



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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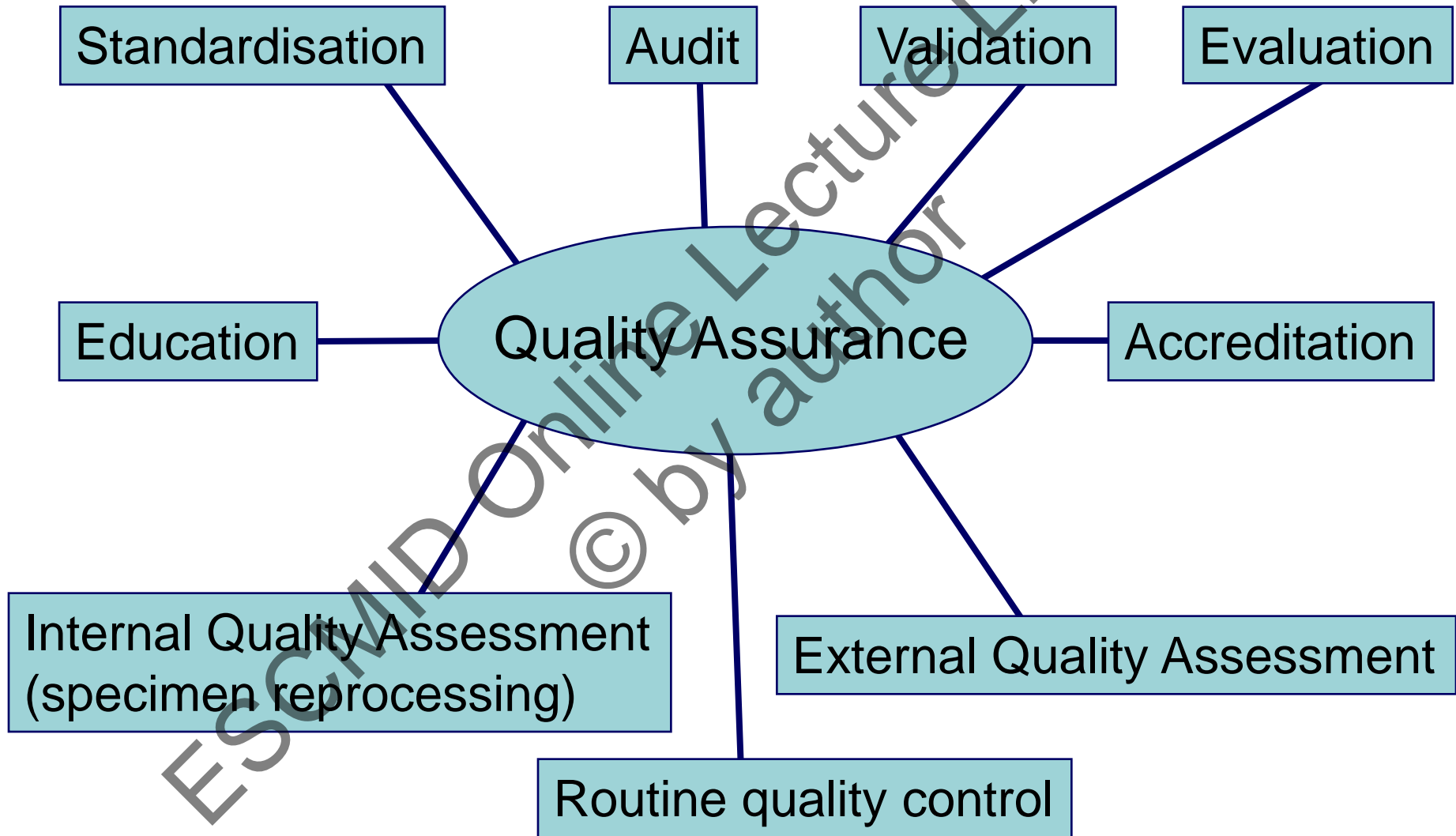
# **Routine quality control**

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

# Quality Assurance in the clinical diagnostic laboratory

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

# Components of quality assurance



# Quality control of disk diffusion antimicrobial susceptibility tests

- Specified routine quality control strains are used to monitor test performance.
- Quality control strains may be purchased from culture collections or from commercial sources.
- See EUCAST website for guidance on storage of control strains.

# 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 $\beta$ -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.

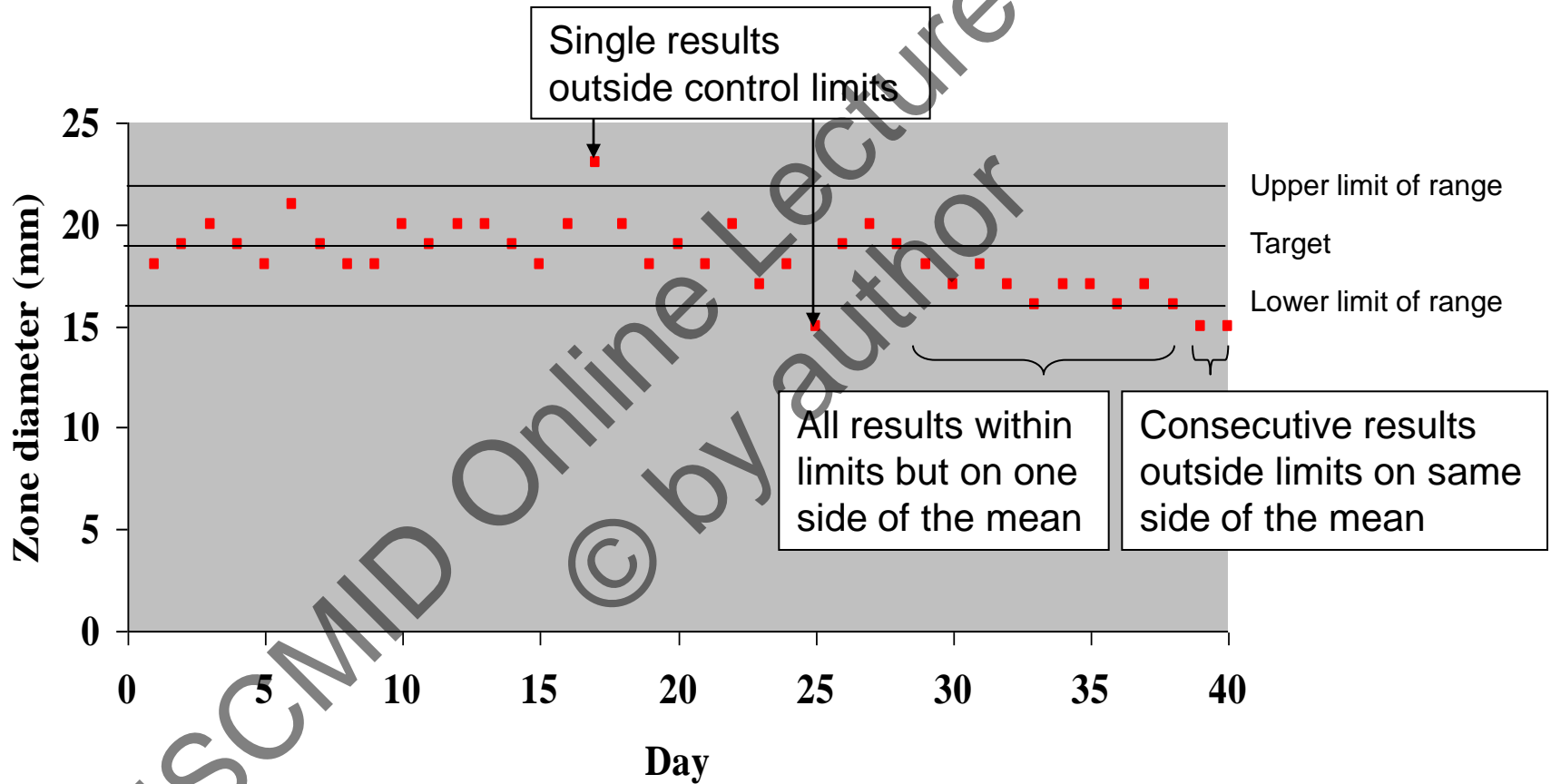
CIP, Collection de l'Institut Pasteur, 25–28 Rue du Docteur Roux, 75724 Paris Cedex 15 France.

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/>

MIC and Zone Diameter Limits for Quality Control Strains						
<i>Escherichia coli</i> ATCC 25922						
Mueller-Hinton agar, McFarland 0.5, air, 35±1°C, 18±2 h. Read complete inhibition from the back of the plates against a black background illuminated with reflected light.						
Antimicrobial agent	MIC (mg/L)		Disk content (µg)	Inhibition zone size (mm)		Comments
	Target	Range <sup>1</sup>		Target	Range <sup>2</sup>	
Amikacin	1-2	0.5-4	30	23	19-26	
Amoxicillin-clavulanic acid	4/2	2/1-8/4	20/10	21	18-24	
Ampicillin	4	2-8	10	19	16-22	Ignore faint growth that appears as inner zone.
Aztreonam	0.12	0.06-0.25	30	32	28-36	
Cefadroxil	-	-	30	18	15-21	EUCAST
Cefepime	0.03-0.06	0.015-0.12	30	34	31-37	
Cefotaxime	0.06	0.03-0.12	5	28	25-31	
Cefotaxime	0.06	0.03-0.12	30	32	29-35	
Cefoxitin	4	2-8	30	26	23-29	
Cefpodoxime	0.5	0.25-1	10	26	23-28	
Ceftazidime	0.12-0.25	0.06-0.5	10	26	23-29	EUCAST
Ceftazidime	0.12-0.25	0.06-0.5	30	29	25-32	
Ceftibuten	0.25	0.12-0.5	30	31	27-35	
Cefuroxime	4	2-8	30	23	20-26	
Chloramphenicol	4	2-8	30	24	21-27	
Ciprofloxacin	0.008	0.004-0.015	5	35	30-40	
Ertapenem	0.008	0.004-0.015	10	33	29-36	
Gentamicin	0.5	0.25-1	10	23	19-26	
Gentamicin	0.5	0.25-1	30	24	21-27	EUCAST
Imipenem	0.12	0.06-0.25	10	29	26-32	
Mecillinam	0.06-0.12	0.03-0.25	10	27	24-30	
Meropenem	0.015-0.03	0.008-0.06	10	31	28-34	
Moxifloxacin	0.015-0.03	0.008-0.06	5	32	28-35	
Nalidixic acid	2	1-4	30	25	22-28	
Nitrofurantoin	8	4-16	100	21	18-24	EUCAST
Nitrofurantoin	8	4-16	300	23	20-25	
Norfloxacin	0.06	0.03-0.12	10	32	28-35	
Piperacillin-tazobactam	2/4	1/4-4/4	30/6	24	21-27	EUCAST
Piperacillin-tazobactam	2/4	1/4-4/4	100/10	27	24-30	
Rifampicin	8	4-16	5	9	8-10	
Tetracycline	1	0.5-2	30	22	18-25	

# 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.

# 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 $\beta$ -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>	$\beta$ -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 enzyme

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

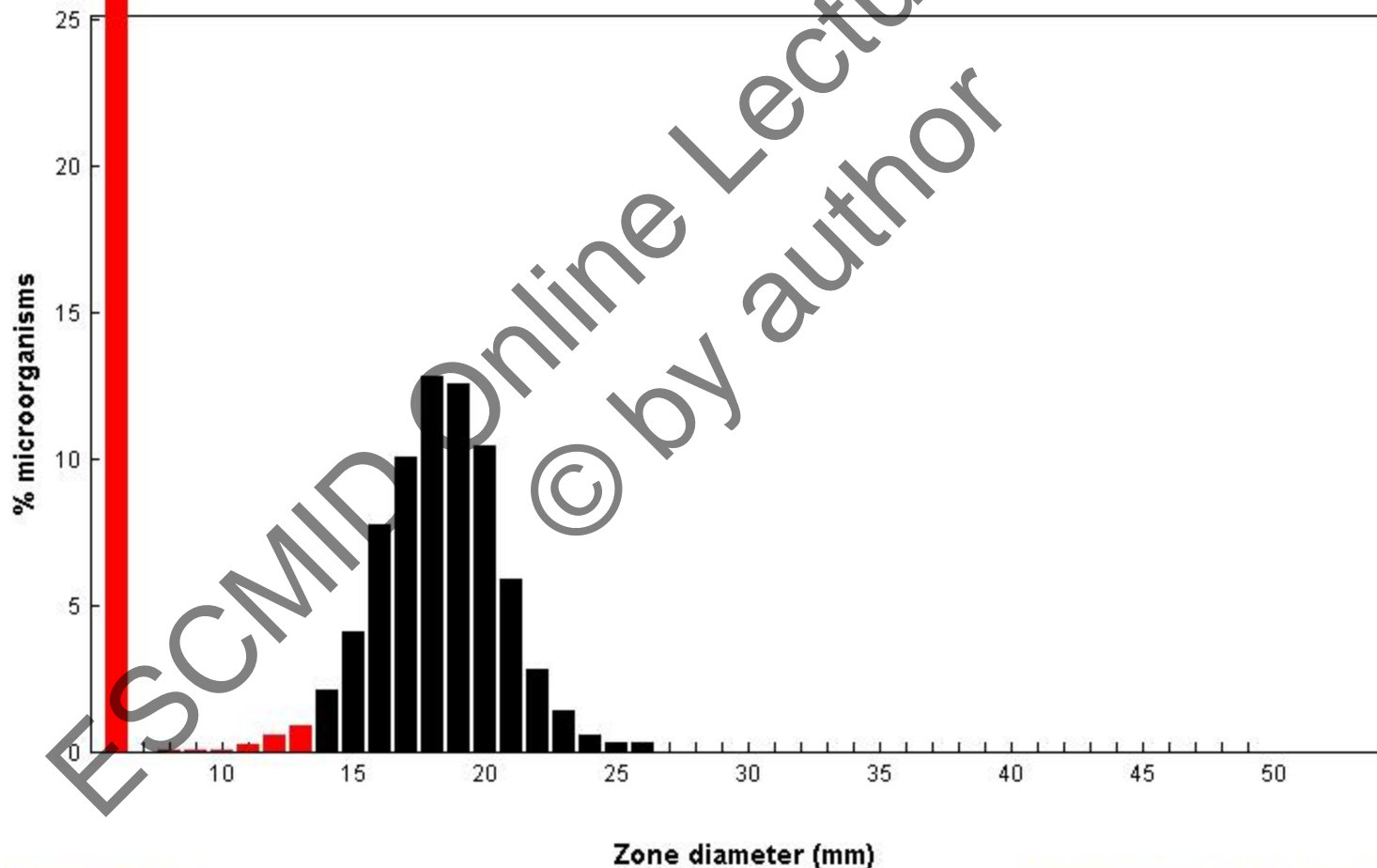
# Quality control by comparison of wild type with reference distributions from EUCAST website

Ampicillin / *Escherichia coli*

EUCAST zone diameter distribution - Reference database 2010-09-24

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: 10

Epidemiological cut-off: WT  $\geq 14$  mm (MIC:  $\leq 8$  mg/L)

9053 observations (2 data sources)

Clinical breakpoints: S  $\geq 14$  mm, R  $< 14$  mm

# Sources of error in disk diffusion

<b>Medium</b>	Storage of plates
	Not prepared to instructions
	Batch to batch variation or change of supplier of agar
	Supplements (batch to batch variations, incorrect amount, expired)
	pH
	Agar depth/Agar volume
	Expiry date
<b>Test conditions</b>	“15-15-15”-rule not adhered to (suspension used within 15 min, disks applied within 15 min, incubation within 15 min)
	Incubation (temperature, atmosphere and time)
	Incorrect inoculation (too light, too heavy or uneven)
	Reading conditions, reading zone edges
<b>Disks</b>	Incorrect disk (wrong agent or wrong disk strength)
	Disk potency (incorrect storage, labile agent, expiry date)
	Disks not at room temperature when containers opened
	Too many disks on plate (interference between agents)
<b>Control organisms</b>	Incorrect QC strain
	Mutation
	Contamination
	Age of culture

# Quality control of MIC testing

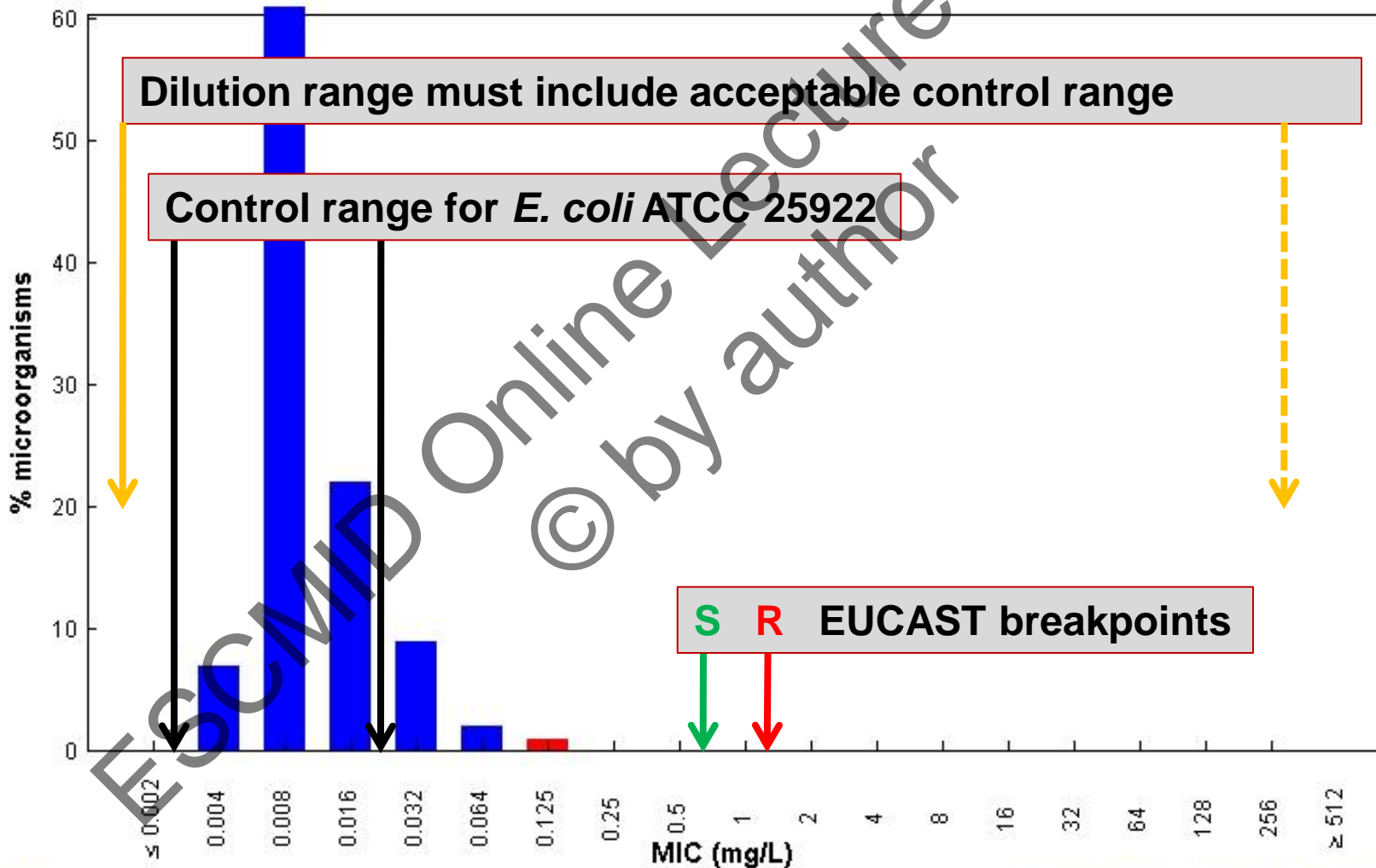
- Use the recommended routine quality control strains to monitor test performance (see EUCAST QC tables).
- Test range must include the MIC of the control strain.

# 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  $\leq 0.064$  mg/L

2181 observations (11 data sources)

Clinical breakpoints: S  $\leq 0.5$  mg/L, R  $> 1$  mg/L

# Quality control of MIC testing

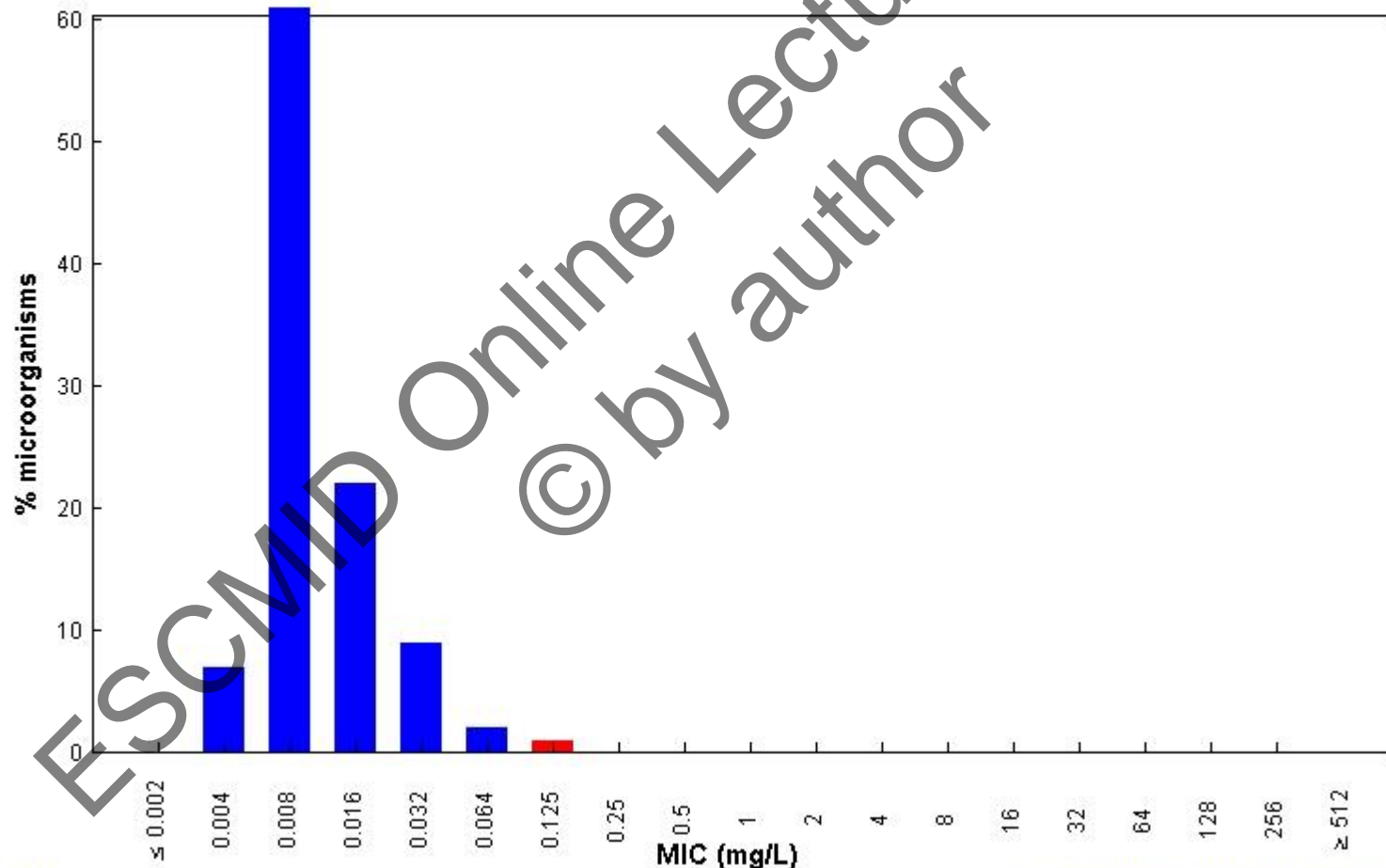
- Use the recommended routine quality control strains to monitor test performance (see EUCAST QC tables).
- Test range must include the MIC of the control strain.
- Include a control without antibiotic to ensure that the test strain grows adequately.
- Test the purity of inoculum by culture on solid 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

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 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

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

# The EQA process (UKNEQAS)

Organising laboratory

Participants

Prepare EQA Samples

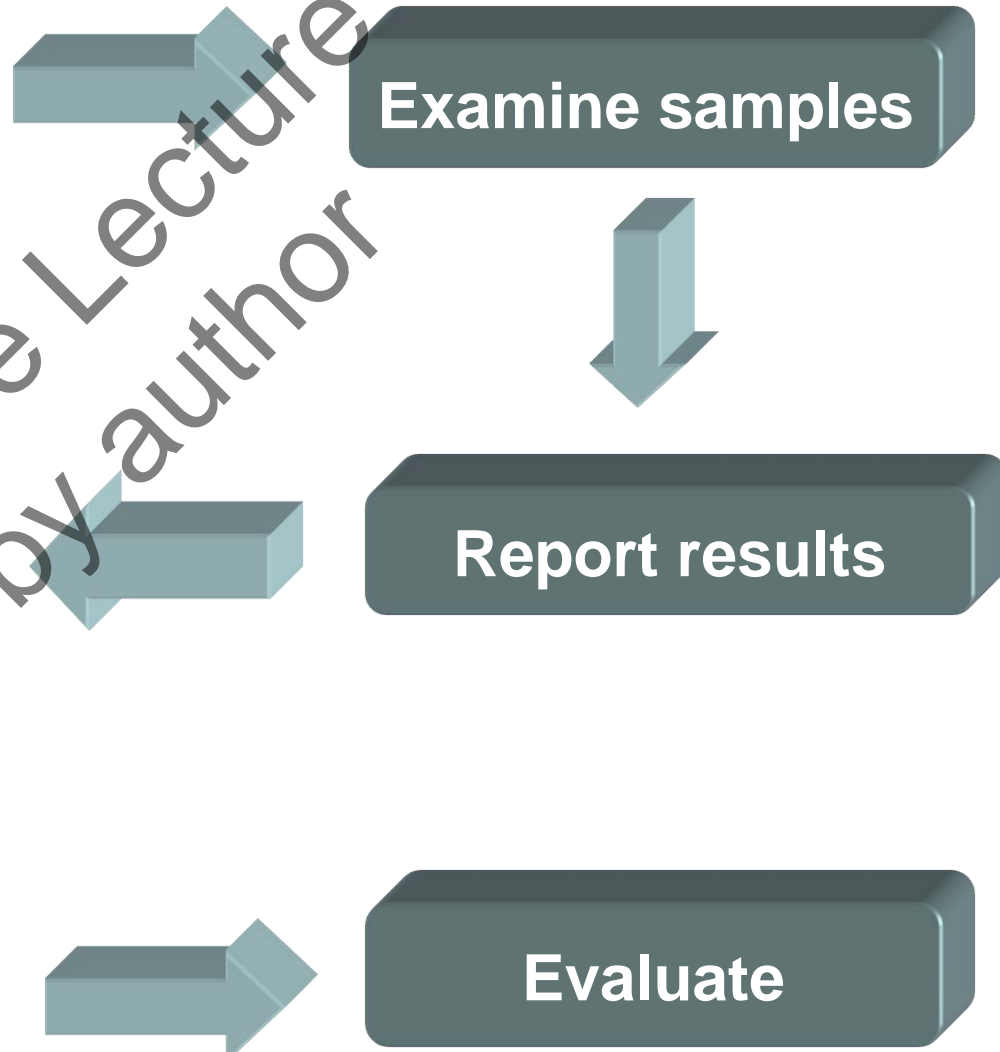
Examine samples

Analyse results

Report results

Prepare report

Evaluate



# EQA Report

The thumbnail shows a page from an EQA report. At the top, it says 'LUP MEDAS for Antimicrobial Susceptibility Laboratory', 'Distribution 1821', and 'Page 1 of 10'. Below that, it says 'Dispatch date: 25-Oct-2004'. The main part of the page is a table with columns for 'Sample ID', 'Year Report', and 'Year Score'. There are two sections of data, one for 'Sample ID 1' and one for 'Sample ID 2'. Each section lists various antimicrobial agents and their corresponding results. Below the table, there is a 'Comments' section with several lines of text.



- Reference MIC results
- Your results
- Scores highlighting your performance
- Cumulative score over time and mean for all laboratories
- Detailed results for labs using the same method as you
- Details of results with different guidelines
- Comments on particular problems

## Evaluate

- Review the results with all staff (include successes and failures)
- Investigate problems
  - How many other participants had problems with the specimen?
  - Are there any relevant comments?
  - Technical or interpretive issues?

# Benefits of EQA in antimicrobial susceptibility testing

- Independent assessment of performance
- Assessment of performance over time
- Comparison with other laboratories
- Performance indicator for accreditation
- Highlights problem areas
- Performance related to methodology
- International differences highlighted
- Education

# **“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

# **Internal Quality Assessment (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
  - Rapid feedback of discrepancy reports
  - Frequent discussion and action in laboratory technical meetings

# Antimicrobial susceptibility testing problems highlighted by IQA

- Different organisms picked from mixture on primary plates
- Wrong disk contents used e.g.
  - low content gentamicin disks used for enterococci
- Borderline susceptibility missed e.g.
  - *S. aureus* erythromycin R changed to S
  - *S. aureus* mupirocin S changed to I
  - *S. aureus* fusidic acid S changed to R
- Discrepancies with “difficult” tests
  - Hetero-resistant MRSA
  - VanB enterococcus
- Typographical errors

# Benefits of IQA in antimicrobial susceptibility testing

- Tests reproducibility of all aspects of processing a specimen
- Covers areas not tested by EQA
- More samples than EQA
- locally responsive
- Rapid turnaround so problems investigated early
- Recognised by accreditation authorities

# 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