



EUCAST

EUROPEAN COMMITTEE  
ON ANTIMICROBIAL  
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

# EUCAST disk diffusion method.

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# EUCAST Development Laboratory (EDL)

- Development and maintenance of EUCAST phenotypic methods
- Evaluation of AST materials (disks, media) – warnings on website.
- Supporting clinical laboratories (collaborative studies, Q&A, education, EUCAST Observerships)
- Educational activities (courses, material on EUCAST website, videos)
- EUCAST Laboratory Networks (bacteria and fungi) with 15 – 25 laboratories around the world



# Antimicrobial susceptibility testing (AST)

## Phenotypic AST – based on MIC and breakpoints

- Predicts resistance and susceptibility
- Quantitative
- Reference method: broth microdilution (CLSI, EUCAST, ISO);  
Surrogate tests: disk diffusion, gradient tests, semiautomated devices, computeraided microscopy, microcalorimetry, flowcytometry

## Genotypic AST – detection of the gene

- Predicts resistance but cannot guarantee susceptibility
- Not quantitative
- Breakpoints not required, but validation does
- No reference method

## Mechanistic AST - detection of a R-mechanism

- Predicts resistance but cannot guarantee susceptibility
- Detection of a R-mechanism indicates resistance.
- Not quantitative
- No breakpoints

## Expert rules AST

- Intrinsic resistance and IF/THEN rules
- Not quantitative

# Phenotypic AST – based on MIC and breakpoints

- Predicts resistance and susceptibility
- Quantitative
- Reference method: MIC broth microdilution (ISO);

## Surrogate tests:

- disk diffusion
- gradient tests
- semiautomated devices
- computeraided microscopy
- microcalorimetry
- flowcytometry

# EUCAST standardised disk diffusion

- Cheap
- Flexible
- Accurate and reproducible – if you use the right material and follow instructions.
- Calibrated to broth micro-dilution MIC for wild type and non-wild type isolates (with a variety of resistance mechanisms)
- New agents available from the date of registration

# Agents under development – disk diffusion criteria published with clinical breakpoints

13 new agents are under development and are currently dealt with by the EUCAST Steering Committee and/or the EUCAST Development laboratory

# Organisms under development

- **Burkholderia pseudomallei**
  - MIC distributions + Zone diameter distributions: MIC and disk diffusion breakpoints.
  - In collaboration with Robert Koch Institute and laboratories in SE Asia and Australia.
- **Nocardia spp.**
  - MIC Breakpoints and disk diffusion.
- **Anaerobe bacteria**
  - Review of breakpoints and disk diffusion for rapidly growing anaerobes.
- ***Neisseria gonorrhoeae*** (disk diffusion?)
- ***Neisseria meningitidis*** (disk diffusion?)

# Each AST test – a SYSTEM

- The system depends on the quality of each of the components.
- Disk diffusion depends on the following parts:
  - MH-medium
    - Powder (manufacturer)
    - Plate production / plate provider / plate storage
  - Antibiotic disk (manufacturer)
  - Inoculum (correctness)
  - Reader of zone diameters (standardization)



# The EUCAST system is calibrated ...

...to accept **some** variation caused by the fact that we need to allow the use of

- several MH media.
- disks from several manufacturers.

The EUCAST recommendations are based on media and disks of tried (and known) quality from 3 manufacturers + the exclusion of disks and media from some manufacturers (where the quality of materials is doubtful or poor).

**We must avoid dependence on one manufacturer**

# Disk quality study

- 9 manufacturers of disks were identified
- 16 disks from each representing agent groups and disks important to the system:
  - Benzylpenicillin 1U (betalactam resistance in H.influenzae)
  - Oxacillin 1 µg (penicillin resistance in S.pneumoniae)
  - Cefoxitin 30 µg (MRSA and MRSE detection)
  - Carbapenem (meropenem, screen for carba-R in Enterobacterales)
  - Aminoglycosides
  - 3<sup>rd</sup> gen cephalosporins (cefotaxime, ceftazidime)
  - F-quinolones
  - Macrolide
  - Tetracycline
- All tested twice, two separate occasions, 2 years apart.
- EUCAST recommended QC-strains were used + QC strains to control inhibitor activity.

Table 2. Results for disks from nine manufacturers vs. EUCAST QC targets and ranges.

| Manufacturer                    | Abba | BD | Bio-analyse | BioRad | HiMedia | Liofil-chem | Mast | Oxoid | SirScan |
|---------------------------------|------|----|-------------|--------|---------|-------------|------|-------|---------|
| <b>Antimicrobial disk</b>       |      |    |             |        |         |             |      |       |         |
| <b>STUDY 2014</b>               |      |    |             |        |         |             |      |       |         |
| Benzympenicillin 1 unit         |      | L  | H           |        | NA      |             |      |       | H       |
| Amoxicillin-clav. 30 µg         | L    |    |             | H      | H       |             |      |       |         |
| Piperacillin-tazo. 36 µg        | L    |    |             |        | NA      |             |      |       | H       |
| Oxacillin 1 µg                  |      | L  | L           |        | H       | L           |      |       | L       |
| Mecillinam 10 µg                | L    |    | H           |        | H       |             |      |       | H       |
| Cefotaxime 5 µg <sup>1</sup>    | NA   |    |             |        | NA      |             |      |       |         |
| Cefoxitin 30 µg                 | NA   |    |             | H*     | L*      | H           |      |       |         |
| Ceftazidime 10 µg               | L    |    |             |        | L       |             |      |       |         |
| Meropenem 10 µg <sup>1</sup>    | L    |    |             | H      | H       | H*          |      | H     |         |
| Ciprofloxacin 5 µg <sup>1</sup> |      | L  |             |        | H       |             | L    |       |         |
| Pefloxacin 5 µg                 | NA   | L  |             |        | H       | L           |      |       | NA      |
| Norfloxacin 10 µg <sup>1</sup>  | L    |    |             |        | H*      |             |      |       | L       |
| Gentamicin 10 µg <sup>1</sup>   |      | H  |             |        | H       |             |      |       | NA      |
| Tobramycin 10 µg                |      |    |             | NA     | H*      | H           |      |       |         |
| Erythromycin 15 µg              | L    | L  | L*          |        | H       | L           |      |       | L       |
| Tetracycline 30 µg              | L    | L* | L           |        |         | L           | L    |       | L*      |
| <b>Antimicrobial disk</b>       |      |    |             |        |         |             |      |       |         |
| <b>STUDY 2017</b>               |      |    |             |        |         |             |      |       |         |
| Benzympenicillin 1 unit         |      | L  |             |        |         |             |      |       | NA      |
| Amoxicillin-clav. 30 µg         | L    |    |             | H      | L       | L           |      |       |         |
| Piperacillin-tazo. 36 µg        | L    |    | H           |        | L       |             | H    |       |         |
| Oxacillin 1 µg                  |      |    |             | H      | H       |             |      |       |         |
| Mecillinam 10 µg                |      |    |             |        |         |             |      |       |         |
| Cefotaxime 5 µg                 |      |    |             |        |         |             |      |       |         |
| Cefoxitin 30 µg                 |      | L  | H           |        | L       |             |      |       |         |
| Ceftazidime 10 µg               | L    |    |             |        | L*      |             |      |       |         |
| Meropenem 10 µg                 |      |    |             |        | L*      |             |      |       |         |
| Ciprofloxacin 5 µg              |      |    |             |        |         |             |      |       |         |
| Pefloxacin 5 µg                 |      |    |             |        | H       | L           |      |       |         |
| Norfloxacin 10 µg               |      |    |             |        |         |             |      |       |         |
| Gentamicin 10 µg                |      |    |             |        | H       |             | H    |       |         |
| Tobramycin 10 µg                |      |    |             |        | H       |             |      |       |         |
| Erythromycin 15 µg              |      |    |             |        |         |             |      |       |         |
| Tetracycline 30 µg              |      |    |             |        | L       |             |      |       |         |

<sup>1</sup>Data reanalyzed due to changes in QC criteria since 2014.

Mean value within ± 1 mm of the target value

Mean value > 1 mm but within ± 2 mm of the target value

Mean value > 2 mm from target value but still within the QC range

Mean value out of the QC range

NA = Not Available

H = High, mean value > 1 mm above target

L = Low, mean value > 1 mm below target

\* One or more readings out of QC range

# MH medium quality study

- 21 different brands of MH powder on the international market
- Plates from each brand were produced in-house simultaneously.
- Antibiotic disks were from a high quality provider (see disk study) as for the disk comparison study.
- All plates/disks were inoculated simultaneously from the same suspension and read by two experienced readers.
- Negative points were given for each QC-strain and antibiotic disk result outside target  $\pm 1$  mm and  $\pm 3$  mm.  
Best result: -4 points and worst result -55 points

# 21 brands of MH media

Results are not yet analysed.

However – the rating of each medium is based on how mean values (30 per agar) from triplicate tests of four QC strains relates to the respective QC criteria (EUCAST QC Tables v. 8.0).

The best theoretical result is 0.

The results for agars vary between +4 (at best) and -55 (at worst)

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# EUCAST methods available on [www.eucast.org](http://www.eucast.org)

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Organization

EUCAST News

New definitions of S, I and R

Clinical breakpoints and dosing

Rapid AST in blood cultures

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

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20 October 2017

## The European Committee on Antimicrobial Susceptibility Testing - EUCAST

EUCAST is a standing committee jointly organized by ESCMID, ECDC and European national breakpoint committees. EUCAST was formed in 1997. It has been chaired by Ian Phillips (1997 - 2001), Gunnar Kahlmeter (2001 - 2016), Rafael Canton (2012 - 2016) and Christian Giske (2016 - ). Its scientific secretary is Derek Brown (1997 - 2016) and John Turnidge (2016 - ). Its webmaster is Gunnar Kahlmeter (2001 - ). From 2016, Rafael Canton is the Clinical Data Co-ordinator and Gunnar Kahlmeter the Technical Data Co-ordinator.

EUCAST deals with breakpoints and technical aspects of phenotypic in vitro antimicrobial susceptibility testing and functions as the breakpoint committee of EMA and ECDC. EUCAST does not deal with antibiotic policies, surveillance or containment of resistance or infection control. The Steering Committee is the decision making body. It is supported by a General Committee with representatives from European and other countries, FESCI and ISC. The Steering Committee also consults on EUCAST proposals with experts within the fields of infectious diseases and microbiology, pharmaceutical companies and susceptibility testing device manufacturers.

EUCAST has several subcommittees - [see page Subcommittees](#).

Antimicrobial MIC breakpoints in Europe have been harmonised by EUCAST and since a few years all European countries now follow EUCAST guidelines. Many countries outside Europe have also decided to follow EUCAST guidance.

Breakpoints for new agents are set as part of the licensing process for new agents through EMA. EUCAST breakpoints are available in devices for automated susceptibility testing but with some limitations, depending on the system. EUCAST develops a disk diffusion susceptibility test method  $\rightarrow$  calibrated to EUCAST MIC breakpoints. New agents rapidly obtain zone diameter breakpoint correlates but there are some limitations. Previous disk diffusion tests from the Swedish, French and British committees have been abandoned and laboratories advised to switch to EUCAST recommended methods.

EUCAST invites anyone with an interest in antimicrobial agents in general and antimicrobial breakpoints in particular to contact EUCAST, ESCMID or one of the National Breakpoint Committees.

QUICK NAVIGATION

### EUCAST News

13 Dec 2018

**Warning on the azole resistance screening plate from VipCheck**

30 Nov 2018

**Consultation EUCAST Breakpoint table v 9.0**

28 Nov 2018

**Rapid AST directly from positive blood culture bottles**

15 Nov 2018

**New definitions of S, I and R now in Spanish**

07 Nov 2018

**Consultation on H.influenzae and breakpoints for piperacillin/tazobactam**

[About Newsfeed](#)

 **ESCMID** EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES




EUROPEAN MEDICINES AGENCY  
SCIENCE · MEDICINES · HEALTH

## AST of bacteria

- Organization
- EUCAST News
- Clinical breakpoints
- Expert rules and intrinsic resistance
- Resistance mechanisms
- Guidance documents
- Consultations
- MIC distributions and ECOFFs
- Zone distributions and ECOFFs
- AST of bacteria
  - Media preparation
  - MIC determination
  - Disk diffusion methodology**
  - Disk diffusion implementation
  - Compliance of manufacturers
  - Breakpoint tables
  - QC Tables
  - Calibration and validation
  - Warnings!
  - Guidance documents
  - Projects and data submission
  - MIC testing services from EUCAST
  - Previous versions of documents



... Disk diffusion methodology 

## EUCAST Disk Diffusion Test Methodology

EUCAST has developed a disk diffusion test based on MH media and calibrated to EUCAST clinical breakpoints. Updates are published regularly.

- [EUCAST Disk Diffusion - Manual v 6.0](#) (9 January, 2017)
- [EUCAST Disk Diffusion - Slide Shows](#) (9 January, 2017)
- [EUCAST Disk Diffusion - Reading Guide](#) (9 January, 2017)

For translations to other languages - see [Translations](#).  
For previous versions of documents - see [Previous versions](#).

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AST of mycobacteria

AST of fungi



... Warnings!

## EUCAST warnings concerning antimicrobial susceptibility testing products or procedures.

The EUCAST disk diffusion development laboratories, a network of laboratories coordinated from the EUCAST development laboratory in Växjö, Sweden, from time to time discover products (disks, media batches, gradient tests or procedures) which are not performing to the expected standard. When this is the case we inform the manufacturer and publish a warning on this page.

We do not systematically test all products so the lack of a warning does not imply that there is no problem with the product in question.

Laboratories which experience problems with a susceptibility test method, and suspect that this may be related to a particular product, may contact EUCAST for advice.

1. **Problems with meropenem gradient tests from two manufacturers** - the warning to the best of our knowledge still not resolved (10 July, 2018).
2. **Wide variation in disk quality in 16 disks from nine manufacturers** - this warning was issued in 2015 and reiterated 2016 and 2017. There is new information on improved quality November 23, 2017 (see below).
3. **Problems with colistin susceptibility testing and several commercially available products** - the warning initially issued July 2016 was updated 26 June, 2017.
4. **A warning against the use of vancomycin Etest™ (bioMérieux) and MTS™ (Liofilchem) for vancomycin MIC determination in *Enterococcus faecalis* and *E. faecium* with low-level vancomycin resistance.** Warning issued 10 July, 2018.
5. **A problem with meropenem in a Thermo Fisher Sensititre panel (DKMGN)** was discovered (6 November, 2018).
6. **Variable performance of the Itraconazole agar in the azole resistance screening plate from VlpCheck.**



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## Videos from EUCAST

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## Instruction videos from EUCAST

In collaboration with the World Health Organisation (WHO), EUCAST publishes instruction videos on how to perform antimicrobial susceptibility testing (AST) using EUCAST recommended methods and interpretation. During 2016, five videos have been completed and 5 more are under construction in 2017.

The videos are published on Youtube™ and have an English speaker voice and English subtitles. There is a mechanism by which subtitles can be translated to other languages.

1. Preparation of inoculum (English).
2. Inoculation of agar plates or disk diffusion (English).
3. Application of antibiotic disks and incubation of plates (English).
4. Reading of inhibition zone diameters (English).
5. Guidance on the use of the breakpoint table (English).

Instruction videos on EUCAST susceptibility testing with subtitles in other languages than English

- Instruction videos in German.
- Instruction videos in Russian.
- Instruction videos in Turkish.
- Instruction videos in French.
- Instruction videos in Spanish.
- Instruction videos in Portuguese.
- Instruction videos in Arabic.
- Instruction videos in Czech.

Instruction videos in ... (more to follow)

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www.youtube.com



# EUCAST disk diffusion antimicrobial susceptibility testing method

## Preparation of inoculum

0:02 / 2:33

### EUCAST videos (English)

Gunnar Kahlmeter · 1/5 videos



- 1 Preparation of inoculum (english)  
Gunnar Kahlmeter
- 2 Inoculation english  
Gunnar Kahlmeter
- 3 Application of disks and incubation english  
Gunnar Kahlmeter
- 4 Reading of zones english  
Gunnar Kahlmeter
- 5 Guidance on breakpoint table english  
Gunnar Kahlmeter

All videos are with English speaker, but subtitles are available in Arabic, Chinese, Czech, French, German, Portuguese, Russian, Spanish and Turkish.

# EUCAST susceptibility testing media

- **Mueller-Hinton agar (MH)**

For Enterobacterales, Pseudomonas, Staphylococci and enterococci.

- 21 products available.



- **Mueller-Hinton agar with 5% mechanically defibrinated horse blood and 20 mg/L  $\beta$ -NAD (MH-F)**

- for fastidious organisms: *S. pneumoniae* and other streptococci, Haemophilus, Moraxella, Pasteurella, Listeria, Campylobacter, Corynebacterium, Aerococcus and *Kingella kingae* (but NOT for Neisseria).

- **FAA** (4 manufacturers) developed for disk diffusion of fast growing anaerobes.

# MH-F agar media

## “Mueller-Hinton Fastidious”

- **MH-F:** MH agar with 5% **mechanically** defibrinated horse blood and 20 mg/L  $\beta$ -NAD
  - sheep blood can not be used!
  - Beta-NAD purity of  $\geq 98\%$  recommended
- MH-F plates should have a **dry surface** before use (on lab bench over night or incubator lid removed 15 min).

# Agar dilution (AD)

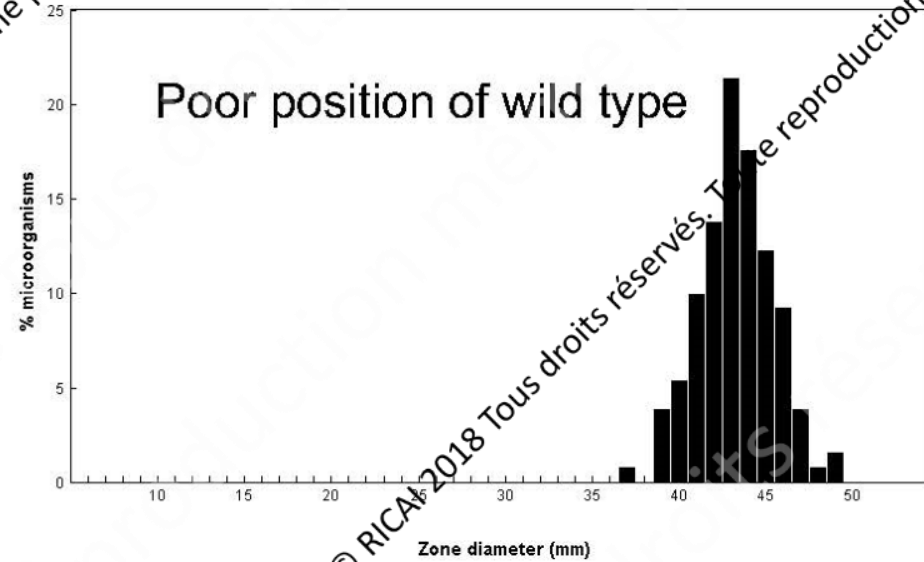
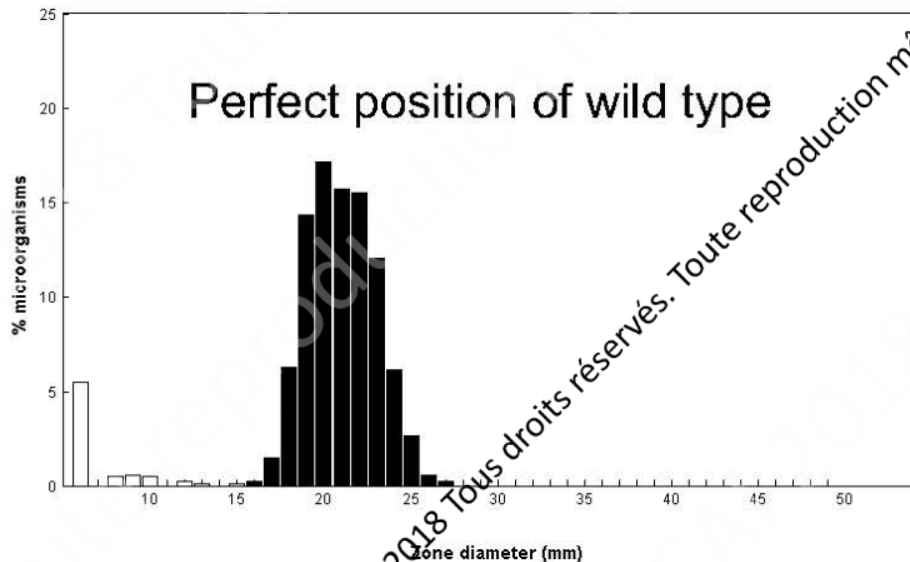
- For some slow growing bacteria
  - Anaerobes
  - *Neisseria gonorrhoeae*
- For some agents when BMD does not give reliable results
  - Fosfomycin
  - Mecillinam

# Criteria for disk diffusion

1. Developing disk mass
2. Developing QC criteria (target and range)
  - Several media
  - Several disk manufacturers
3. Developing zone diameter correlates for broth microdilution MIC-values for relevant micro-organisms.

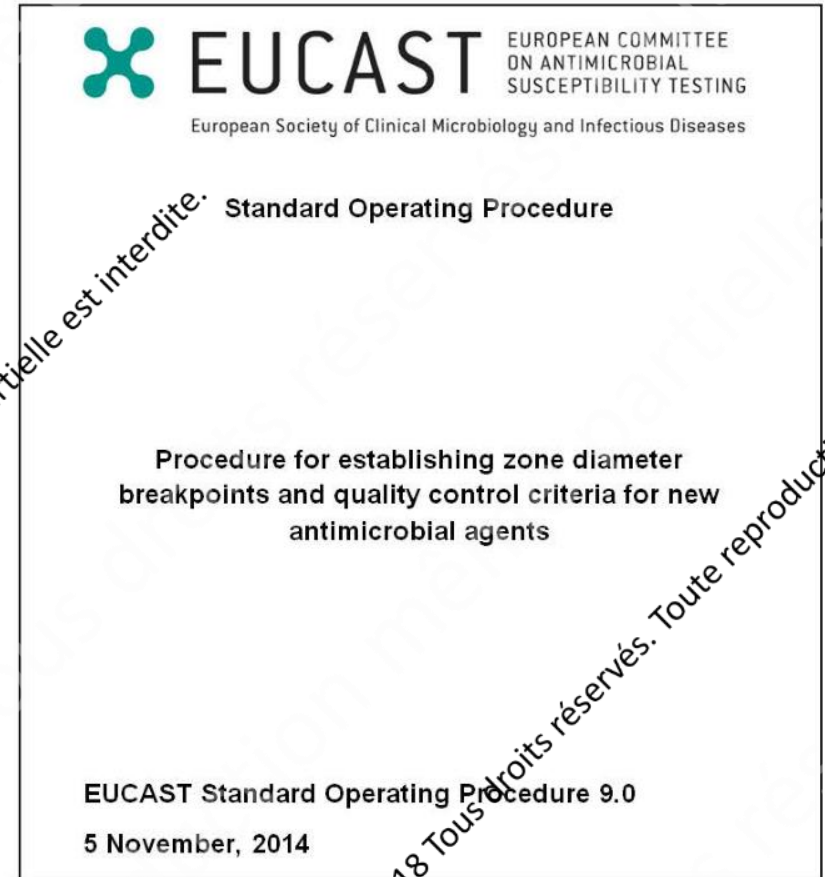
# 1. Developing disk mass

- Optimising inhibition zone size
  - 15-35 mm for wild-type isolates of relevant species
- No overlap between wild type and non-wild type organisms and no overlap between S, I and R.





# 2. Developing QC criteria



<http://www.eucast.org/documents/sops/>

# QC studies

- CLSI (M23)

- One multi-lab study: 7 labs x 3 media x 10 replicates
- Disks from 2 manufacturers

- EUCAST (SOP 9.0)

- Initial two-site study: 2 labs x 3-4 media x 15 replicates
- Validation study:  $\geq 4$  labs x 1 local media x 10 replicates
- Disks from 2-3 disk manufacturers

# QC ranges and targets

## *Escherichia coli* ATCC 25922

(NCTC 12247, CIP 76.24, DSM 1103, CCUG 17620, CECT 434)

See EUCAST Breakpoint Tables for short descriptions of MIC and disk diffusion methodology.

| Antimicrobial agent                        | MIC (mg/L)          |                    | Disk content (µg) | Inhibition zone diameter (mm) |                    |
|--|---------------------|--------------------|-------------------|-------------------------------|--------------------|
|  | Target <sup>1</sup> | Range <sup>2</sup> |                   | Target <sup>1</sup>           | Range <sup>3</sup> |
| Amikacin                                   | 1-2                 | 0.5-4              | 30                | 22-23                         | 19-26              |
| Amoxicillin                                | 4                   | <b>2-8</b>         | -                 | -                             | -                  |
| Amoxicillin-clavulanic acid <sup>4,5</sup> | 4                   | 2-8                | 20-10             | 21                            | 18-24 <sup>6</sup> |
| Ampicillin                                 | 4                   | 2-8                | 10                | 18-19                         | 15-22 <sup>6</sup> |
| Ampicillin-sulbactam <sup>5,7</sup>        | <b>2</b>            | <b>1-4</b>         | 10-10             | 21-22                         | 19-24 <sup>6</sup> |
| Aztreonam                                  | 0.125               | 0.06-0.25          | 30                | 32                            | 28-36              |
| Cefadroxil                                 | -                   | -                  | 30                | <b>17</b>                     | <b>14-20</b>       |
| Cefalexin                                  | 8                   | 4-16               | 30                | <b>18</b>                     | <b>15-21</b>       |
| Cefepime                                   | 0.03-0.06           | 0.016-0.125        | 30                | 34                            | 30-37              |
| Cefixime                                   | 0.5                 | 0.25-1             | 5                 | <b>23</b>                     | <b>20-26</b>       |
| Cefotaxime                                 | 0.06                | 0.03-0.125         | 5                 | <b>28</b>                     | <b>25-31</b>       |

### Range

Used to allow random variation ( $\pm 2SD$ )

### Target

Mean values from repeated measurements should optimally be on target  $\pm 1$  mm (mode MIC on target)

**Escherichia coli ATCC 25922**

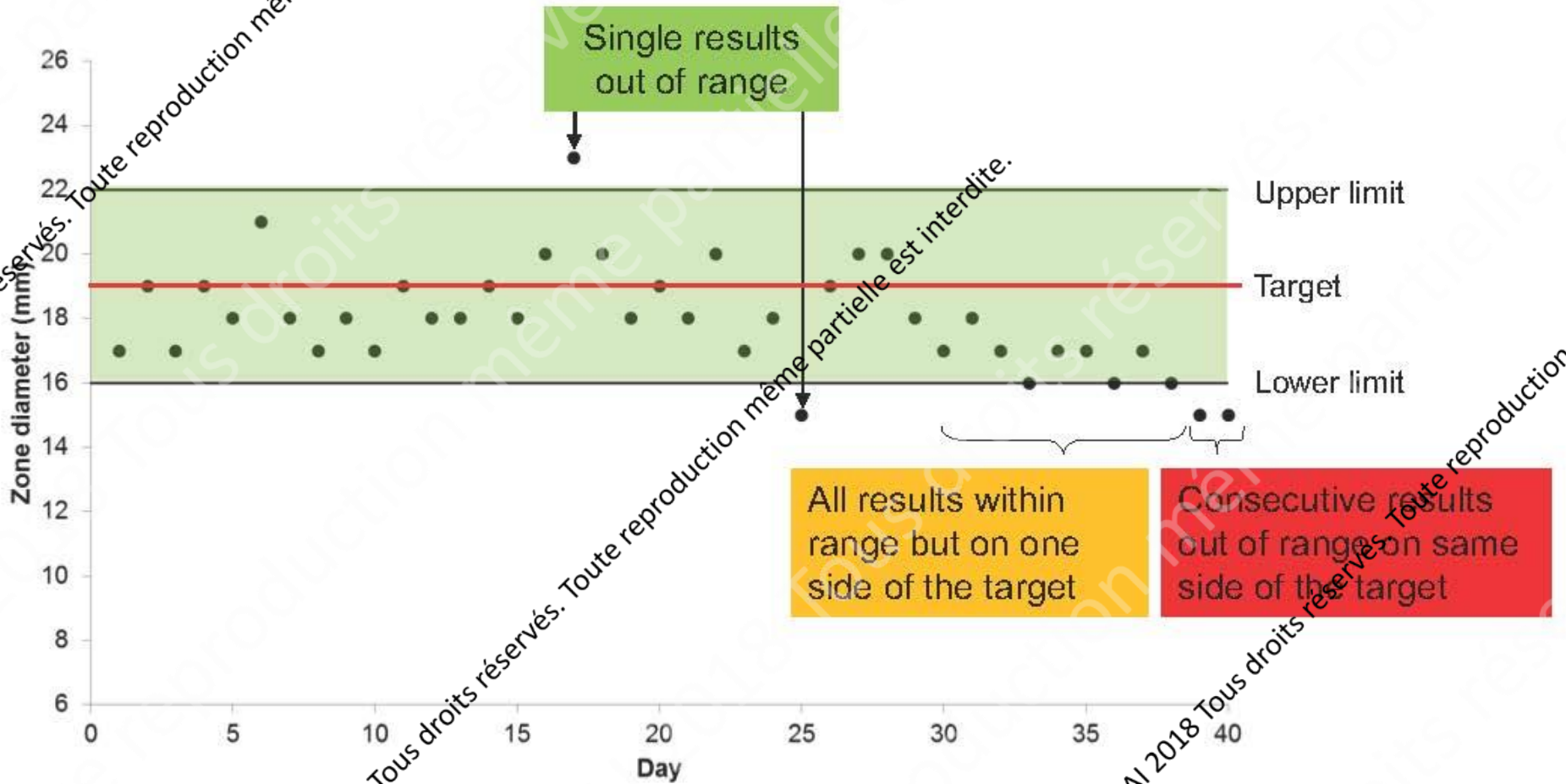
(NCTC 12241, CIP 76.91, DSM 1103, CCUG 17620, CECT 434)

In bold – criteria specific for EUCAST

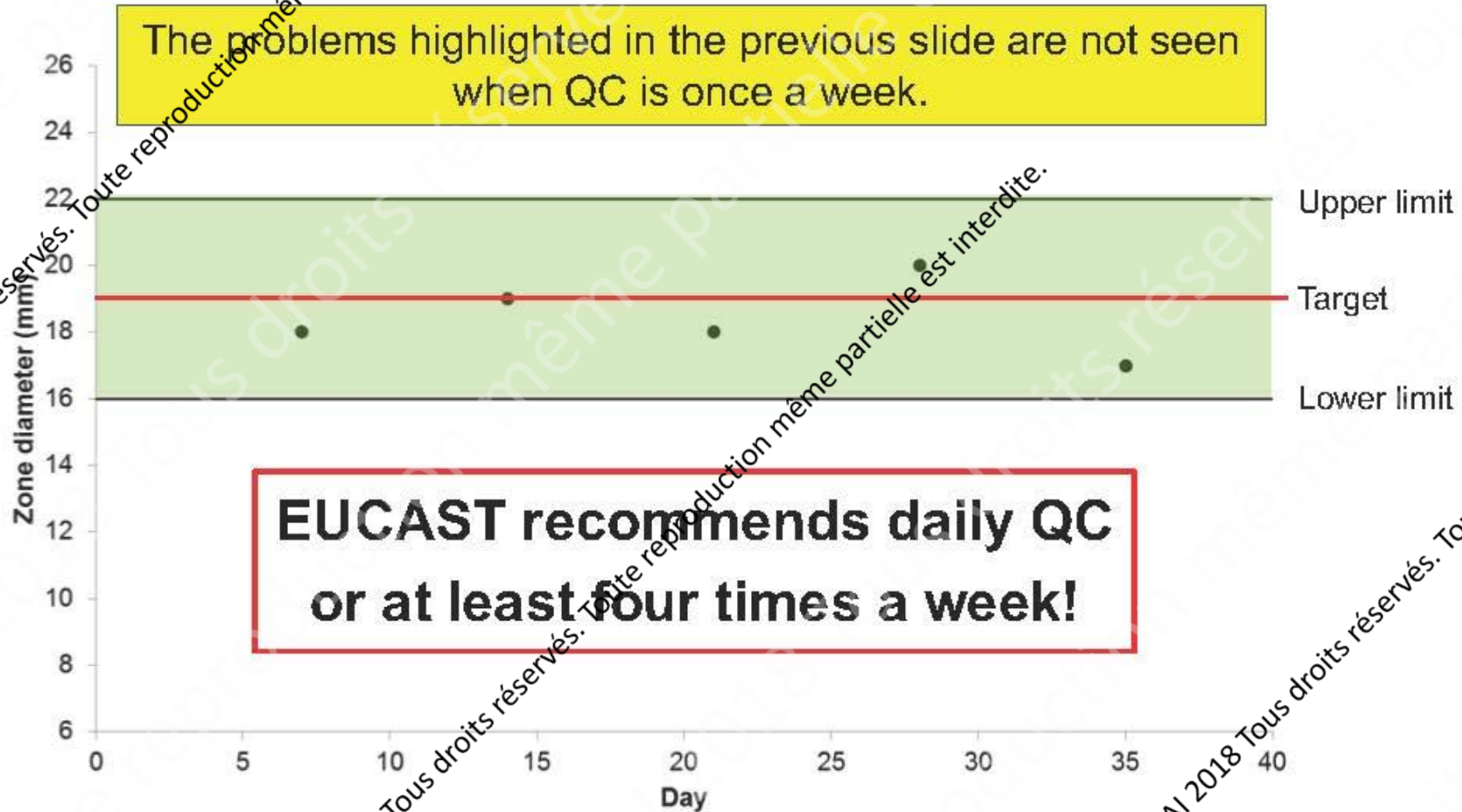
See EUCAST Breakpoint Tables for short descriptions of MIC and disk diffusion methodology.

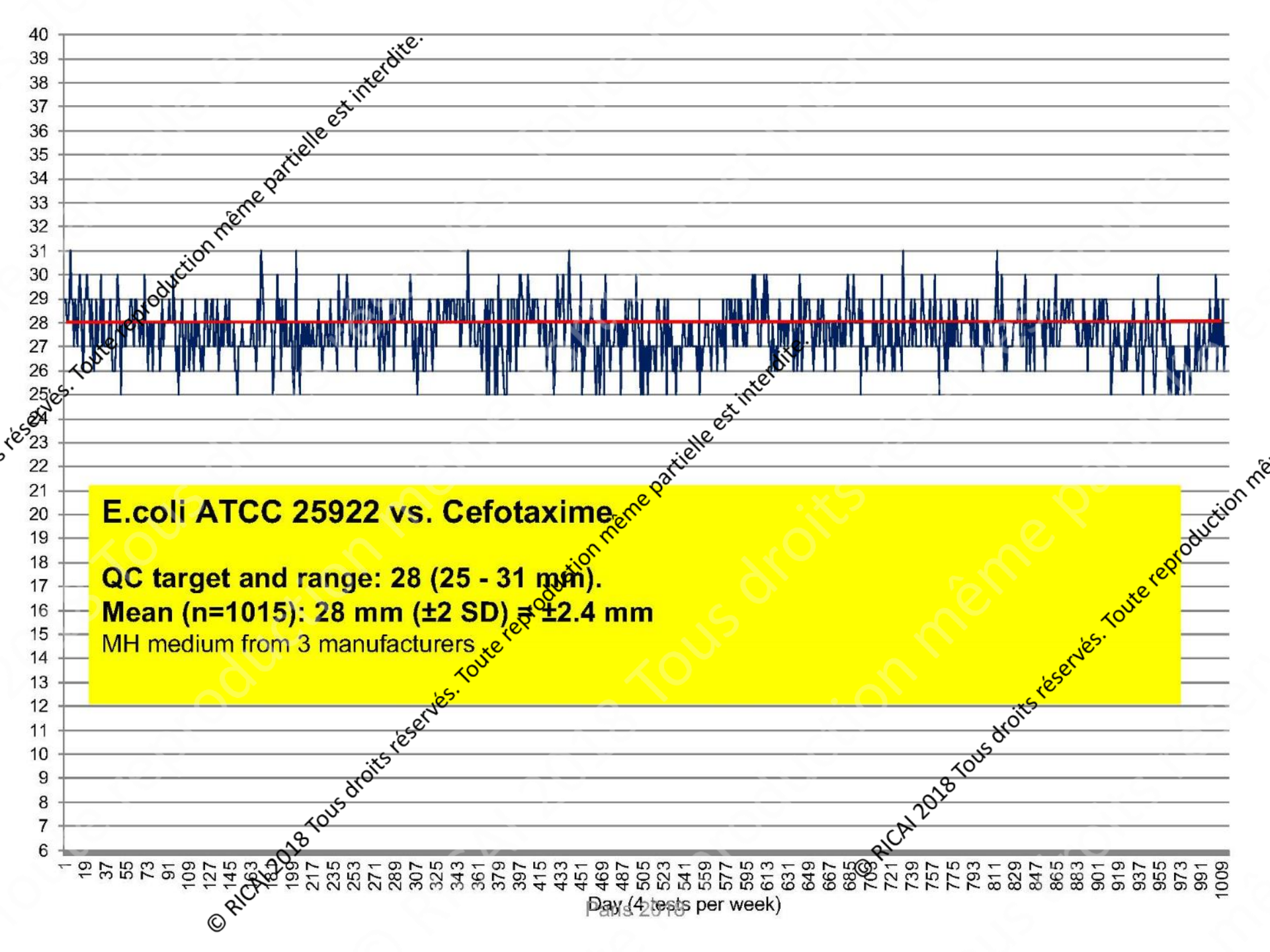
| Antimicrobial agent                        | MIC (mg/L)          |                    | Disk content (µg) | Inhibition zone diameter (mm) |                    |
|--|---------------------|--------------------|-------------------|-------------------------------|--------------------|
|  | Target <sup>1</sup> | Range <sup>2</sup> |                   | Target <sup>1</sup>           | Range <sup>3</sup> |
| Amikacin                                   | 1-2                 | 0.5-4              | 30                | 22-23                         | 19-26              |
| Amoxicillin                                | 4                   | <b>2-8</b>         | -                 | -                             | -                  |
| Amoxicillin-clavulanic acid <sup>4,5</sup> | 4                   | 2-8                | 20-10             | 21                            | 18-24 <sup>6</sup> |
| Ampicillin                                 | 4                   | 2-8                | 10                | 18-19                         | 15-22 <sup>6</sup> |
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| Cefadroxil                                 | -                   | -                  | 30                | <b>17</b>                     | <b>14-20</b>       |
| Cefalexin                                  | 8                   | 4-16               | 30                | <b>18</b>                     | <b>15-21</b>       |
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| Cefixime                                   | 0.5                 | 0.25               | 5                 | <b>23</b>                     | <b>20-26</b>       |
| Cefotaxime                                 | 0.06                | 0.03-0.125         | 5                 | <b>28</b>                     | <b>25-31</b>       |
| Cefoxitin                                  | 4                   | 2-8                | 30                | 26                            | 23-29              |
| Cefpodoxime                                | 0.5                 | 0.25-1             | 10                | 25-26                         | 23-28              |
| Ceftaroline                                | 0.06                | 0.03-0.125         | 5                 | <b>27</b>                     | <b>24-30</b>       |
| Ceftazidime                                | 0.125-0.25          | 0.06-0.5           | 10                | <b>26</b>                     | <b>23-29</b>       |
| Ceftazidime-avibactam <sup>8,9</sup>       | 0.125-0.25          | 0.06-0.5           | 10-4              | <b>27</b>                     | <b>24-30</b>       |
| Ceftibuten                                 | 0.2                 | 0.125-0.5          | 30                | 31                            | 27-35              |
| Ceftobiprole                               | 0.06                | 0.03-0.125         | 5                 | <b>28</b>                     | <b>25-31</b>       |
| Ceftolozane-tazobactam <sup>10,11</sup>    | 0.25                | 0.125-0.5          | 30-10             | 28                            | 24-32              |
| Ceftriaxone                                | 0.06                | 0.03-0.125         | 30                | 32                            | 29-35              |
| Cefuroxime                                 | 4                   | 2-8                | 30                | 23                            | 20-26              |
| Chloramphenicol                            | 4                   | 2-8                | 30                | 24                            | 21-27              |
| Ciprofloxacin                              | 0.008               | 0.004-0.016        | 5                 | <b>33</b>                     | <b>29-37</b>       |

# Monitoring Laboratory QC results



# Monitoring Laboratory QC results





### E.coli ATCC 25922 vs. Cefotaxime

QC target and range: 28 (25 - 31 mm).

Mean (n=1015): 28 mm ( $\pm 2$  SD)  $\pm 2.4$  mm

MH medium from 3 manufacturers

1 19 37 55 73 91 109 127 145 163 181 199 217 235 253 271 289 307 325 343 361 379 397 415 433 451 469 487 505 523 541 559 577 595 613 631 649 667 685 703 721 739 757 775 793 811 829 847 865 883 901 919 937 955 973 991 1009  
Day (4 tests per week)  
Paris 2016

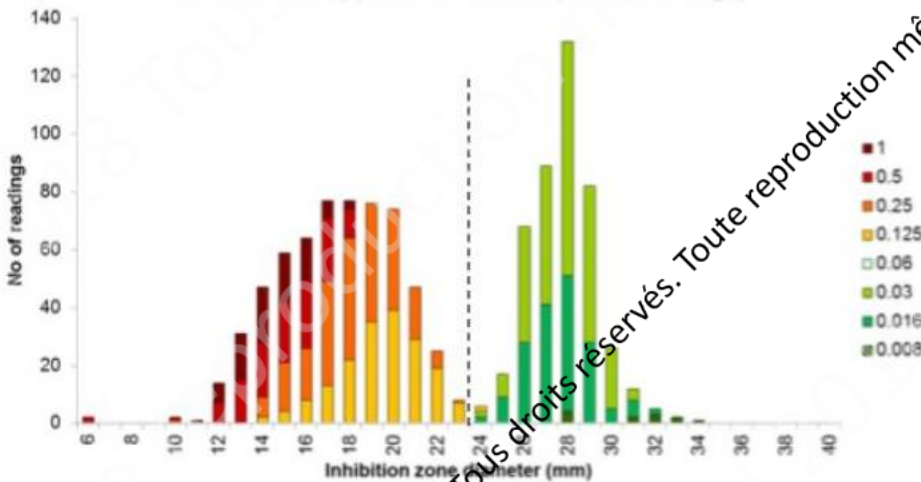
# Establishment of zone diameter breakpoints



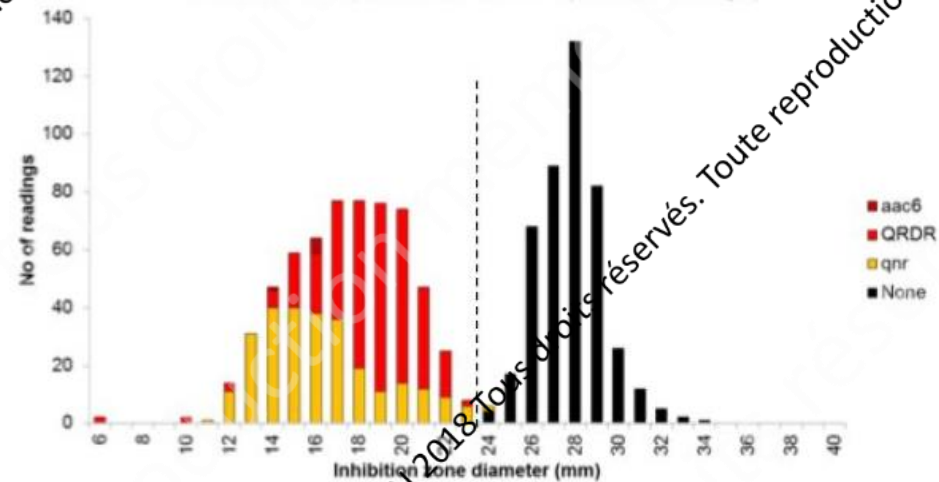
# 3. Establishing zone diameter breakpoints

- Correlation of zone diameters to corresponding MIC values and/or defined resistance mechanisms.

Pefloxacin 5 µg vs. Ciprofloxacin MIC  
*Salmonella* spp., 126 isolates (1044 readings)

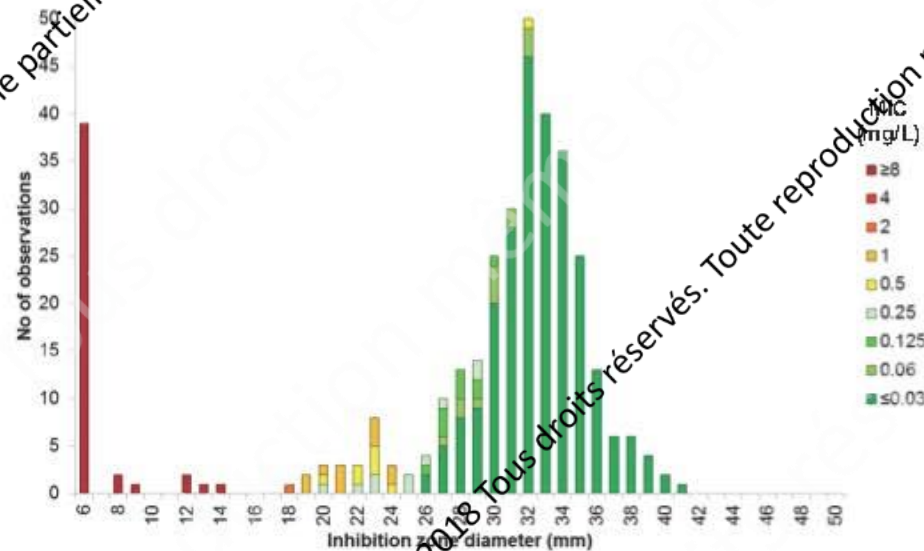
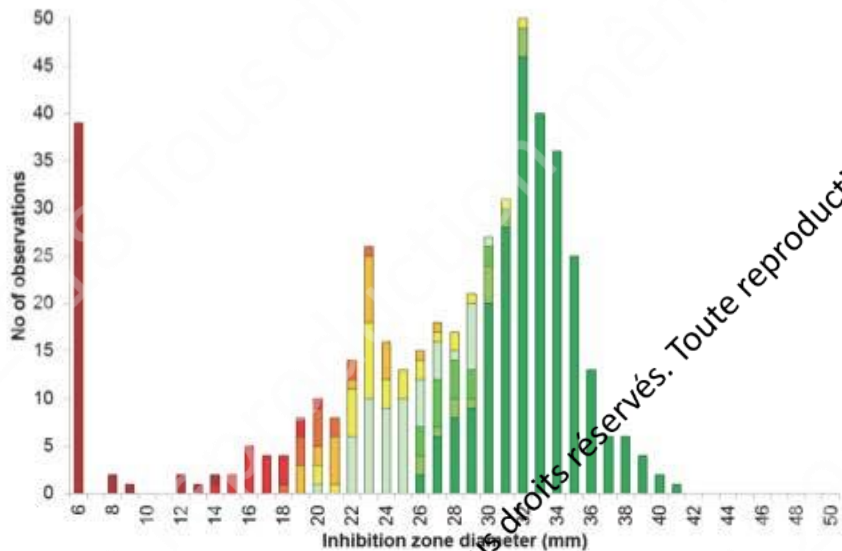


Pefloxacin 5 µg vs. FQ resistance mechanism  
*Salmonella* spp., 126 isolates (1044 readings)



# Isolates for MIC-zone correlation studies

- The composition of the isolate collection greatly affects the results!
  - We can manipulate the numbers of VME:s and ME:s by including more or less difficult isolates.



VME = Very Major Error (false susceptible)  
 ME = Major Error (false resistant)

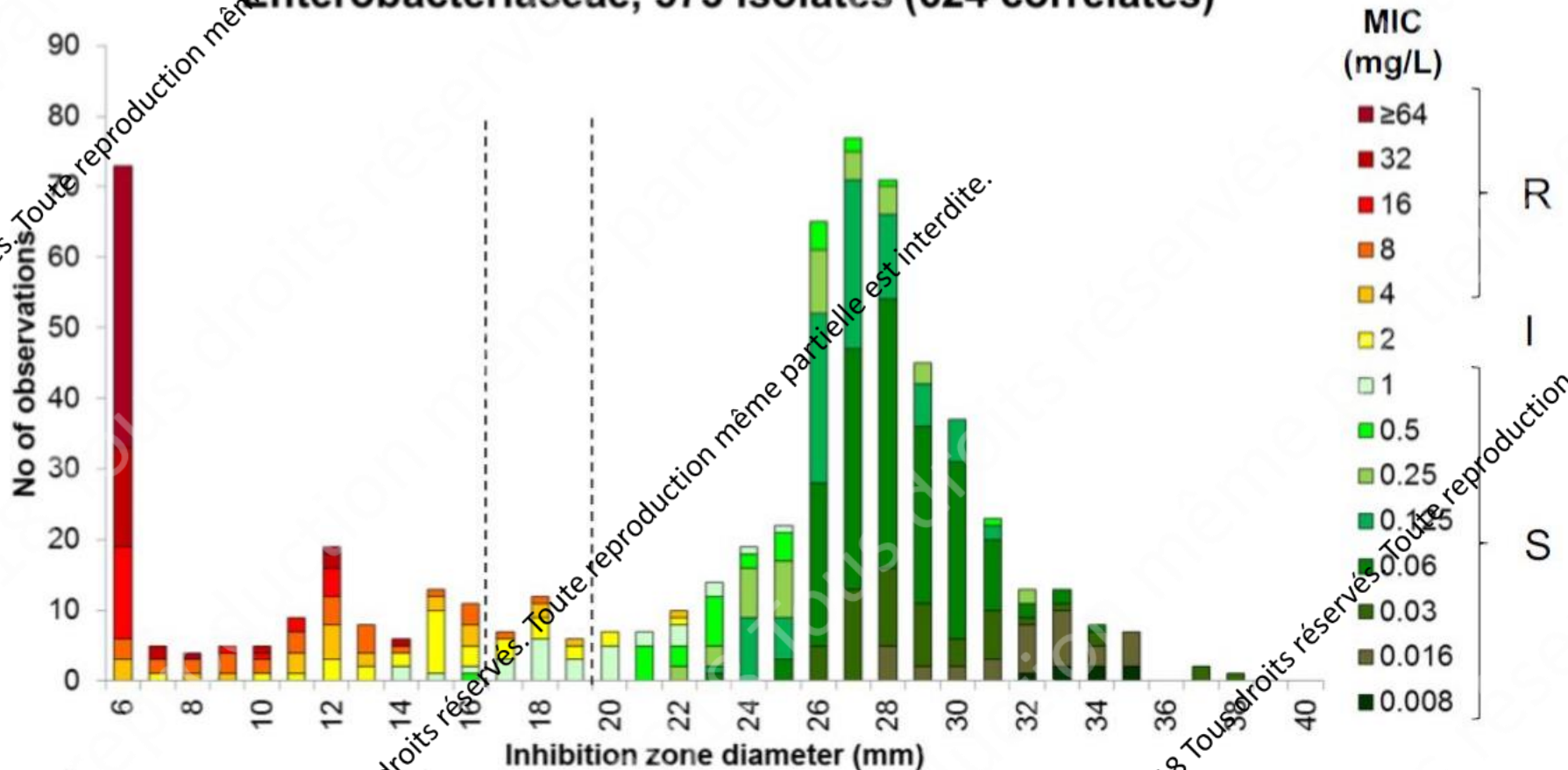
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# MIC-zone diameter correlation studies

## Study layout

- CLSI (M23)
  - No specifications on number of media and disk manufacturers or number of test sites
- EUCAST (SOP 9.0)
  - Media from  $\geq 2$  manufacturers
  - Disks from  $\geq 2$  manufacturers
  - 1-2 laboratories for MIC-zone diameter correlation studies
  - Validation by  $\geq 4$  additional laboratories

## Cefotaxime 5 $\mu$ g vs. MIC Enterobacteriaceae, 573 isolates (624 correlates)



### Breakpoints

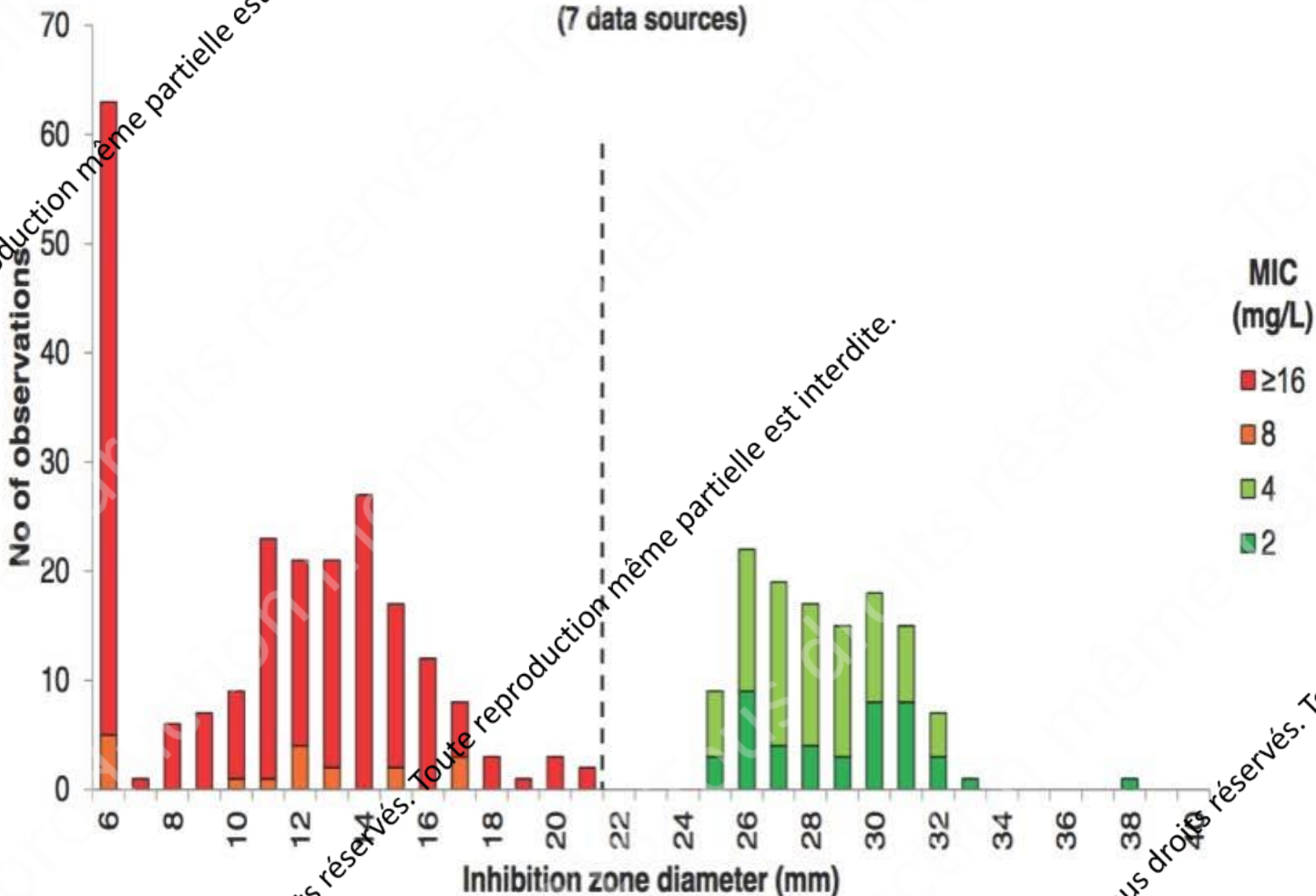
$S \leq 1$ ,  $R > 2$  mg/L

$S \geq 20$ ,  $R < 17$  mm

# Cefoxitin 30 µg vs. MIC

## *S. aureus*, 287 isolates (348 correlates)

(7 data sources)



### Breakpoints

MIC (screen)

Zone diameter (screen)

S ≤ 4, R > 4 mg/L

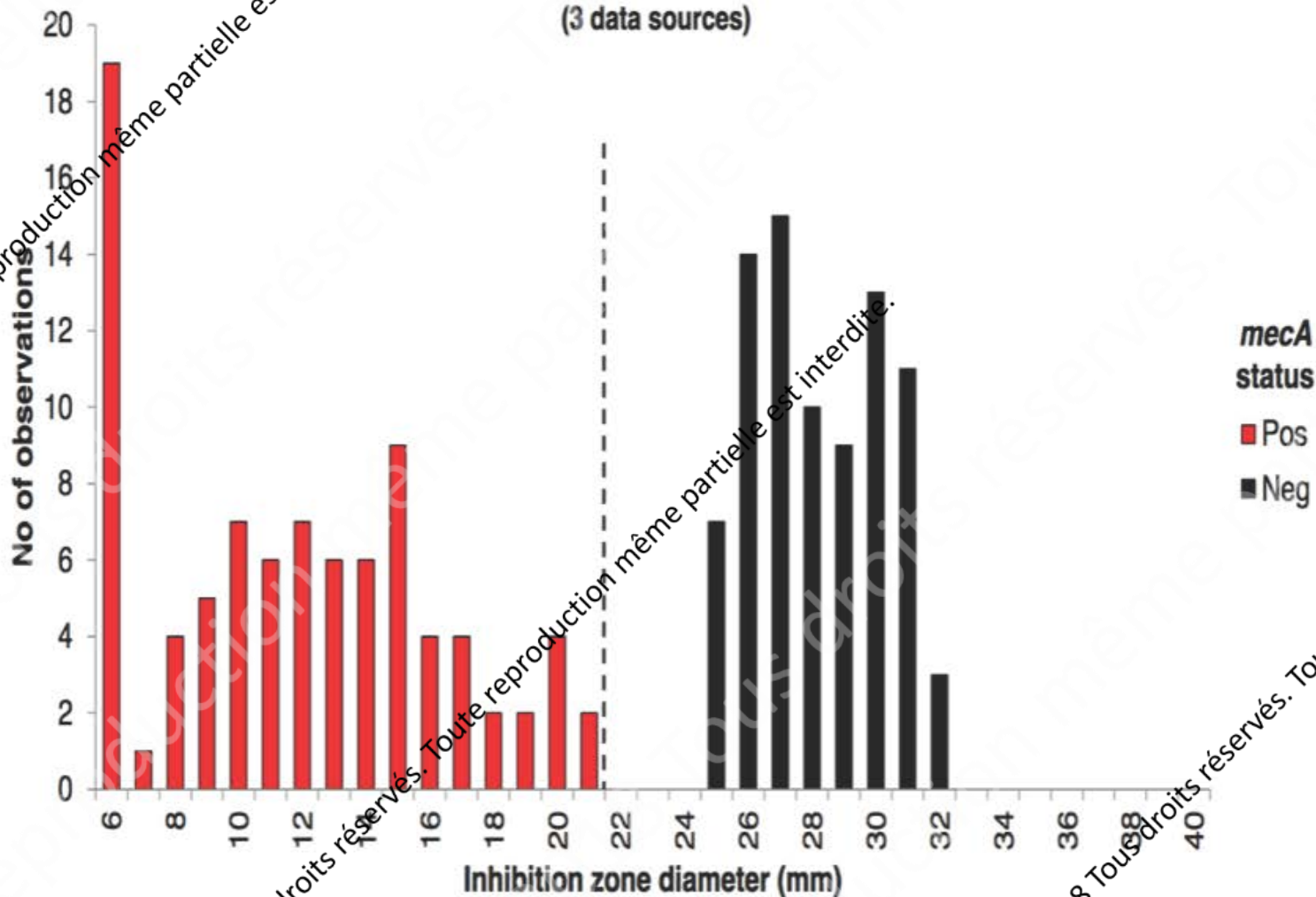
S ≥ 22, R < 22 mm

### ECOFF

4 mg/L

# Cefoxitin 30 µg vs. *mecA* status *S. aureus*, 170 isolates

(3 data sources)



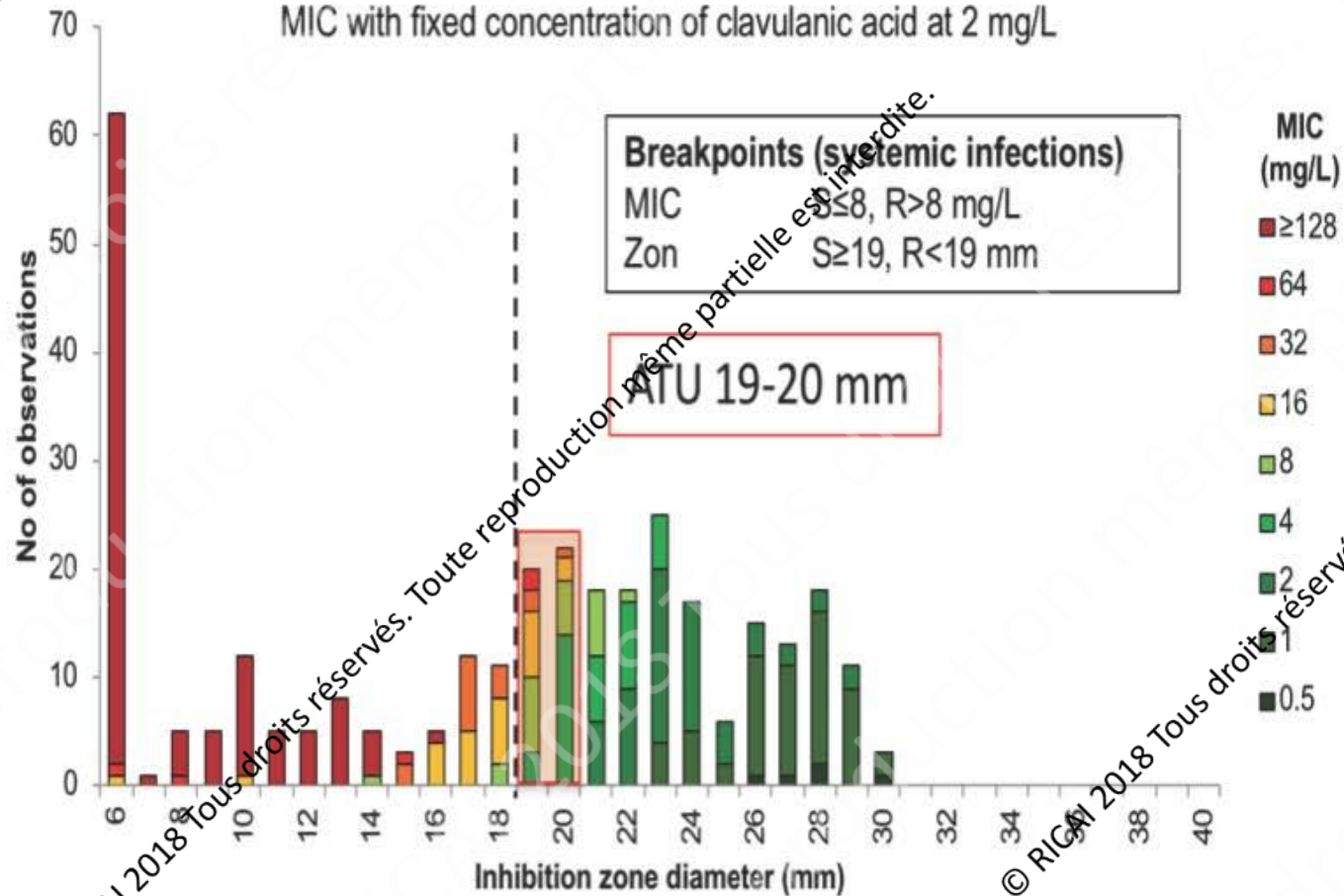
## Breakpoints

Zone diameter (screen)  $S \geq 22$ ,  $R < 22$  mm

# Amoxicillin-clavulanic acid vs. Enterobacterales with breakpoints for systemic infections

## Amoxicillin-clavulanic acid 20-10 µg vs MIC Enterobacterales, 325 isolates

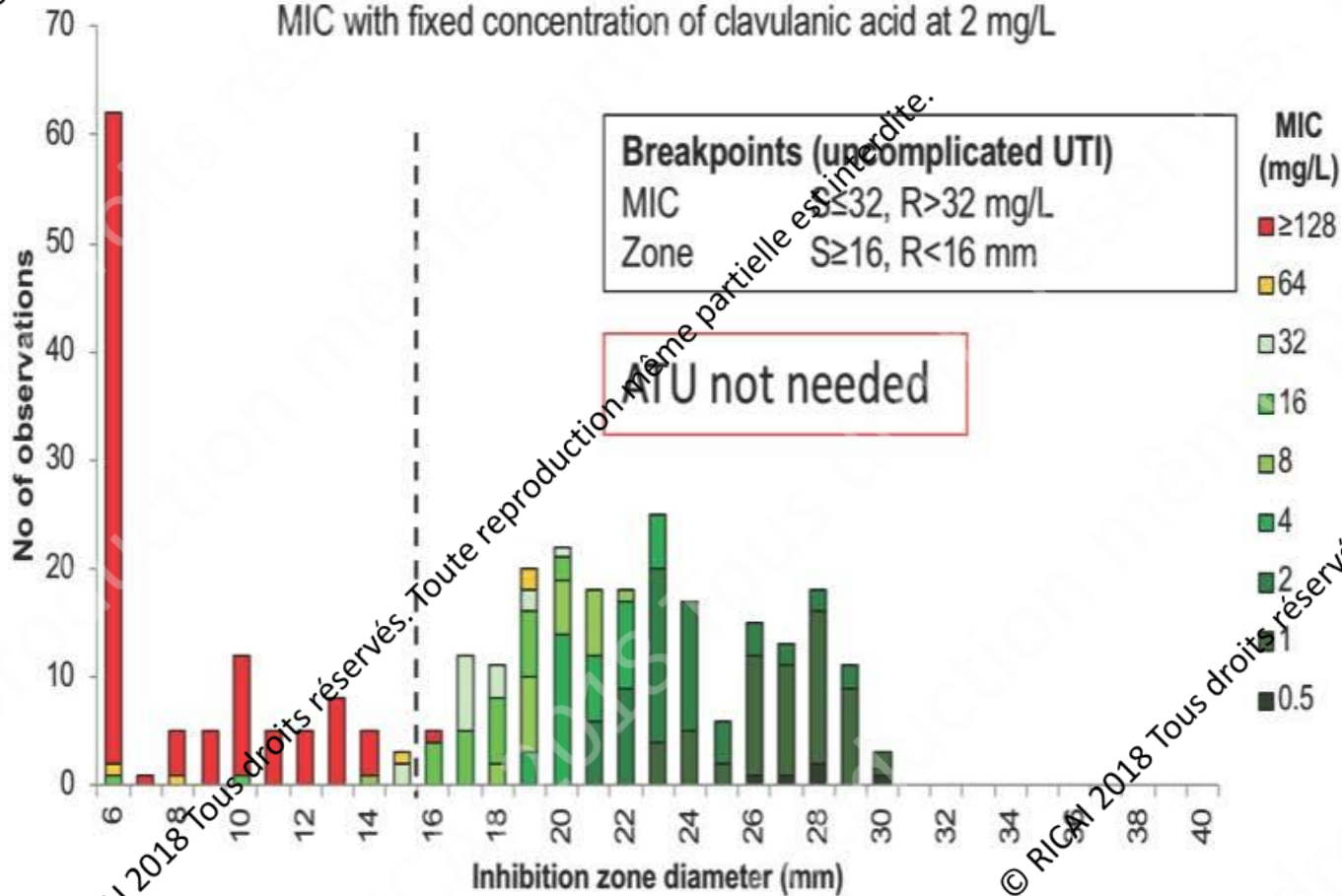
MIC with fixed concentration of clavulanic acid at 2 mg/L



# Amoxicillin-clavulanic acid vs. Enterobacterales with breakpoints for uncomplicated UTI

## Amoxicillin-clavulanic acid 20-10 µg vs MIC Enterobacterales, 325 isolates

MIC with fixed concentration of clavulanic acid at 2 mg/L





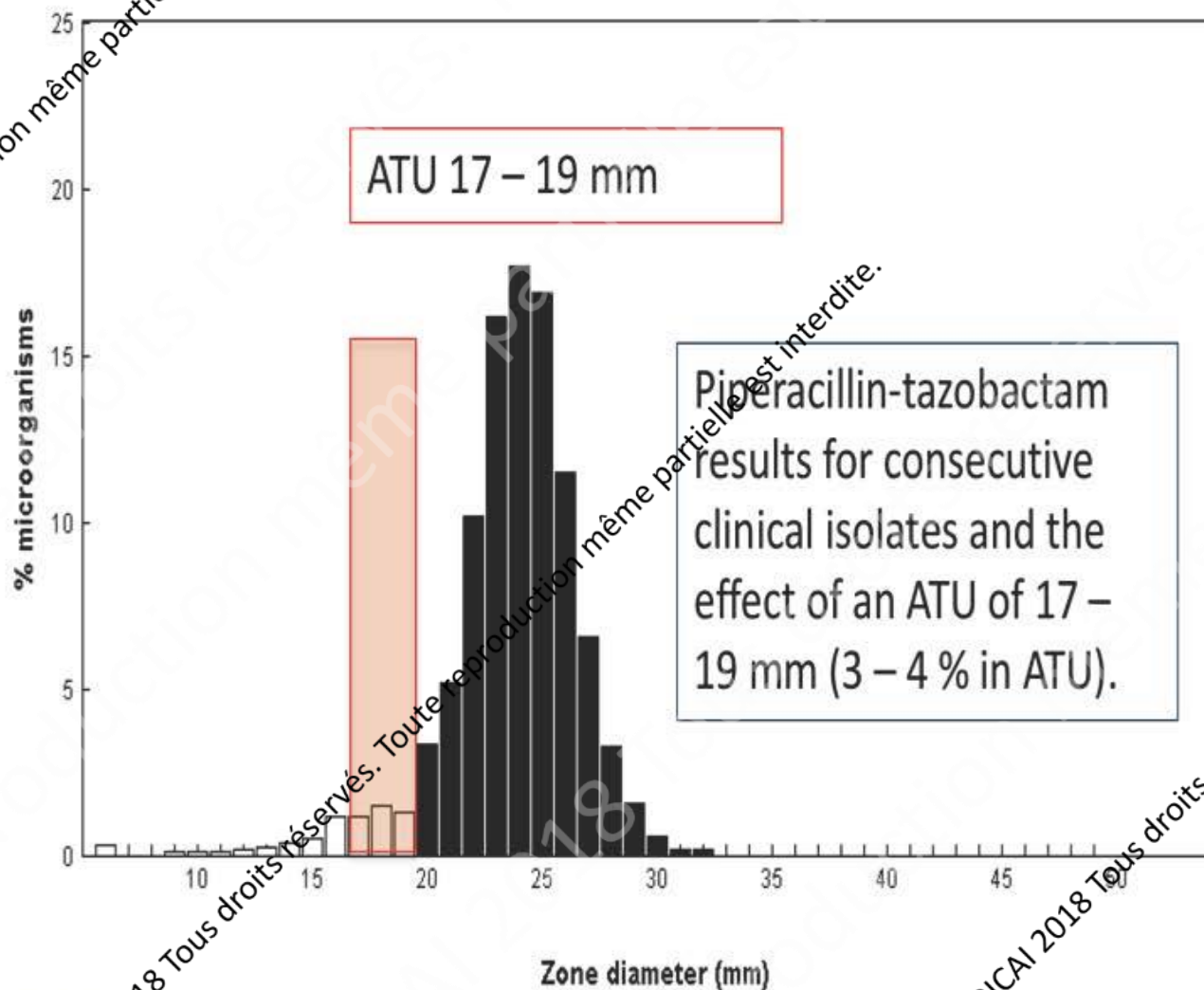


# Piperacillin-tazobactam / Escherichia coli

International wild type zone diameter distribution - Reference database 2017-04-21

EUCAST disk diffusion method

Distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



Disk content: 36

Epidemiological cut-off (ECOFF): 20 mm (MIC = 8 mg/L)

Wild type (WT) organisms:  $\geq 20$  mm (MIC = 8 mg/L)

Paris 2018

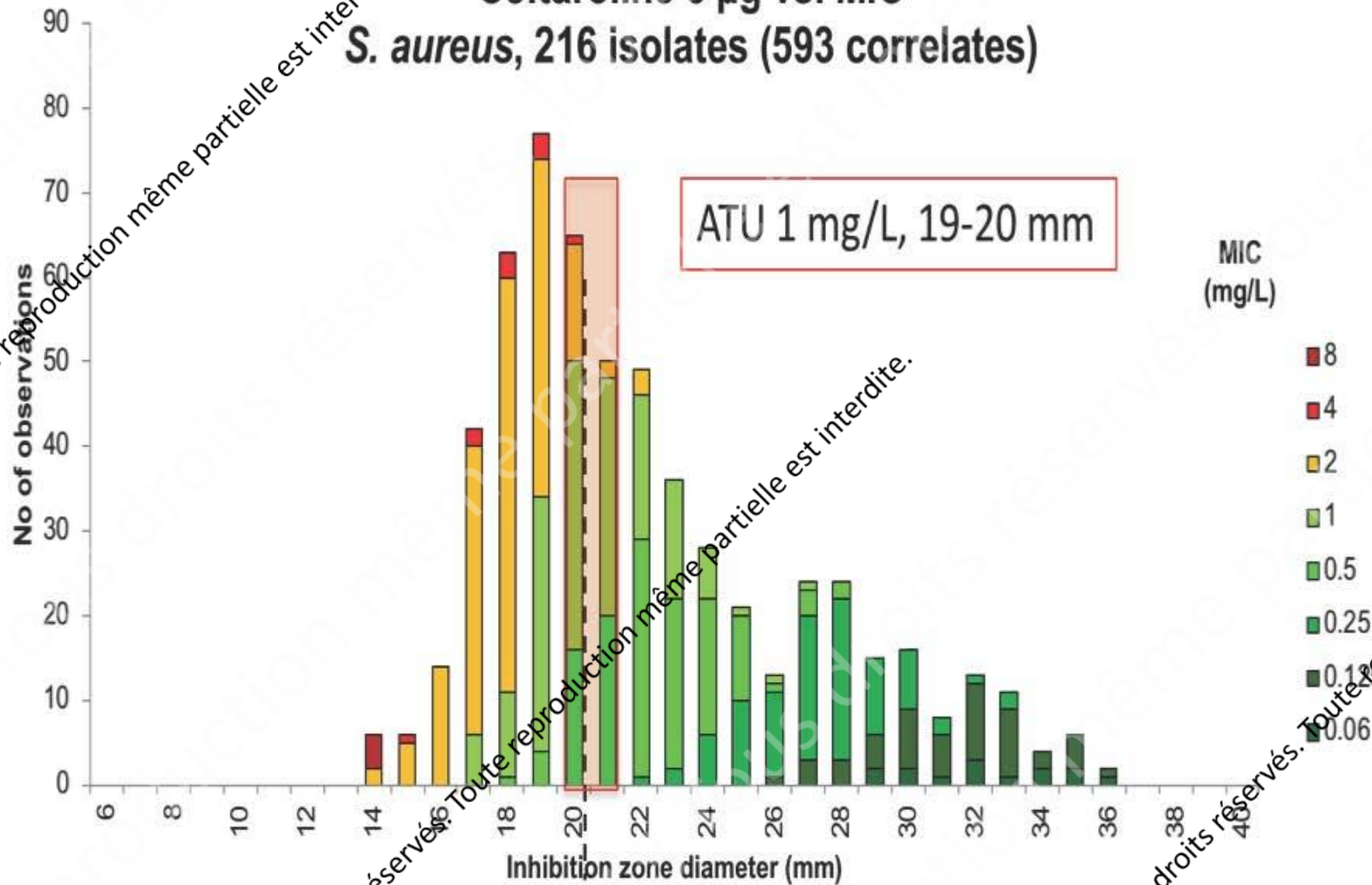
6033 observations (9 data sources)

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# Ceftaroline 5 µg vs. MIC

## *S. aureus*, 216 isolates (593 correlates)



### Breakpoints (pneumonia)

MIC  $S \leq 1, R > 1 \text{ mg/L}$   
 Zone diameter  $S \geq 20, R < 20 \text{ mm}$

Paris 2018

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**AST of bacteria**

- Organizations
- EUCAST News
- How definitions of S, I and R
- Clinical breakpoints and dosing
- Rapid AST in blood cultures
- Expert rules and intrinsic resistance
- Resistance mechanisms
- Guidance documents
- Consultations - New!
- MIC and zone distributions and ECOFFs
- AST of bacteria**
  - Media preparation
  - MIC determination
  - Disk diffusion methodology
  - Disk diffusion implementation
  - Breakpoint tables
  - QC Tables
  - Calibration and validation**
  - Archive
  - Warnings!
  - Guidance documents
  - Projects and data submission
  - MIC testing services from EUCAST
  - Previous versions of documents



... Calibration and validation

**Development and validation of EUCAST Disk Diffusion breakpoints**

The EUCAST Disk Diffusion test was developed by EUCAST during 2009 - 2013 under the auspices of ESCMID and with the help of many laboratories. The help of these laboratories is gratefully acknowledged. The EUCAST disk diffusion method is continuously developed and updated for new agents and for new species. Furthermore, changes in MIC breakpoints merit updates in the validation/calibration files.

The files below list material and graphs used for determining zone diameter breakpoints to match MIC breakpoints (Example 1).

Updated (all) and added (*Aeromonas*, *Plesiomonas*, *Stenotrophomonas* and *Yersinia*) files on 7 June, 2018. The order in which files are presented matches the order in which species are presented in the breakpoint table. All MIC values are with broth microdilution. All presentations are against MIC breakpoints in the EUCAST breakpoint Table 8.1.

- Enterobacteriaceae
- *Salmonella* spp.
- *Yersinia enterocolitica*
- *Plesiomonas shigelloides*
- *Pseudomonas aeruginosa*
- *Pseudomonas fluorescens*
- *Stenotrophomonas maltophilia*
- *Acinetobacter* spp.
- *Staphylococcus aureus*
- *Staphylococcus, coagulase-negative*
- *Staphylococcus saprophyticus*
- *Staphylococcus pseudintermedius*
- *Enterococcus* spp.
- *Streptococcus pyogenes* (group A)
- *Streptococcus agalactiae*
- *Streptococcus pneumoniae*
- *Streptococcus pneumoniae* - screen for beta-lactam resistance
- Viridans Group Streptococci
- *Haemophilus influenzae*
- *Haemophilus influenzae* - screen for beta-lactam resistance
- *Moraxella catarrhalis*
- *Listeria monocytogenes*
- *Pasteurella multocida*
- *Campylobacter jejuni* and *C.coli*
- *Corynebacterium* spp.
- *Aerococcus sanguinicola* and *A. anophagephaga*
- *Kingella kingae*
- *Aeromonas* spp.

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# AST – you need to get it right!

## Difficult AST (irrespective of method):

- Piperacillin/tazobactam in Enterobacteriaceae (ATU)
- Amoxicillin/clavulanic acid systemic breakpoint in Enterobacteriaceae .
- Betalactams in *H. influenza* with PBP3-mutations.
- Ceftaroline and ceftobiprole in Staphylococci.
- Ceftaroline in Enterobacteriaceae.
- Colistin (not so difficult but BMD is mandatory)
- Fosfomycin – all methods (AD, Disk diffusion)

# AST – you need to get it right!

## Difficult AST (irrespective of method):

- Piperacillin/tazobactam in Enterobacteriaceae (ATU)
- Amoxicillin/clavulanic acid in Enterobacteriaceae
- Betalactams in Enterobacteriaceae
- Ceftazidime in Enterobacteriaceae
- Ceftazidime/avopiban in Enterobacteriaceae
- Ceftazidime/avopiban in Enterobacteriaceae
- Colistin in Enterobacteriaceae
- Fosfomycin in Enterobacteriaceae

## Not difficult AST:

- Amoxicillin/clavulanic acid with UTI breakpoints
- Cefotaxime/ceftazidime/cefepime Enterobacteriaceae
- Screening for RBP3-resistance mechanisms in *H. influenzae*
- Meropenem/imipenem in Enterobacteriaceae
- Penicillin resistance in *S. pneumoniae*
- Erythromycin resistance
- Trimethoprim and trimethoprim-sulfamethoxazole in any bacterium (except *S. maltophilia* and *B. pseudomallei*)
- ....and most others

# Problems and/or questions?

Please contact us!

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