Investigational New Drug - Groundwork for *in vitro* antimicrobial susceptibility testing

Erika Matuschek, Ph D
Lead Scientist/Operational Manager
EUCAST Development Laboratory (EDL)
Växjö, Sweden

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Disclosures

- The work at EUCAST Development Laboratory is financed by ESCMID.

- During 2014-2017, development of QC criteria and zone diameter for new antimicrobial agents have been supported by:
  - Basilea, Cempra, Cubist, GSK, Merck, Nabriva, Paratek and Tetraphase.

EUCAST Development Laboratory for bacteria (EDL)

- Development and maintenance of EUCAST methods

- Evaluation of AST materials

- Support to clinical laboratories

- Educational activities

- Collaborations with several other laboratories
  - "EUCAST Network Laboratories"

c/o Clinical Microbiology
Central Hospital
351 85 Växjö
Sweden

Standardization of AST

- Results change with changed parameters.
  - Standardization is crucial to get reproducible and reliable results!

- Standardization of:
  - Potency of antimicrobial agent (disk potency)
  - Media
    - Type of media, supplements, pH, agar depth etc.
  - Inoculum
  - Incubation
    - Time and atmosphere
  - Reading of results

Reference methodology for MIC testing

ISO standard 20776-1, 2006

Clinical laboratory testing and in vitro diagnostic test systems —
Susceptibility testing of infectious agents and evaluation of
performance of antimicrobial susceptibility test devices —
Part 1:
Reference method for testing the in vitro activity of antimicrobial
agents against rapidly growing aerobic bacteria involved in
infectious diseases

Broth microdilution
- standard methodology

- Mueller-Hinton (MH) broth

- Inoculum: 5×10^5 CFU/mL

- Incubation of sealed panels in ambient air at 35°C for 16-20 h

- The MIC is recorded as the lowest concentration of the agent that completely inhibits visible growth

Special test situations (I)

- Daptomycin
  - Addition of 50 mg/L Ca^{2+}

- Tigecycline
  - Freshly prepared (<12 h) test medium

- Lipoglycopeptides (dalbavancin, telavancin and oritivancin)
  - Addition of 0.002% polysorbate-80

- Cefiderocol
  - Iron-depleted MH broth

Special test situations (II)

- *Streptococcus* species
  - Addition of 2.5-5% lysed horse blood (CAMHB-LHB)

- Other fastidious organisms are not covered by the ISO standard.
  - Two methods used internationally for *H. influenzae*:
    - CLSI: Haemophilus Test Medium (HTM)
    - EUCAST: MH-F broth (MH broth with 5% lysed horse blood and 20 mg/L β-NAD)
      - EUCAST recommends MH-F broth as a common medium for several other fastidious organisms, including streptococci.

Specific reading instructions

- Sulphonamides and trimethoprim (ISO 20776-1)
  - The MIC should be read at the lowest concentration that inhibits approximately 80% of growth as compared with the growth control well.

- Other specific reading instructions may have to be agreed e.g. to handle trailing endpoints.

Example trimethoprim-sulfamethoxazole: ≥80% reduction in growth as compared to the growth control
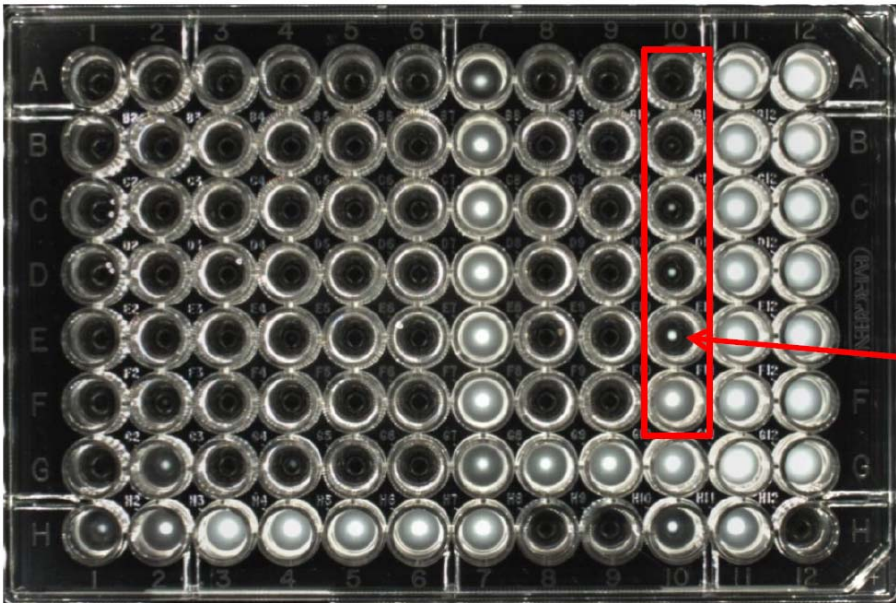
Example linezolid: Read the MICs at the first spot were trailing begins (ignore pin-point growth)
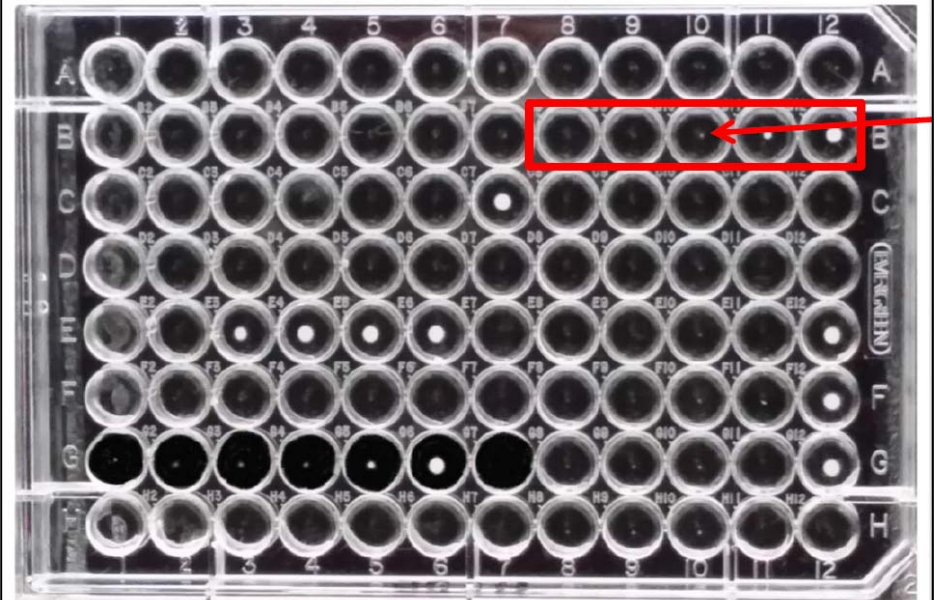
CLSI, M07-A10, 2015: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically.
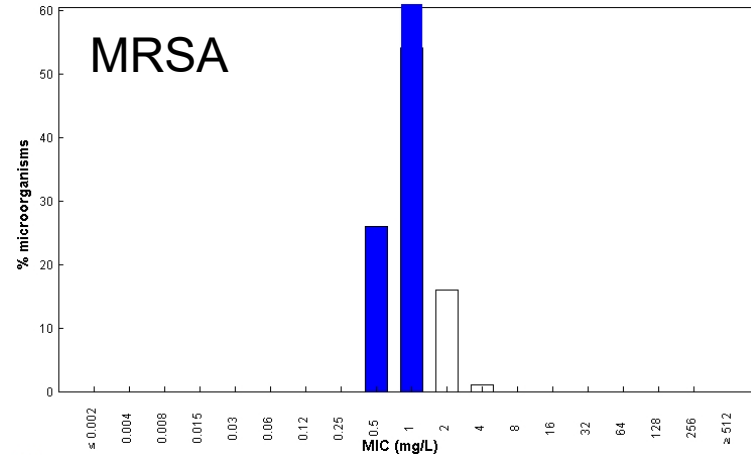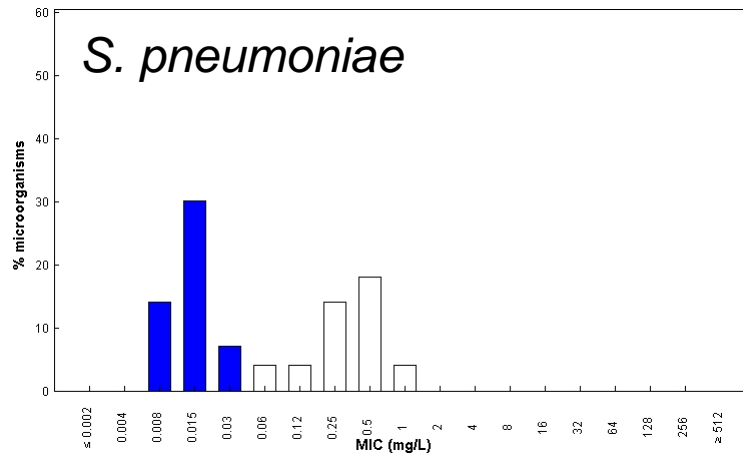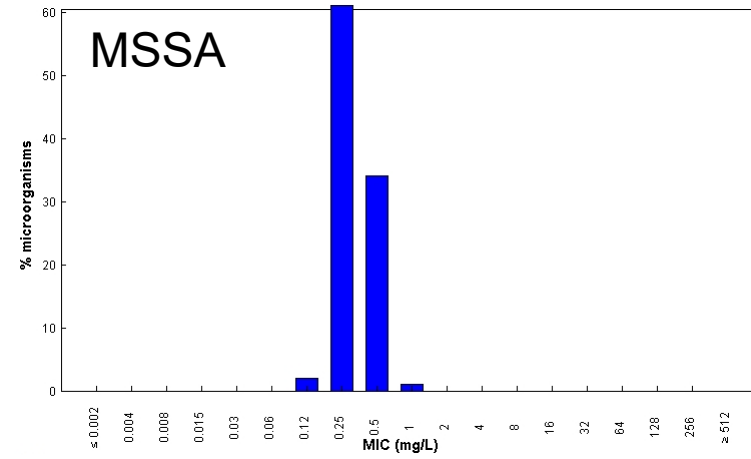
MIC testing during drug development

- Reference methodology must be defined before producing MIC distributions and performing potency determinations
  - Special test situations or supplements?
  - Specific reading instructions?
  - Fastidious organisms?
    - Differences between EUCAST and CLSI recommendations
  - Agent-inhibitor combinations
    - Ratio or fixed concentration?

- QC ranges must be defined beforehand to allow reliable testing during clinical trials and to detect resistance
  - Reduce patient risk

Microbiological activity

- MIC distributions for relevant Gram-negative and Gram-positive bacteria

  – Define target species

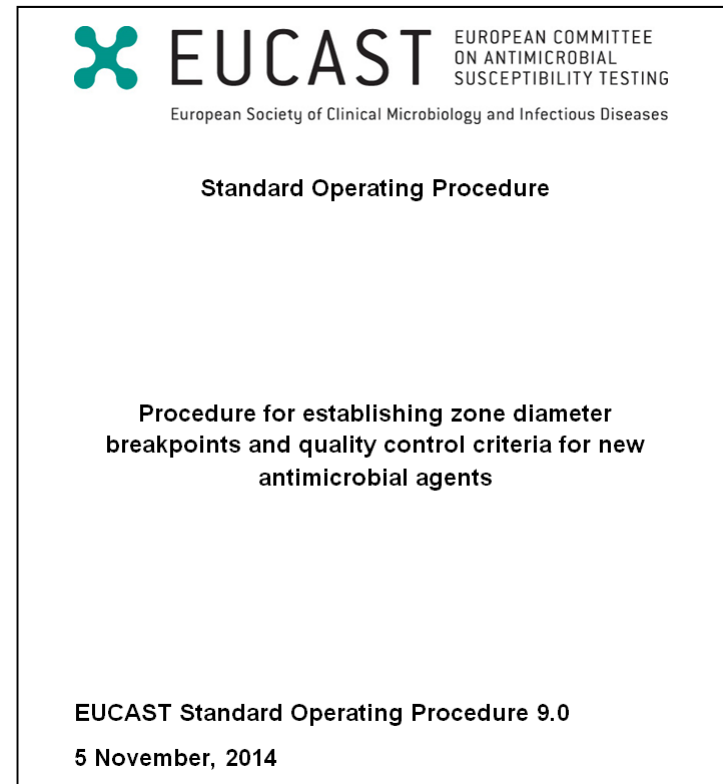 – Identify wild-type isolates

  – Identify isolates with known resistance mechanisms
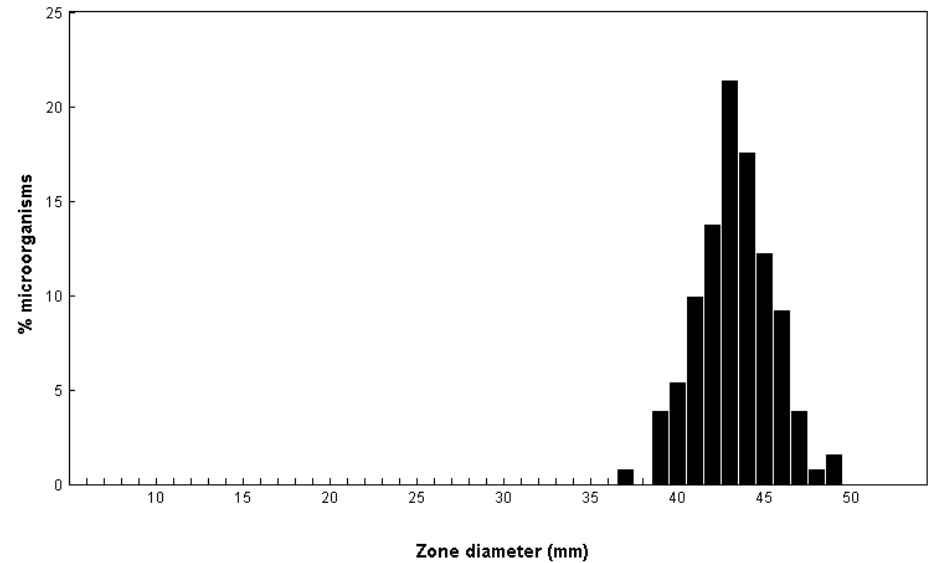
Example: ceftobiprole
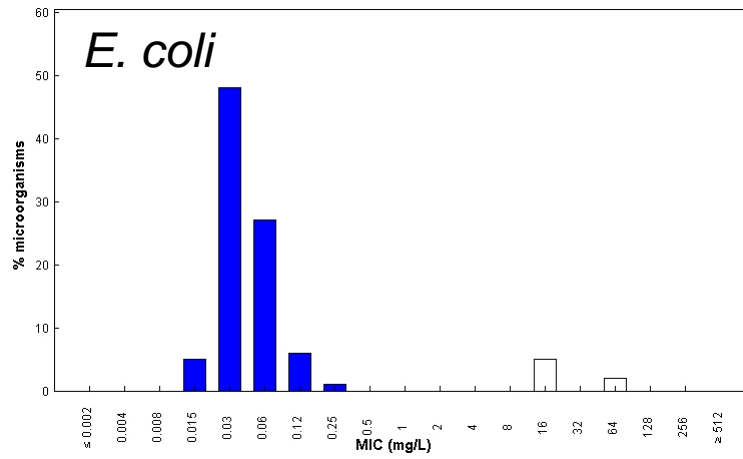
Development of quality control (QC) criteria and zone diameter breakpoints

M23-A3
Vol. 28 No. 27
Replaces M23-A2
Vol. 21 No. 7

Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline—Third Edition

This document addresses the required and recommended data needed for the selection of appropriate interpretive criteria and quality control ranges for antimicrobial agents. A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

EUCAST — EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Standard Operating Procedure

Procedure for establishing zone diameter breakpoints and quality control criteria for new antimicrobial agents

EUCAST Standard Operating Procedure 9.0

5 November, 2014

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

Selecting disk potency

- Optimized inhibition zone size

Selecting disk potency

- Optimized inhibition zone size

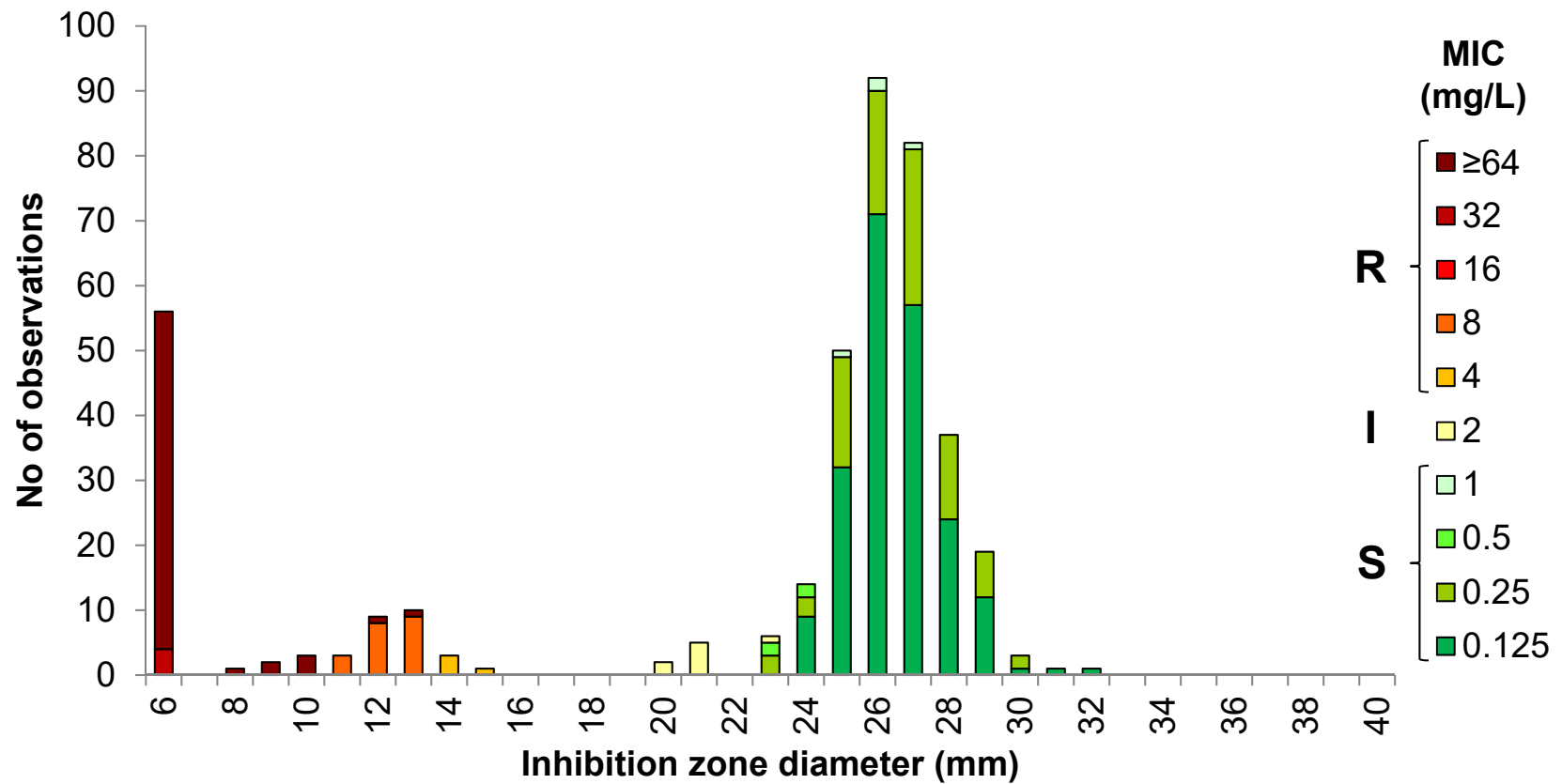- Calibration of inhibition zones to reference MIC
    - Correlation between zone diameters and MIC
    - Separation between wild-type and non-wild type isolates
    - Prediction of susceptibility and resistance

Agent X, 5 µg disk vs. MIC
S. aureus

Differences in disk potencies between CLSI and EUCAST

Antimicrobial agent	Disk potency (µg)	
	CLSI	EUCAST
Amoxicillin-clavulanic acid	20-10	20-10 for Enterobact. 2-1 for HI and Gram-pos
Ampicillin	10	10 for Enterobact. 2 for HI and Gram-pos
Benzylpenicillin	10 units	1 unit
Piperacillin	100	30
Piperacillin-tazobactam	100-10	30-6
Cefotaxime	30	5
Ceftaroline	30	5
Ceftazidime	30	10
Ceftazidime-avibactam	30-20	10-4
Ceftobiprole	30	5
Gentamicin HLAR screen	120	30
Netilmicin	30	10
Vancomycin	30	5
Linezolid	30	10
Nitrofurantoin	300	100

…and several new drugs are developed with different disk potencies.

Selecting relevant QC strains
(MIC testing and disk diffusion)

- Strains representing target organisms

- On-scale MIC values

- Optimized inhibition zone size

- Resistant strain(s) needed for control?
  - E.g. β-lactam-β-lactamase inhibitor combinations

QC studies

- CLSI (M23)
  - One multi-lab study: 7 labs x 3 media (10 replicates)
  - Disks from 2 manufacturers
  - Media from 2-3 manufacturers

- EUCAST
  - Initial two-site study: 2 labs x 3-4 media (15 replicates)
  - Validation study: ≥4 sites x local media (10 replicates)
  - Disks from 2-3 disk manufacturers

Disk QC studies: CLSI (M23) data analysis

Zone (mm)	Lab 1 Lot A	Lot B	Lot C	Lab 2 Lot A	Lot B	Lot C	Lab 3 Lot A	Lot B	Lot C	Lab 4 Lot A	Lot B	Lot C	Lab 5 Lot A	Lot B	Lot C	Lab 6 Lot A	Lot B	Lot C	Lab 7 Lot A	Lot B	Lot C	Lab 8 Lot A	Lot B	Lot C	ALL Labs Lot A	Lot B	Lot C
20																											
21																											
22																											
23																											
24																											
25							1																		1		
26		3					2			3	10						1								5	14	
27	7	12		1	9		10	9		8	16	3	1	12			2		1				2		28	62	3
28	13	11	5	10	11		13	20	1	18	3	13	15	13	14	1	5		3			4	9		77	72	33
29	9	3	10	9	6	2	4		13	1	1	13	11	4	11	7	13	4	7			12	9	3	62	42	56
30	1	1	9	8	4	9			15			1	3	1	5	8	5	7	7	5		10	8	10	37	22	56
31			6	2		12			1							8	3	6	3	10	5	4	2	11	17	15	41
32						6										5	1	6	5	7	10			4	10	8	26
33						1										1		7	4	4	12			2	3	4	22
34																				1	3					1	3
35																											
36																											
37																											
38																											
Count	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	240	240	240

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	ALL Labs
Combined	90	90	90	90	90	90	90	90	720
Mean	28.4	29.3	28.3	27.6	28.3	30.2	31.2	29.7	29.1
SD	1.235	1.492	1.142	0.969	0.850	1.554	1.625	1.245	1.707
Median	28	29	28	28	28	30	31	30	29
Range	6	7	7	5	4	8	8	7	10

|		Rounded Out		All Lab Median	29	Median + 1/2 R	
All Lab Mean	29.1						
Average SD	1.31			Median of Ranges (MR)	7		
+/- 2SD	26.51 31.77	26	32	1/2 MR Rounded up (R)	4.00	25	33

- Gavan statistics (median values and standard deviation).
- Ideally, ≥95% of the data should be included in the range.

Disk QC studies: EUCAST data analysis

- Mean and median values

- Range (minimum to maximum value)

- Data analyzed per

  – Testing site

  – Disk manufacturer

  – Media manufacturer

- Normal distribution Gaussian shaped?

Disk QC studies: EUCAST data analysis

- Range often median ± 3 mm.

Initial two-site study

Validation study
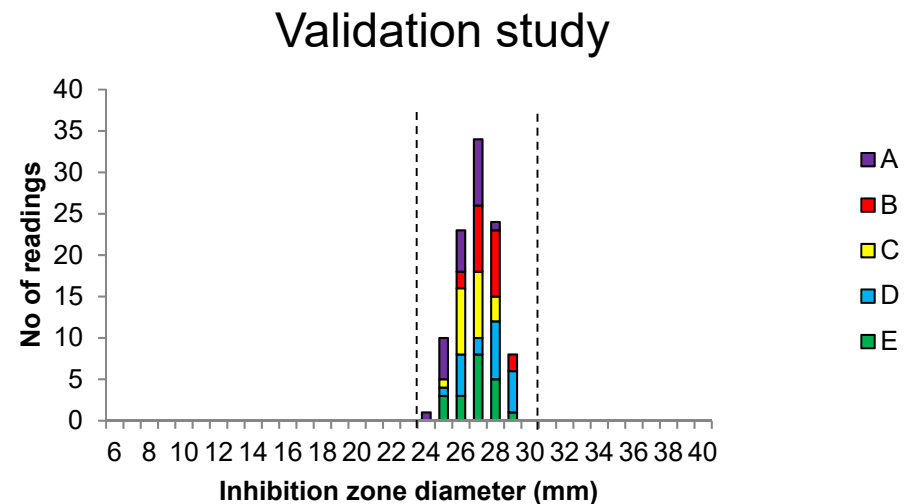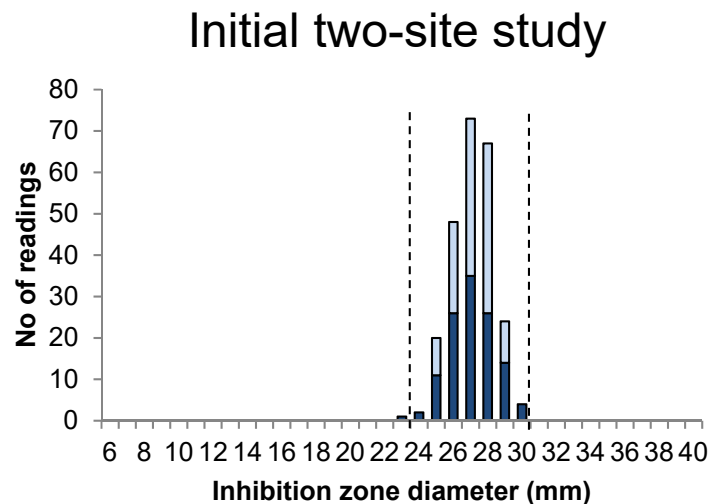
Range 24-30 mm, target 27 mm

EUCAST QC ranges and targets

Routine QC   EUCAST QC Tables v. 6.1, valid from 2016-03-01

Escherichia coli ATCC 25922
(NCTC 12241, CIP 76.24, DSM 1103, CCUG 17620, CECT 434)

Disk diffusion methodology: Mueller-Hinton agar, McFarland 0.5, air, 35±1°C, 18±2h. Read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.

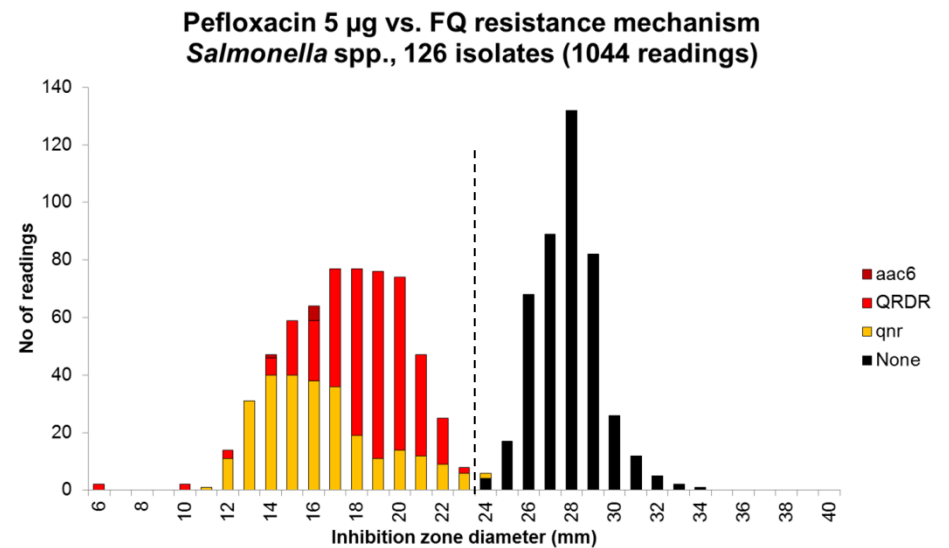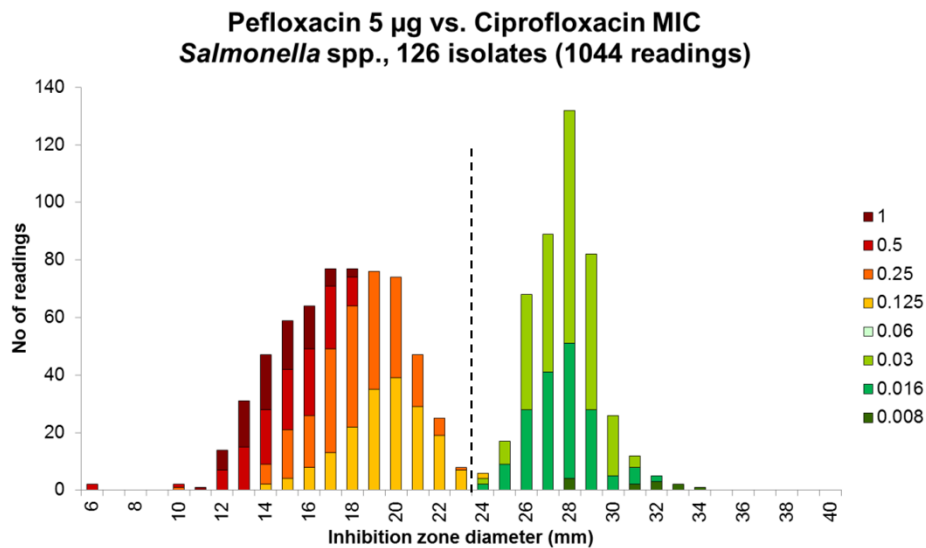| Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone diameter (mm)	
	Target[1]	Range[2]		Target[1]	Range[3]
Amikacin	1-2	0.5-4	30	22-23	19-26
Amoxicillin	4	*2-8*	-	-	-
Amoxicillin-clavulanic acid[4,5]	4	2-8	20-10	21	18-24[6]
Ampicillin	4	2-8	10	18-19	15-22[6]
Ampicillin-sulbactam[5,7]	*2*	*1-4*	10-10	21-22	19-24[6]
Aztreonam	0.125	0.06-0.25	30	32	28-36
Cefadroxil	-	-	30	*17*	*14-20*
Cefalexin	8	4-16	30	*18*	*15-21*
Cefepime	0.03-0.06	0.016-0.125	30	34	31-37
Cefixime	0.5	0.25-1	5	25	23-27
Cefotaxime	0.06	0.03-0.125	5	*28*	*25-31*
Cefoxitin	4	2-8	30	26	23-29

Range
Used to allow occasional variation

Target
Mean values from repeated measurements should optimally be on target ± 1 mm
(mode MIC on target)

Establishment of zone diameter breakpoints

- Correlation of inhibition 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)

Establishment of zone diameter breakpoints

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

  - USA: MIC and zone diameter breakpoints are established in parallel and included in the CLSI/FDA submission.

  - Europe: Zone diameter breakpoints are established after the MIC breakpoints are set.

    - Disk diffusion data not part of the package submitted to EMA/EUCAST by the pharmaceutical company.

EUCAST clinical MIC breakpoints are based on

- Available formulations

- Standard and maximum dosing

- Clinical indications and target organisms

- MIC distributions for individual species

- Pharmacokinetic data (PK)

- Pharmacodynamic data (PD)

- Information from modelling processes (Monte Carlo simulations)

- Clinical data relating outcome to MIC values

- Information on resistance mechanisms

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

EUCAST controlled document	EUCAST SOP 1.2
Date of issue: 21 November 2016	Page 1 of 15

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Standard Operating Procedure

Setting breakpoints for new antimicrobial agents
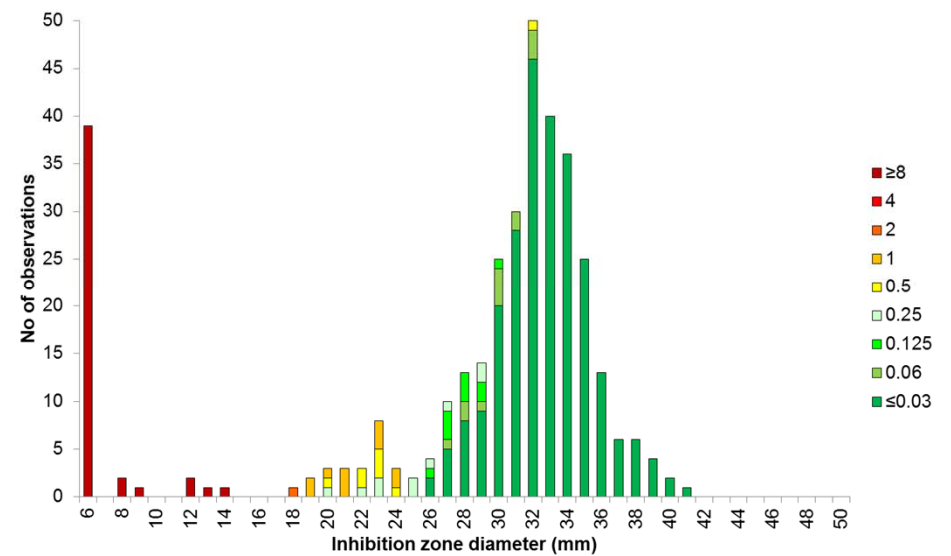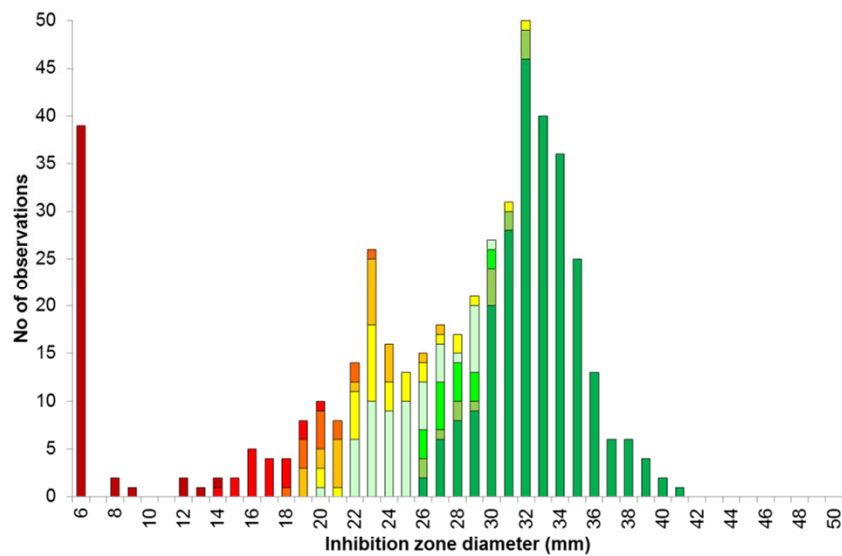
EUCAST SOP 1.2

21 November 2016

Isolates for MIC-zone correlation studies

- ~100 isolates per relevant species

  - Wild-type isolates

  - Isolates with relevant resistance mechanisms

  - Isolates with MICs close to the breakpoint

Isolates for MIC-zone correlation studies

- The composition of the isolate collection greatly affects the results!

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 ≥2 manufacturers
  - Disks from ≥2 manufacturers
  - 1-2 laboratories for MIC-zone diameter correlation studies
  - Validation by ≥4 additional laboratories

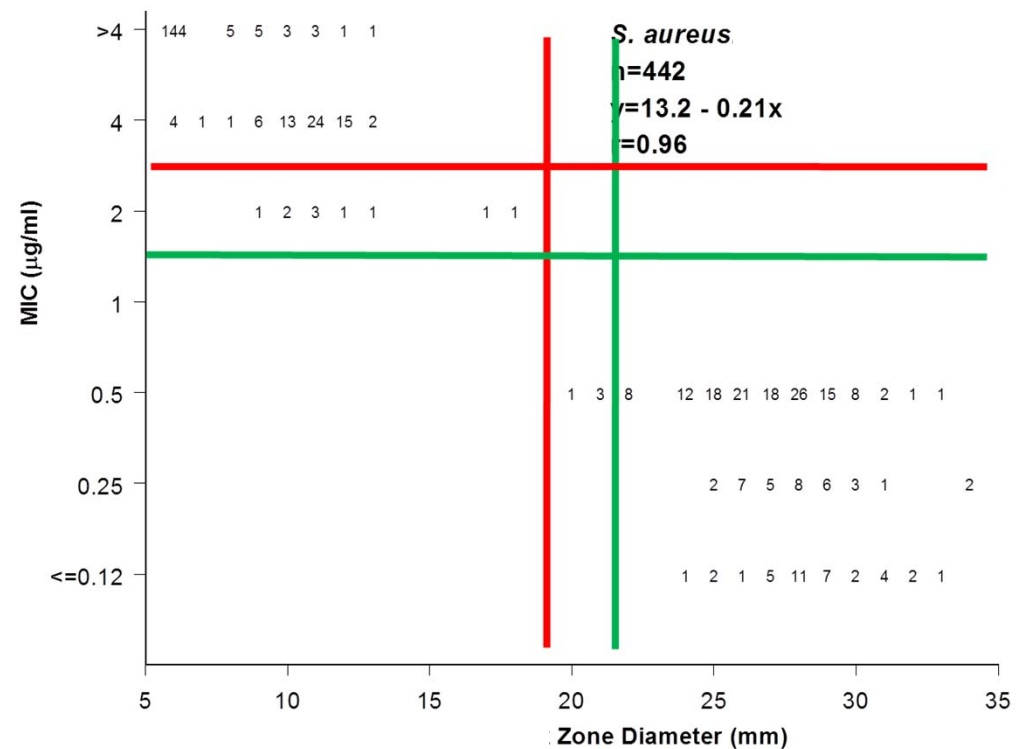# MIC-zone diameter correlation studies
CLSI data analysis

Error rate-bounded method

The zone diameter interpretive criteria are adjusted to minimize:
- False susceptible results (very major discrepancies)
- False resistant results (major discrepancies)

A higher level of minor discrepancies (any discrepancy including intermediate) is accepted.

MIC-zone diameter correlation studies EUCAST data analysis

Inhibition zone diameter distributions with corresponding MIC values as different colours of the bars:

- Wild-type population defined

- Zone diameter breakpoints set to minimize the number of false susceptible results (very major discrepancies)

- An intermediate category is only included if there is an intermediate MIC category (intermediate never used as a buffer zone)

Cefotaxime 5 μg vs. MIC
Enterobacteriaceae, 573 isolates (624 correlates)
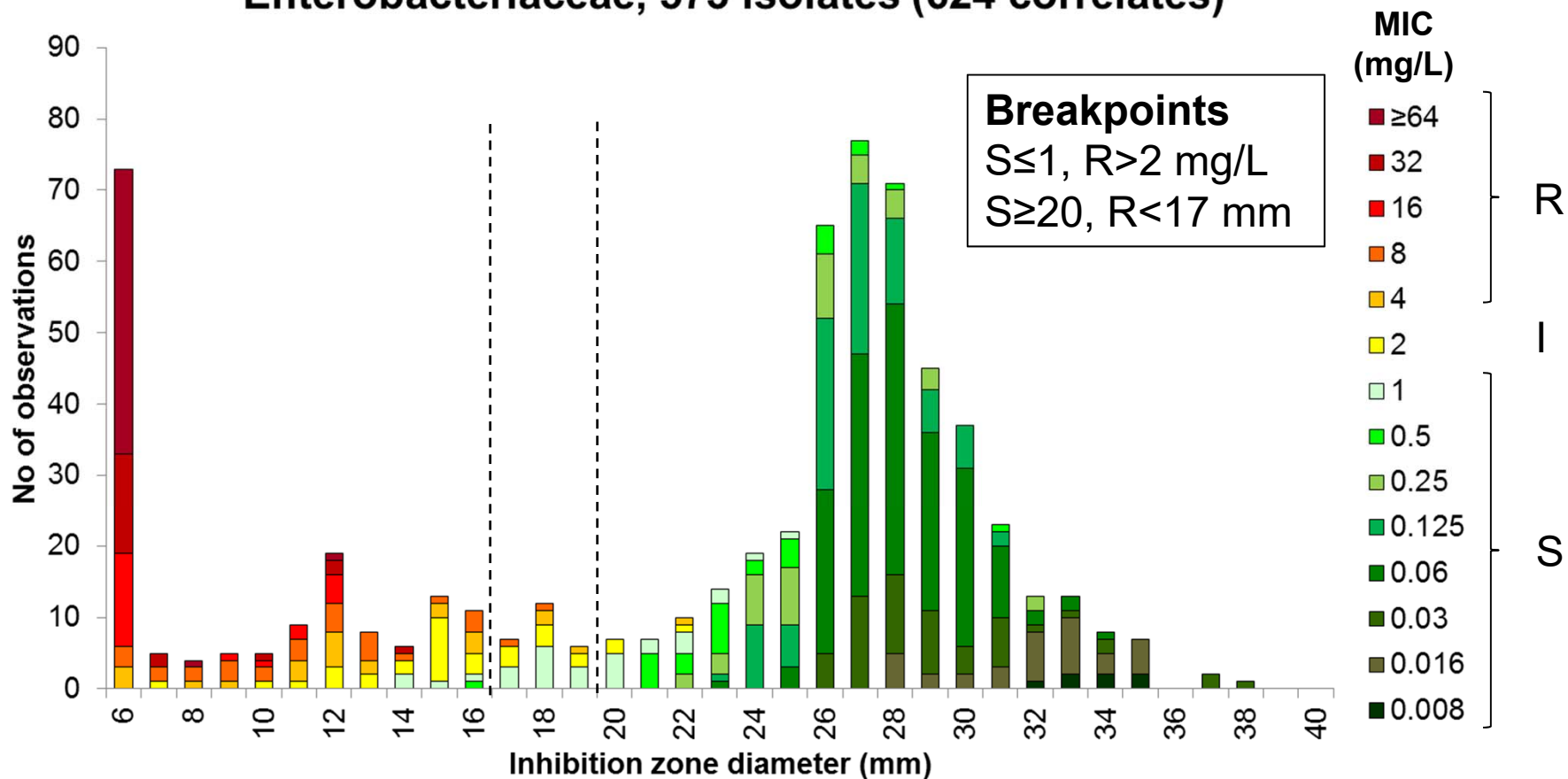
Conclusions

Groundwork for *in vitro* testing during drug development:

- To get robust MIC data and to reduce patient risk during clinical trials:
  - Standardized reference methods
  - Validation of AST materials from different manufacturers
  - Quality control criteria

- For development of zone diameter breakpoints also:
  - Optimal disk potency
  - Well chosen isolate collection for MIC-zone diameter correlation studies

Thanks for your attention!

erika.matuschek@escmid.org