



EUCAST

EUROPEAN COMMITTEE
ON ANTIMICROBIAL
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Quality assurance of antimicrobial susceptibility testing

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Q1

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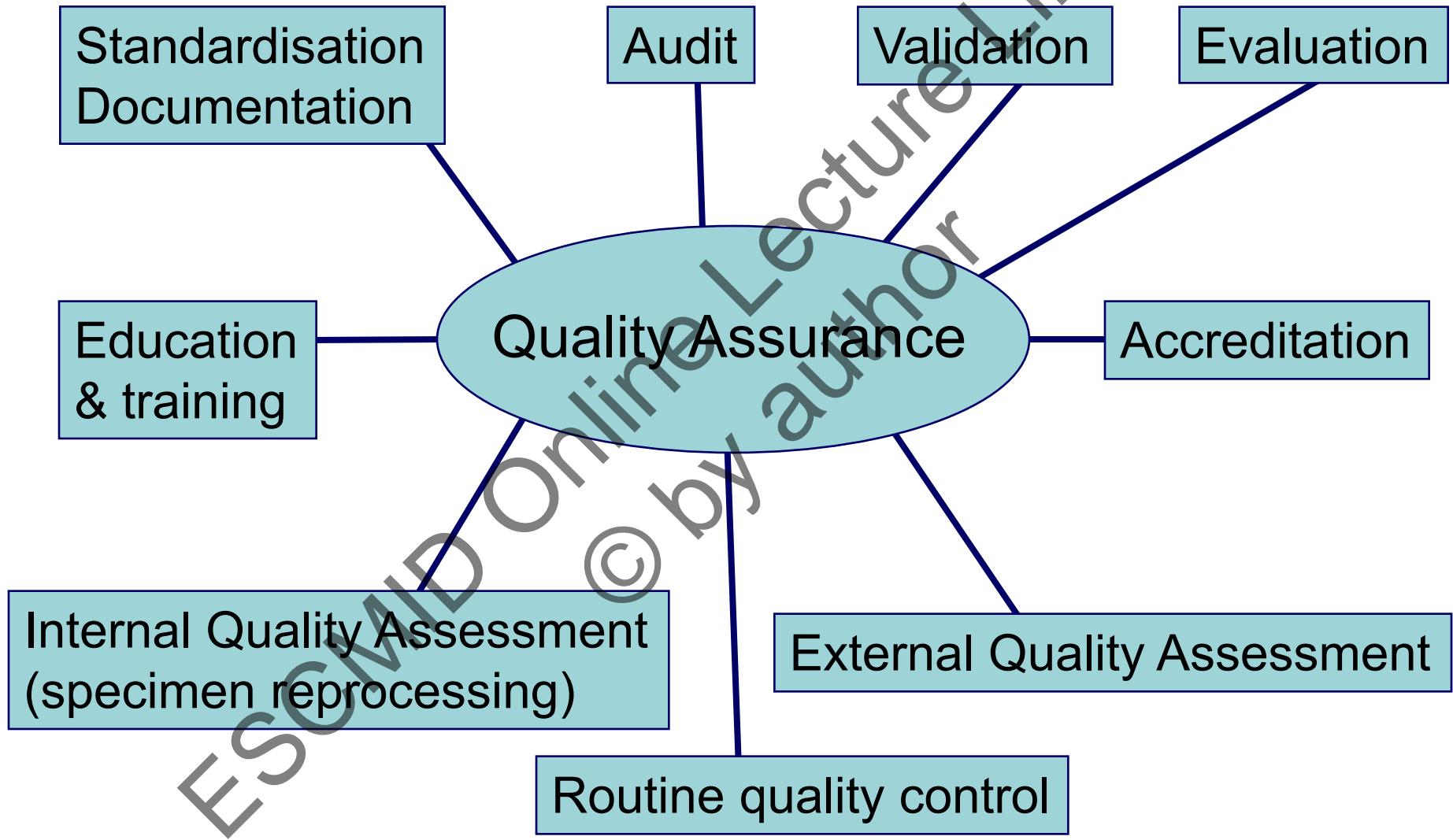
Quality Assurance in the clinical diagnostic laboratory

The total process by which the quality of laboratory reports can be guaranteed

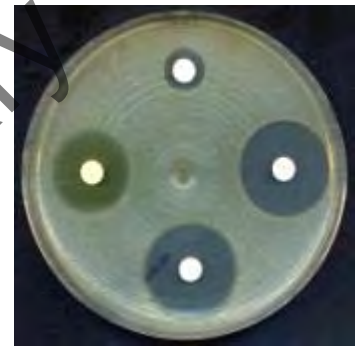
Not just routine quality control

(repeated testing of controls in parallel with tests to ensure that the test system is performing reproducibly within defined limits)

Components of quality assurance



QC of disk diffusion tests



- Specified routine quality control strains are used to monitor test performance
- Quality control strains must be from a reliable source (culture collections or from commercial sources)
- Store control strains correctly to maintain characteristics (see EUCAST website for guidance)

EUCAST routine quality control strains

Use the recommended routine quality control strains daily to monitor test performance with agents in routine test panels

Organism	Culture collection numbers	Characteristics
<i>E. coli</i>	ATCC 25922; NCTC 12241; CIP 7624 DSM 1103; CCUG 17620	Susceptible, wild-type
<i>P. aeruginosa</i>	ATCC 27853; NCTC 12903; CIP 76110 DSM 1117; CCUG 17619	Susceptible, wild-type
<i>S. aureus</i>	ATCC 29213; NCTC 12973; CIP 103429 DSM 2569; CCUG 15915	Weak β -lactamase producer
<i>E. faecalis</i>	ATCC 29212; NCTC 12697; CIP 103214 DSM 2570; CCUG 9997	Susceptible, wild-type
<i>S. pneumoniae</i>	ATCC 49619; NCTC 12977; CIP 104340 DSM 11967; CCUG 33638	Penicillin intermediate
<i>H. influenzae</i>	NCTC 8468; CIP5494, CCUG 23946	Susceptible, wild-type

ATCC, American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, USA.

NCTC, National Collection of Type Cultures, Health Protection Agency Centre for Infections, 61 Colindale Avenue, London NW9 5HT, UK.

CIP, Collection de Institut Pasteur, 25–28 Rue du Docteur Roux, 75724 Paris Cedex 15 France.

DSMZ, Deutsche Stammsammlung für Mikroorganismen und Zellkulturen, Mascheroder Weg 16, D-38124 Braunschweig, Germany.

CCUG, The Culture Collection University of Gothenburg <http://www.ccug.se/>

Quality control limits

Escherichia coli ATCC 25922

(NCTC 12241, CIP 76.24, DSM 1103, CCUG 17620, CECT 434)

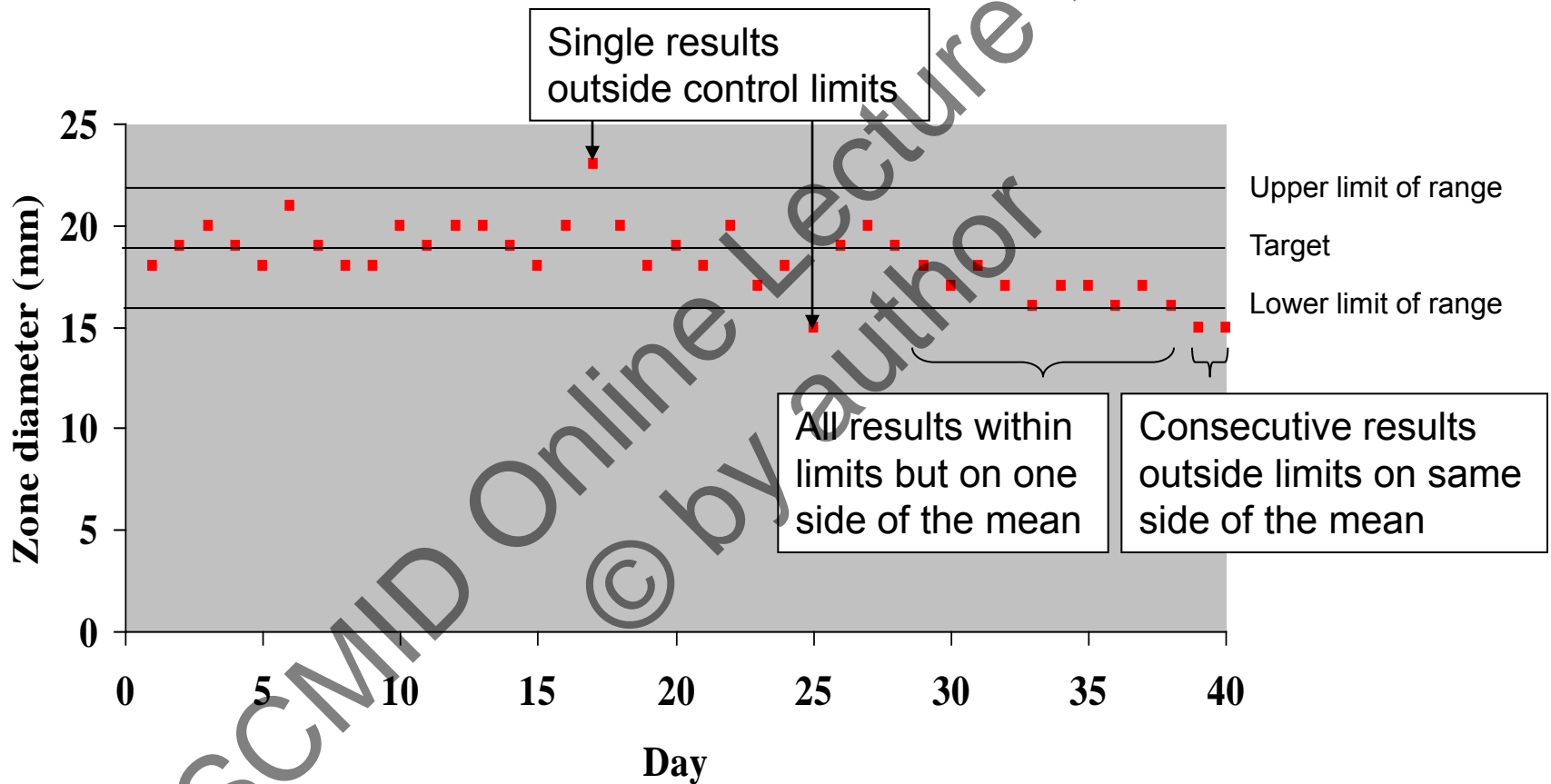
Mueller-Hinton agar, McFarland 0.5, air, 35±
no growth from the back of the plate against

Target
23

Range
19-26

Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone size (mm)	
	Target ¹	Range ²		Target ¹	Range ³
Amikacin	1-2	0.5-4	30	23	19-26
Amoxicillin	8	4-16	10	17	14-20 ⁴
Amoxicillin-clavulanic acid	4/2	2/2-8/2	20-10	21	18-24 ⁴
Ampicillin	4	2-8	10	19	16-22 ⁴
Ampicillin-sulbactam	4/2	2/2-8/2	10-10	22	19-24 ⁴
Aztreonam	0.12	0.06-0.25	30	32	28-36
Cefadroxil	-	-	30	17	14-20

Monitoring disk diffusion test performance



Response to disk diffusion QC results out of range

- Single test out of range – report susceptibility if no obvious problem.
- Each day that tests are set up, examine the results of the last 20 consecutive tests. If two non-consecutive control zone diameters of 20 tests are out of range – then report results if no obvious problem but investigate.
- If two consecutive control zone diameters are outside the acceptable range – then investigate before reporting results. The tests may have to be repeated.
- If multiple antibiotics (>2) are out of range on one day – then investigate before reporting results. The tests may have to be repeated.

Sources of error in disk diffusion tests

- Medium
- Disks
- Test conditions
- Control strains

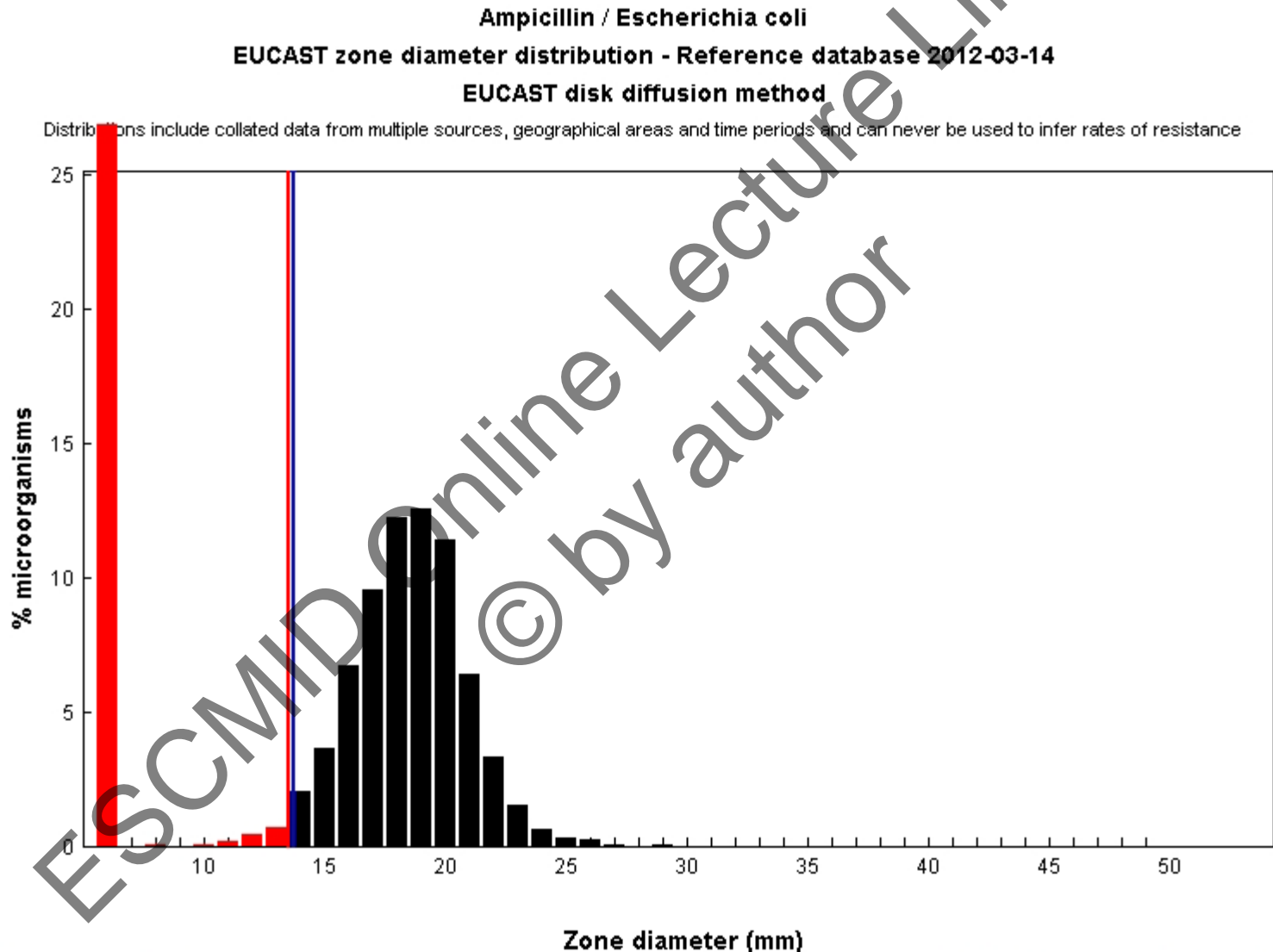
EUCAST strains for detection of resistance mechanisms (in progress)

Quality control strains with defined resistance mechanisms may be used to confirm the ability to detect resistance.

Organism	Characteristics
<i>E. coli</i>	TEM-1 β -lactamase producer
<i>S. aureus</i>	Oxacillin hetero-resistant, <i>mecA</i> positive
<i>E. faecalis</i>	VanA (low teicoplanin MIC) and VanB (low vancomycin MIC)
<i>S. pneumoniae</i>	Penicillin MIC 4 mg/L
<i>H. influenzae</i>	β -lactamase negative, ampicillin-resistant (BLNAR)
<i>E. coli</i>	ESBL, cefotaxime S, ceftazidime R
<i>E. coli</i>	ESBL, ceftazidime R, cefotaxime S
<i>E. coli</i>	Plasmid AmpC
<i>E. coli</i>	Carbapenemase producer
<i>K. pneumoniae</i>	KPC producer

If resistance in a resistant control strain is not recognised suppress test results, retest and investigate.

Quality control by comparison of wild type with reference distributions from EUCAST website



Disk content: 10

Epidemiological cut-off: WT ≥ 14 mm (MIC ≤ 8 mg/L)

18733 observations (4 data sources)

Clinical breakpoints: S ≥ 14 mm, R < 14 mm (S ≤ 8 mg/L, R > 8 mg/L,)

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Quality control of MIC testing



- Use the recommended routine quality control strains to monitor test performance (see EUCAST QC tables).
- Test range must include the MIC of the control strain.

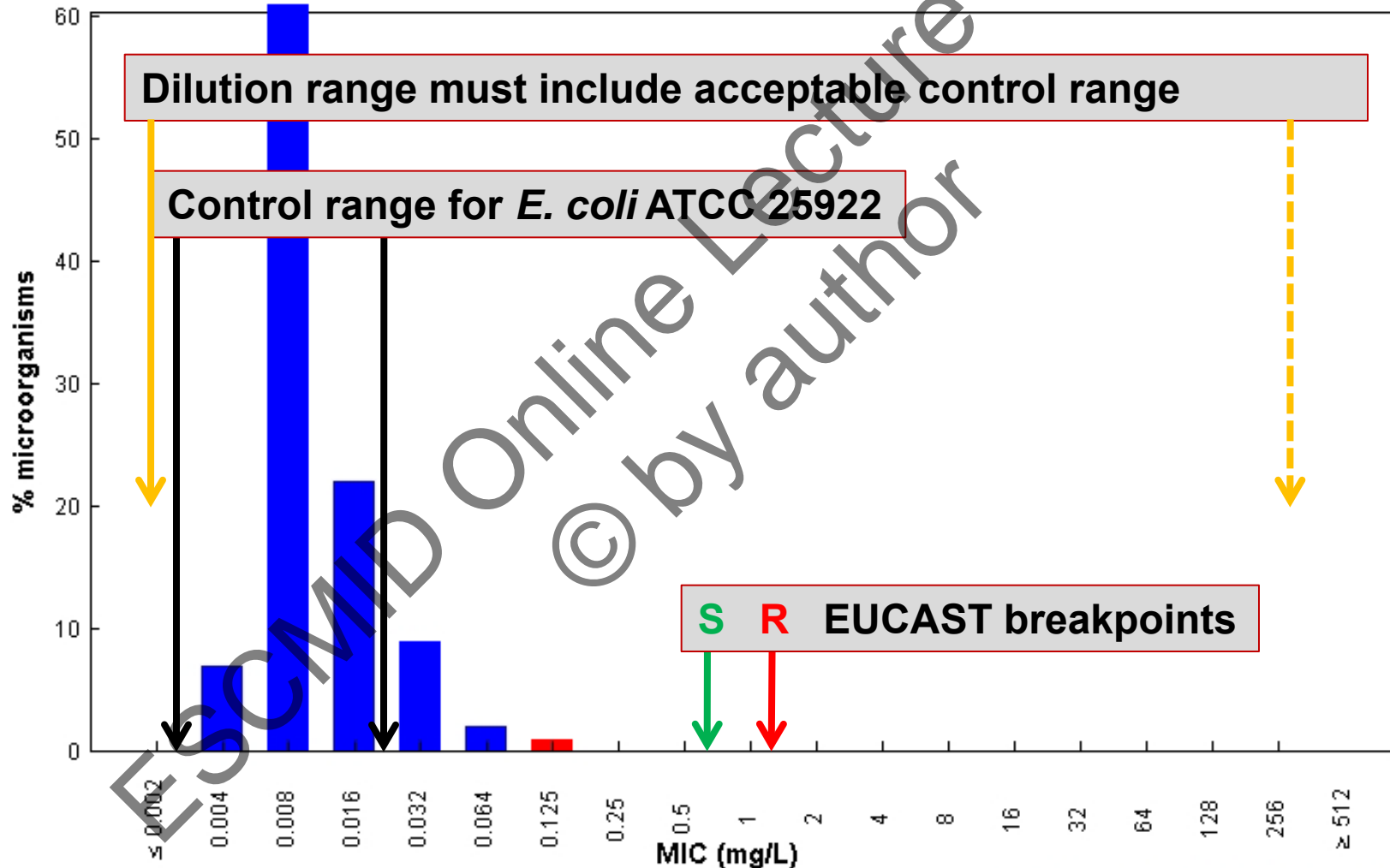
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Quality control of MIC testing

Ertapenem / *Escherichia coli*

EUCAST MIC Distribution - Reference Database 2010-09-24

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



MIC
Epidemiological cut-off: WT ≤ 0.064 mg/L

2181 observations (11 data sources)
Clinical breakpoints: S ≤ 0.5 mg/L, R > 1 mg/L

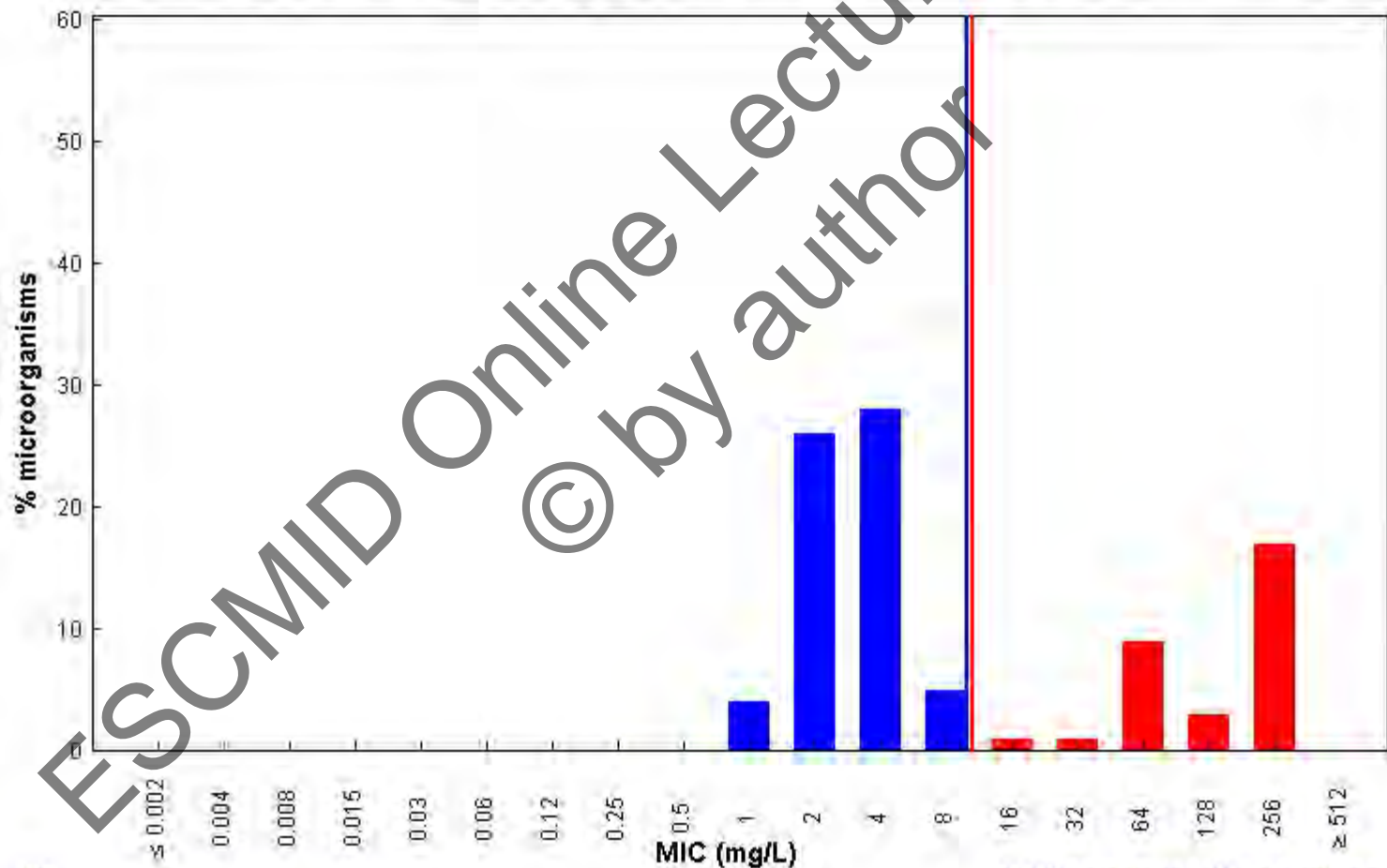
Quality control of MIC testing

- Include a control without antibiotic to ensure that the test strain grows adequately.
- Test the purity of inoculum by culture on solid medium to obtain isolated colonies.
- If MIC of control is out of range the source of error must be sought and the test repeated.
- Check wild type distribution against EUCAST distribution on website.

Quality control by comparison of wild type with reference distributions from EUCAST website

Ampicillin / *Escherichia coli*
EUCAST MIC Distribution - Reference Database 2012-03-14

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



MIC
Epidemiological cut-off: WT ≤ 8 mg/L

39220 observations (48 data sources)
Clinical breakpoints: S ≤ 8 mg/L, R > 8 mg/L

QC of automated systems



- Use the recommended routine quality control strains to monitor test performance (see manufacturer's instructions).
- Restricted range of test concentrations mean that the range may not include the MIC of the control strain.
- Purity of inoculum tested by culture on solid medium to obtain isolated colonies.
- If control is out of range the source of error must be sought and the test repeated.

External Quality Assessment (EQA)

The challenge of laboratory procedures with specimens of known but undisclosed content

The EQA process (UKNEQAS)

Organising laboratory

Participants

Prepare EQA Samples

Examine samples

Analyse results

Report results

Prepare report

Evaluate



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Benefits of EQA in antimicrobial susceptibility testing

- Independent assessment of performance
- Assessment of performance over time
- Comparison with other laboratories
- Highlights problem areas
- Performance related to guidelines and methods
- International differences highlighted
- Gives practical experience of difficult tests (especially if resistance is uncommon)
- Provides background information and guidance on appropriate methods
- Performance indicator for accreditation

“Limitations” of EQA in antimicrobial susceptibility testing

- Number of specimens distributed is small
- May be considered inappropriate to send some organisms
- Specimens do not reflect routine isolates
- Laboratories may not treat specimens as routine

Performance may be affected by breakpoint guidelines used

E. faecalis

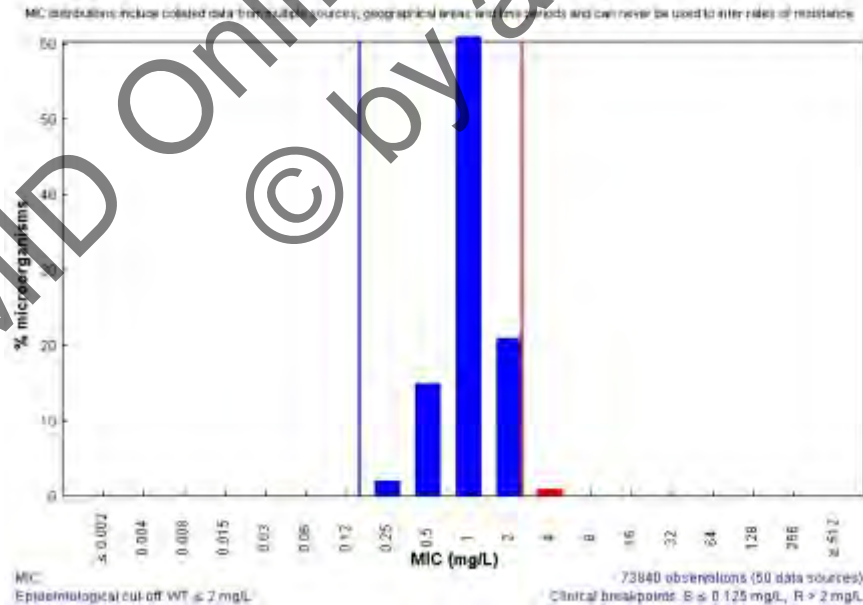
vancomycin MIC 8-16 mg/L (VanB)

Guideline	Breakpoints (mg/L)		Percent reporting		
	S \leq	R $>$	S	I	R
EUCAST (n=316)	4	4	5.1	1.9	93.0
CLSI (n=314)	4	16	10.2	35.0	54.8

Guidelines are not always followed

S. pneumoniae ciprofloxacin MIC 0.5-1 mg/L

Guideline	Breakpoints (mg/L)		Percent reporting		
	S ≤	R >	S	I	R
EUCAST (n=202)	0.12	2	30.2	67.8	2.0
CLSI (n=181)	-	-	92.9	14.9	2.2



Performance may be affected by the method used

E. faecalis vancomycin MIC 8-16 mg/L

VanB EUCAST resistant, CLSI intermediate

Method	Percent reporting		
	S	I	R
Automated (n=333)	3.9	6.2	89.9
MIC (n=71)	2.8	13.9	83.3
Disk diffusion (n=262)	15.5	11.6	72.9

Borderline susceptibility leads to variable reporting

Organism	Agent	Expected result	% reporting		
			S	I	R
<i>E faecalis</i> 0138	Vancomycin	I/R	7.3	18.4	74.3
<i>E coli</i> 0270	Piperacillin-tazobactam	S/I	32.7	23.6	43.6

Uncertainty in reporting e.g. *S. aureus* with dissociated (MLSB- inducible) resistance to clindamycin

clindamycin MIC 0.12-0.5 mg/L,
resistance induced by erythromycin



Percent reporting (n=775)		
S	I	R
24.0	1.8	74.2

- EUCAST expert rules recommend reporting resistant, or susceptible with warning of possible failure due to selection of resistant mutants. Avoid use in serious infections
- CLSI – report resistant with note that some may respond

Internal Quality Assessment (IQA) -specimen reprocessing

The challenge of laboratory procedures by repeat testing of specimens of unknown content

Internal quality assessment (IQA) process

- Specimens split and both processed on same day, or same specimen processed twice on the same day, with identification of repeat test blinded
- For susceptibility testing the same organism could be processed twice on same day or repeated on different days
- Reports compared and discrepancies investigated
- Feedback

Antimicrobial susceptibility testing problems highlighted by IQA

- Different organisms picked from mixture on primary plates
- Wrong disk contents used
- Borderline susceptibility leads to variation
- Discrepancies with “difficult” tests
- Typographical errors

Quality assurance of antimicrobial susceptibility testing

- Quality assurance is essential to ensure reliable results
- Multiple components contribute to maintaining the quality of antimicrobial susceptibility testing