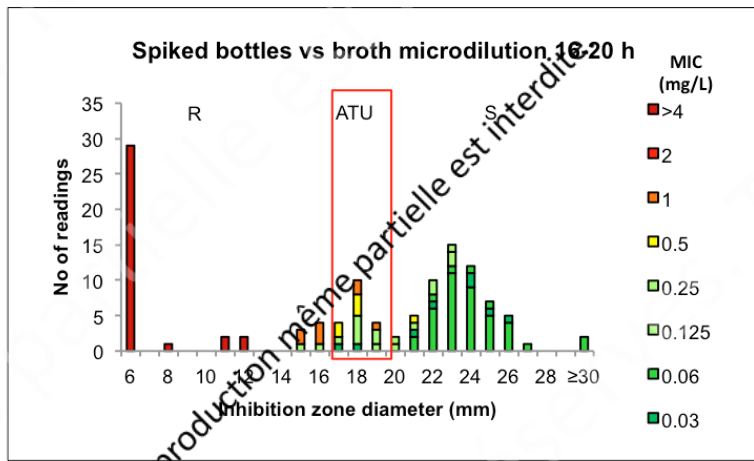


EUCAST disk diffusion 16-20 h vs. broth microdilution 16-20 h

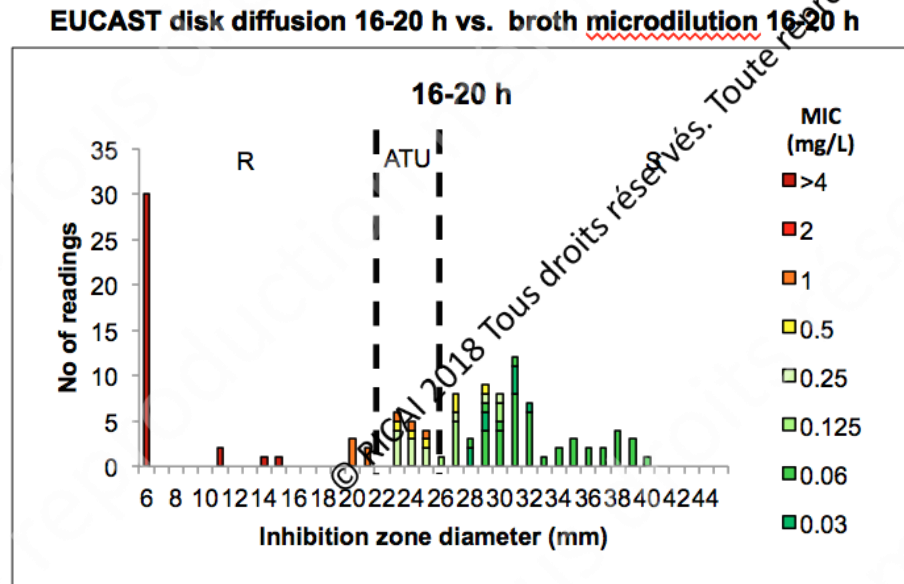
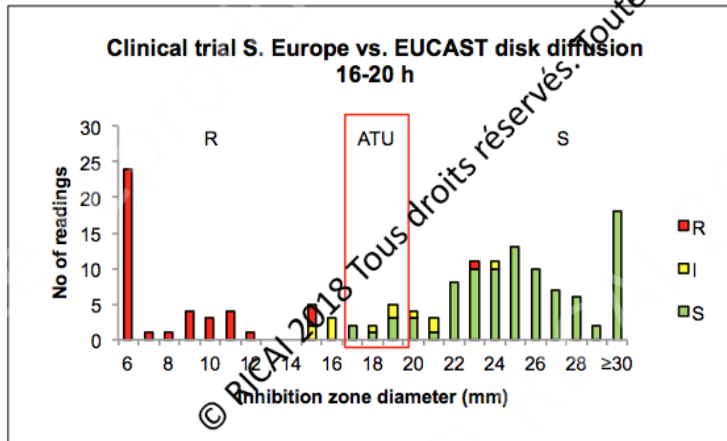
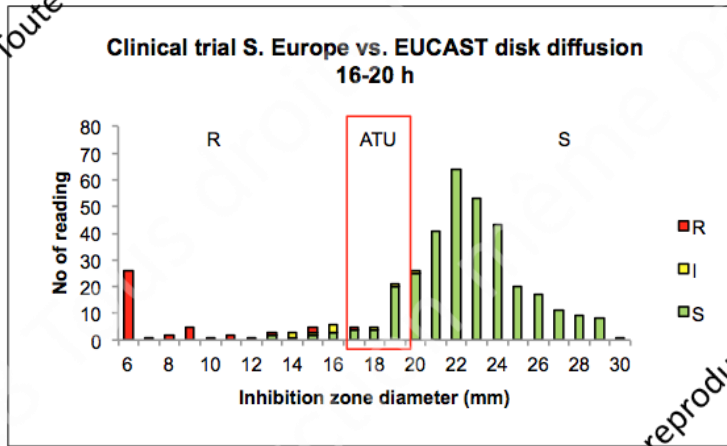
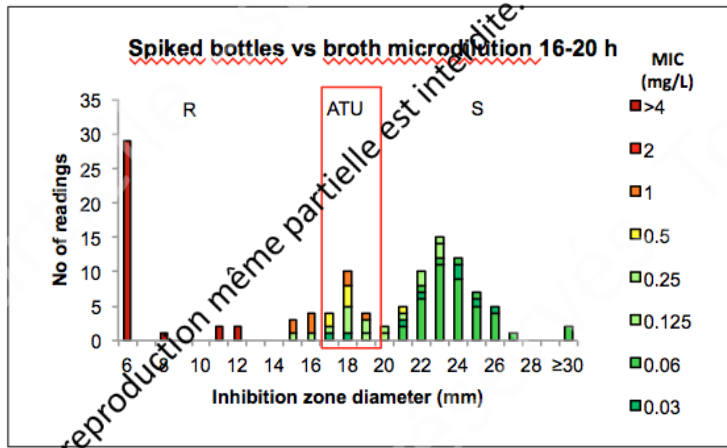


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E. coli and ciprofloxacin 5 µg RAST 6 h



E. coli and ciprofloxacin 5 µg RAST 8 h



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S. aureus

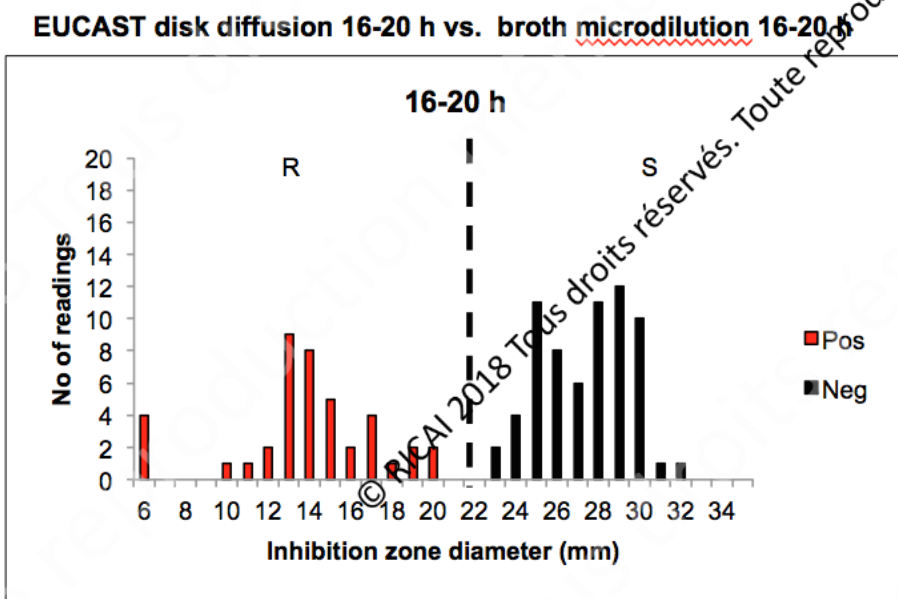
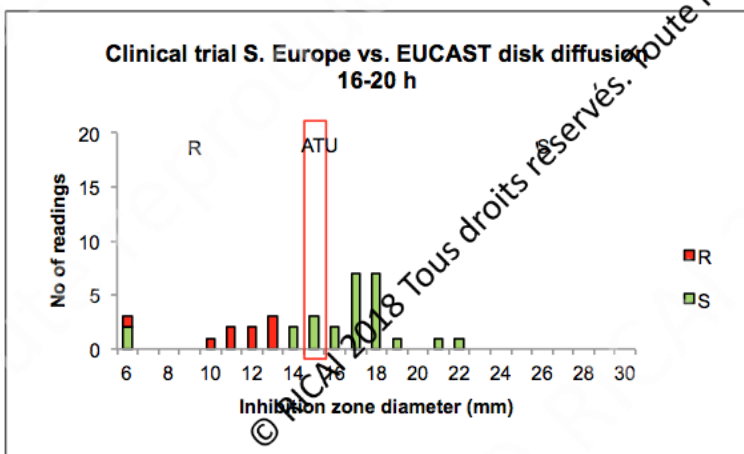
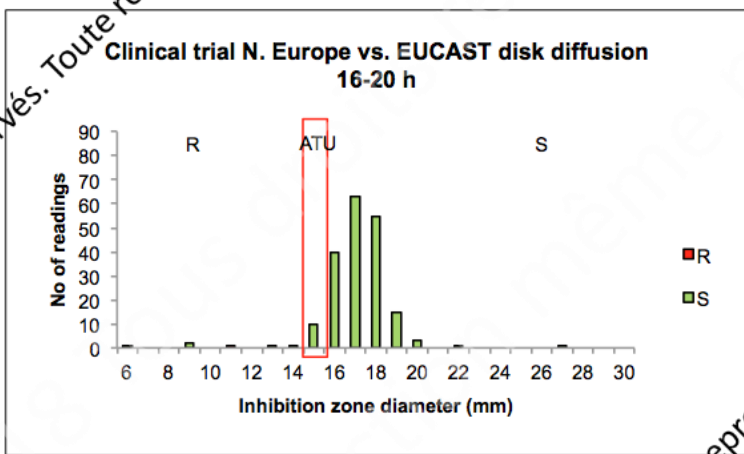
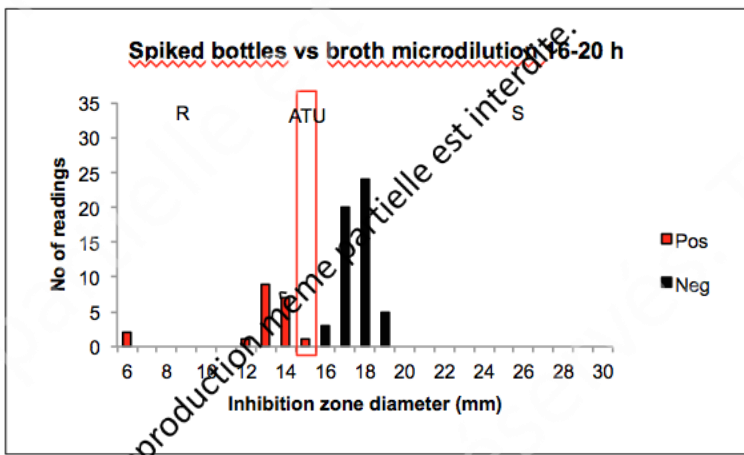
Spiked bottles, n=60 (each isolate is tested on MH from two manufacturers)

Clinical trial northern Europe, n=267

Clinical trial southern Europe, n=70

S. aureus	4h* (%)	6h (%)	8h (%)
Spiked bottles	58	89	91
Clinical trial northern Europe	66	93	96
Clinical trial southern Europe	43	94	99

S. aureus and cefoxitin 30 µg RAST 4 h

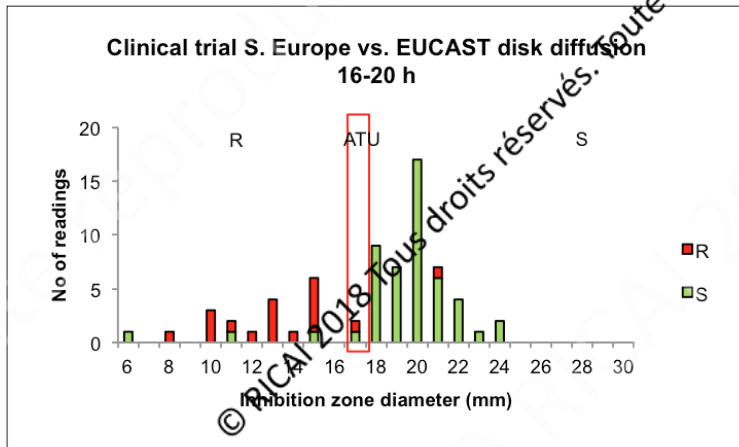
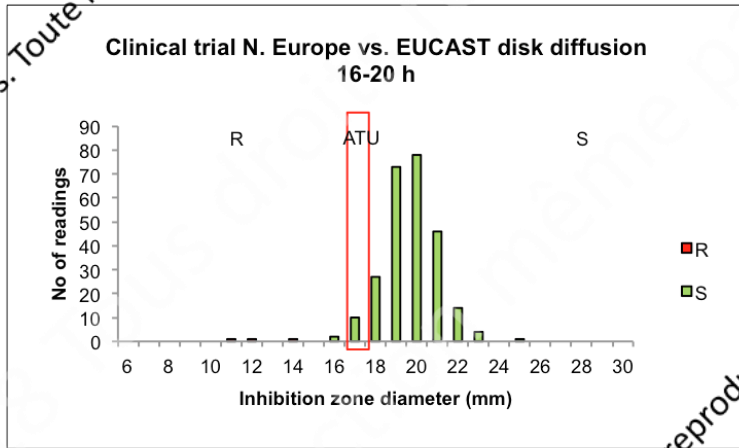
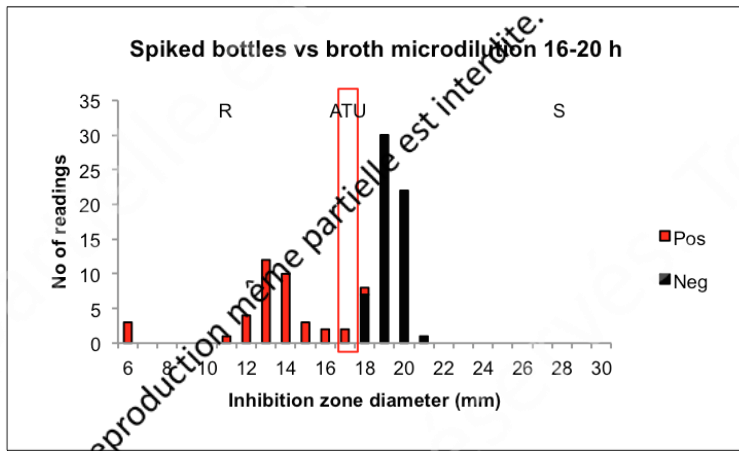


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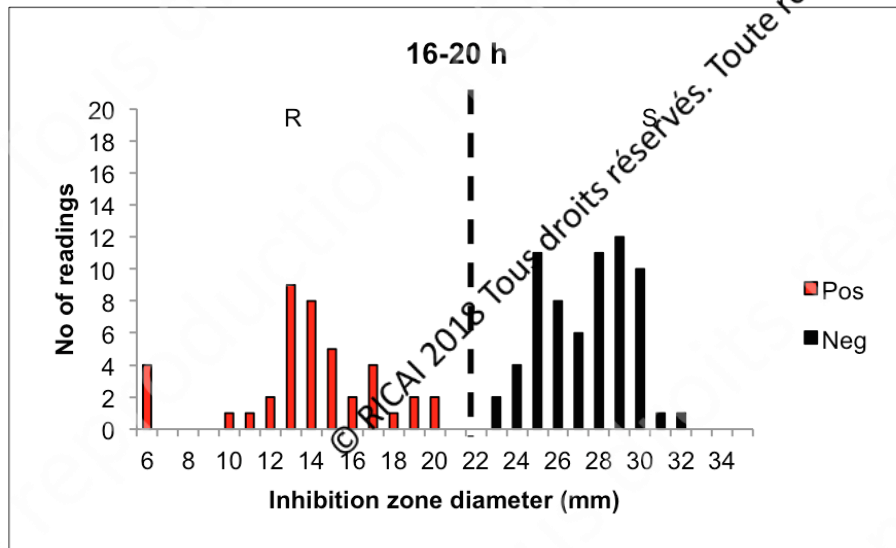
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S. aureus and cefoxitin 30 µg RAST 6 h

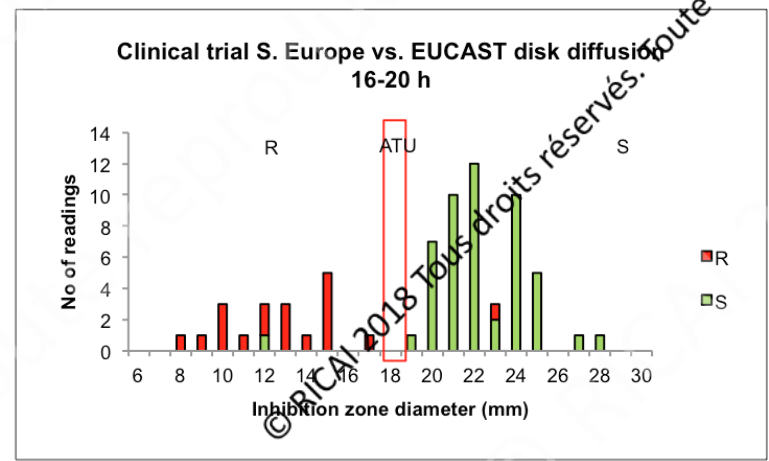
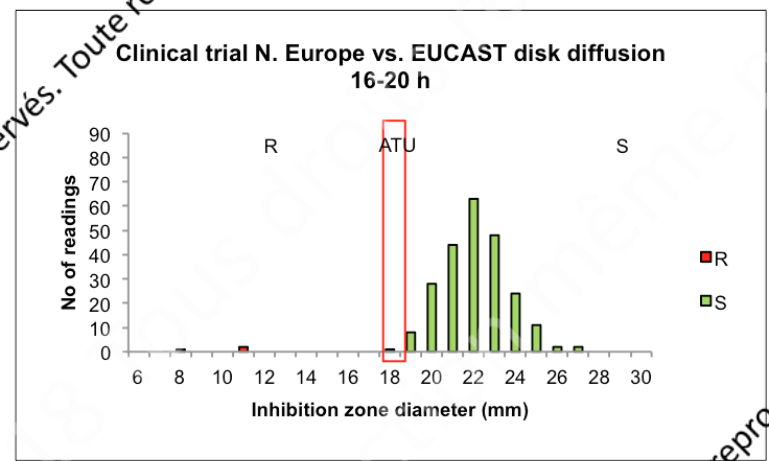
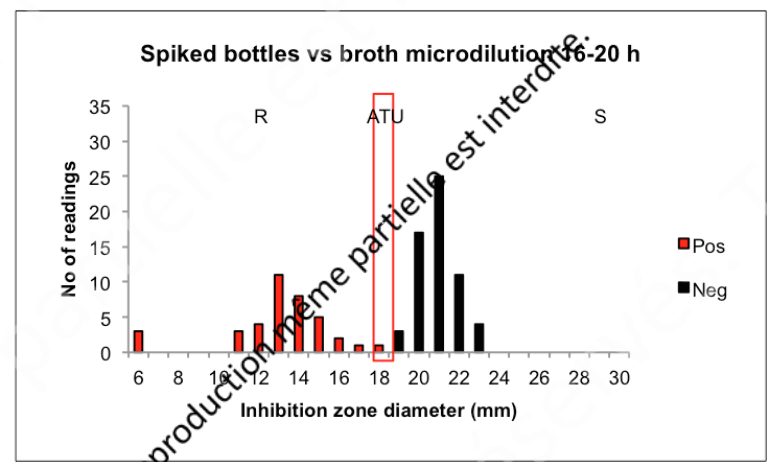


EUCAST disk diffusion 16-20 h vs. broth microdilution 16-20 h

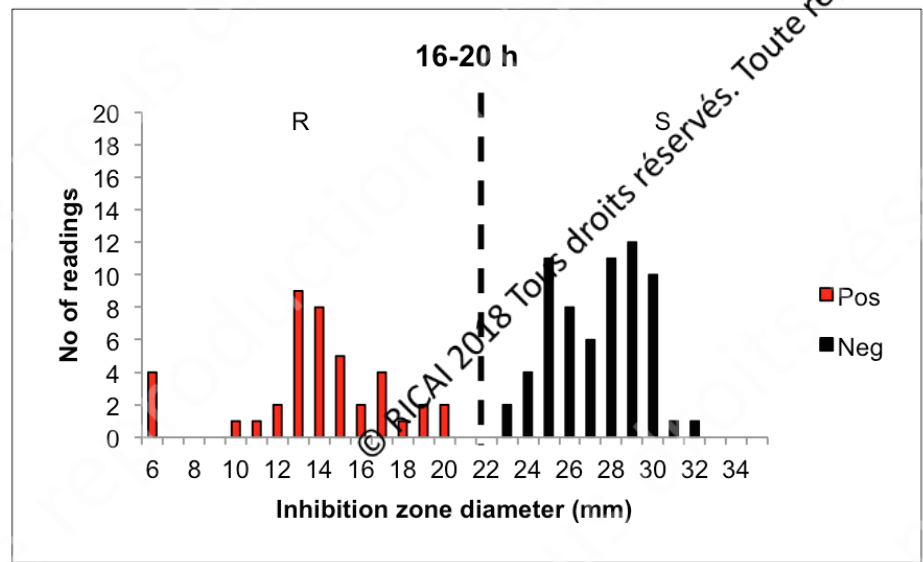


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S. aureus and ceftaxime 30 µg RAST 8 h



EUCAST disk diffusion 16-20 h vs. broth microdilution 16-20 h



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Of the 40 north European laboratories...

- 35 laboratories had $\leq 3\%$ ME/VME errors

Of the 15 south European laboratories...

- Analysis not yet finalised.

Summary

- Rapid AST directly from positive blood culture bottles can be performed without the standardised inoculum and with short incubation.
- Breakpoints must be adjusted to each reading times (4h, 6h and 8h)
- Four bottles from three manufacturers have been validated.
- Variation is absorbed and errors avoided by including an Area of Technical Uncertainty (ATU), where interpretation is not permitted.
- The proportion of tests inside the ATU decreases after 6 and 8 h.
- The I-group was sacrificed to achieve reproducible results
- EUCAST recently publish recommendations for rapid AST from blood cultures on the EUCAST website.

Warning

- Do not use EUCAST standard breakpoint tables with zone diameters obtained after short incubation.
- Do not assume that an MIC resulting from a short incubation (gradient tests or broth micro dilution) is on par with a standard incubation MIC.

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Merci!

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On behalf of EUCAST and the EDL

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Acknowledgements

- Emma Jonasson – performed most of the hard labor, much of the analyses and prepared all graphs.
- Anna Åkerlund – laboratory work and data analysis.
- Erika Matuschek, the EUCAST Development Laboratory. Planning and data analysis.
- Martin Sundqvist, Clinical microbiology, Örebro. Planning and data analysis.