



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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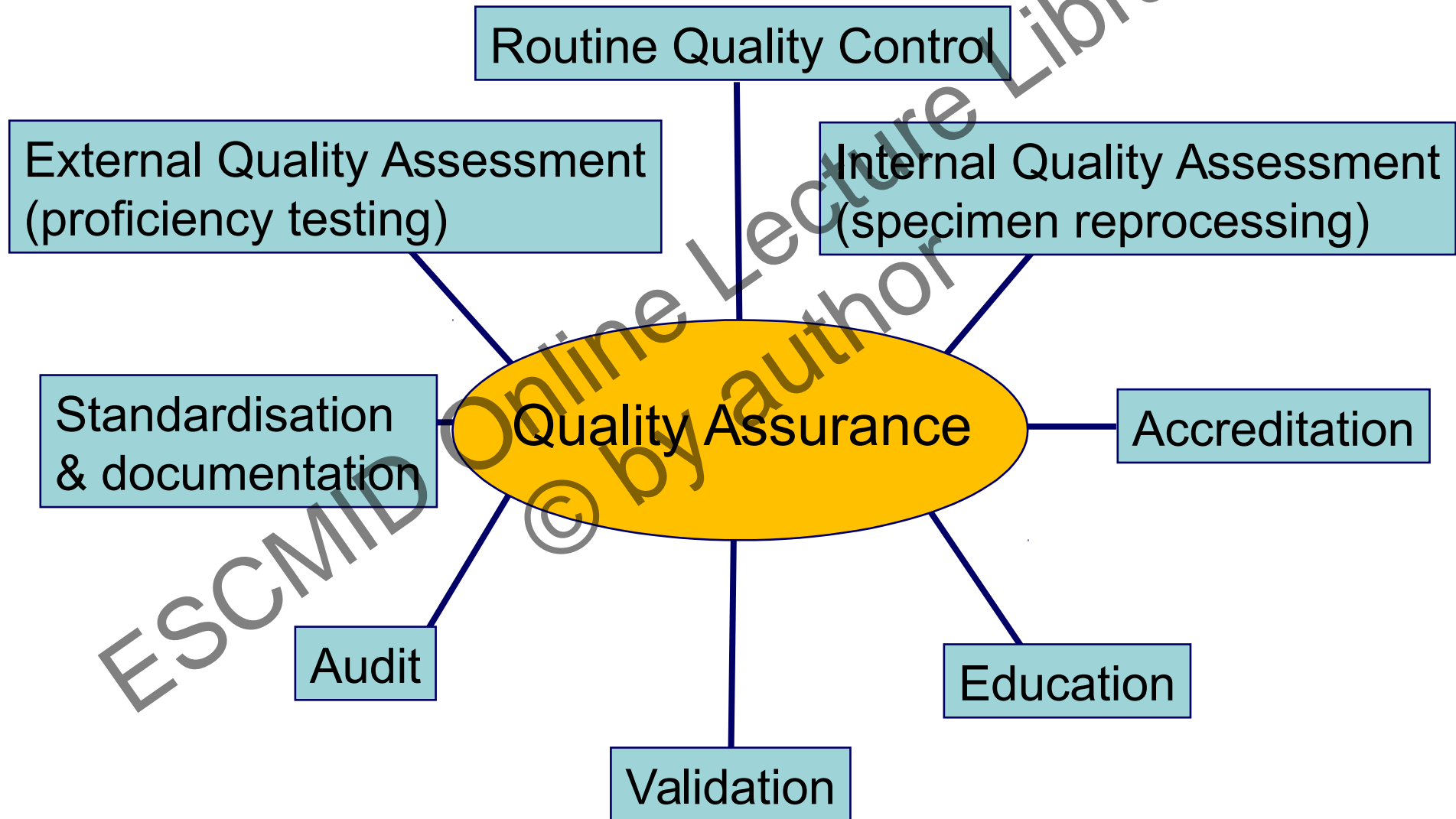
EUCAST Scientific Secretary

Quality Assurance

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

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Components of quality assurance



Components of quality assurance

Routine quality control

External Quality Assessment
(proficiency testing)

Internal Quality Assessment
(specimen reprocessing)

Standardisation
& documentation

Quality Assurance

Accreditation

Audit

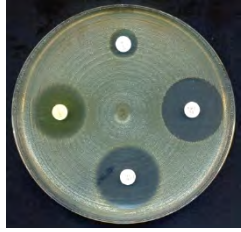
Education

Validation

Routine quality control

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

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

Organism	Culture collection numbers	Characteristics
<i>E. coli</i>	ATCC 25922; NCTC 12241; CIP 7624 DSM 1103; CCUG 17620, CECT 434	Susceptible, wild-type
<i>E. coli</i>	ATCC 35218; NCTC 11954; CIP 102181; DSM 5564; CCUG 30600; CECT 943	TEM-1 β -lactamase producer
<i>P. aeruginosa</i>	ATCC 27853; NCTC 12903; CIP 76110 DSM 1117; CCUG 17619; CECT 108	Susceptible, wild-type
<i>S. aureus</i>	ATCC 29213; NCTC 12973; CIP 103429 DSM 2569; CCUG 15915; CECT 794	Weak β -lactamase producer
<i>E. faecalis</i>	ATCC 29212; NCTC 12697; CIP 103214 DSM 2570; CCUG 9997; CECT 795	Susceptible, wild-type
<i>S. pneumoniae</i>	ATCC 49619; NCTC 12977; CIP 104340 DSM 11967; CCUG 33638	Penicillin intermediate
<i>H. influenzae</i>	ATCC 49766; NCTC 12975 CIP 103570; DSM 11970 CCUG 29539	Susceptible, wild-type
<i>Campylobacter jejuni</i>	ATCC 33560; NCTC 11351; CIP 702 DSM 4688; CCUG 11284	Susceptible, wild-type

Staphylococcus aureus ATCC 29213*

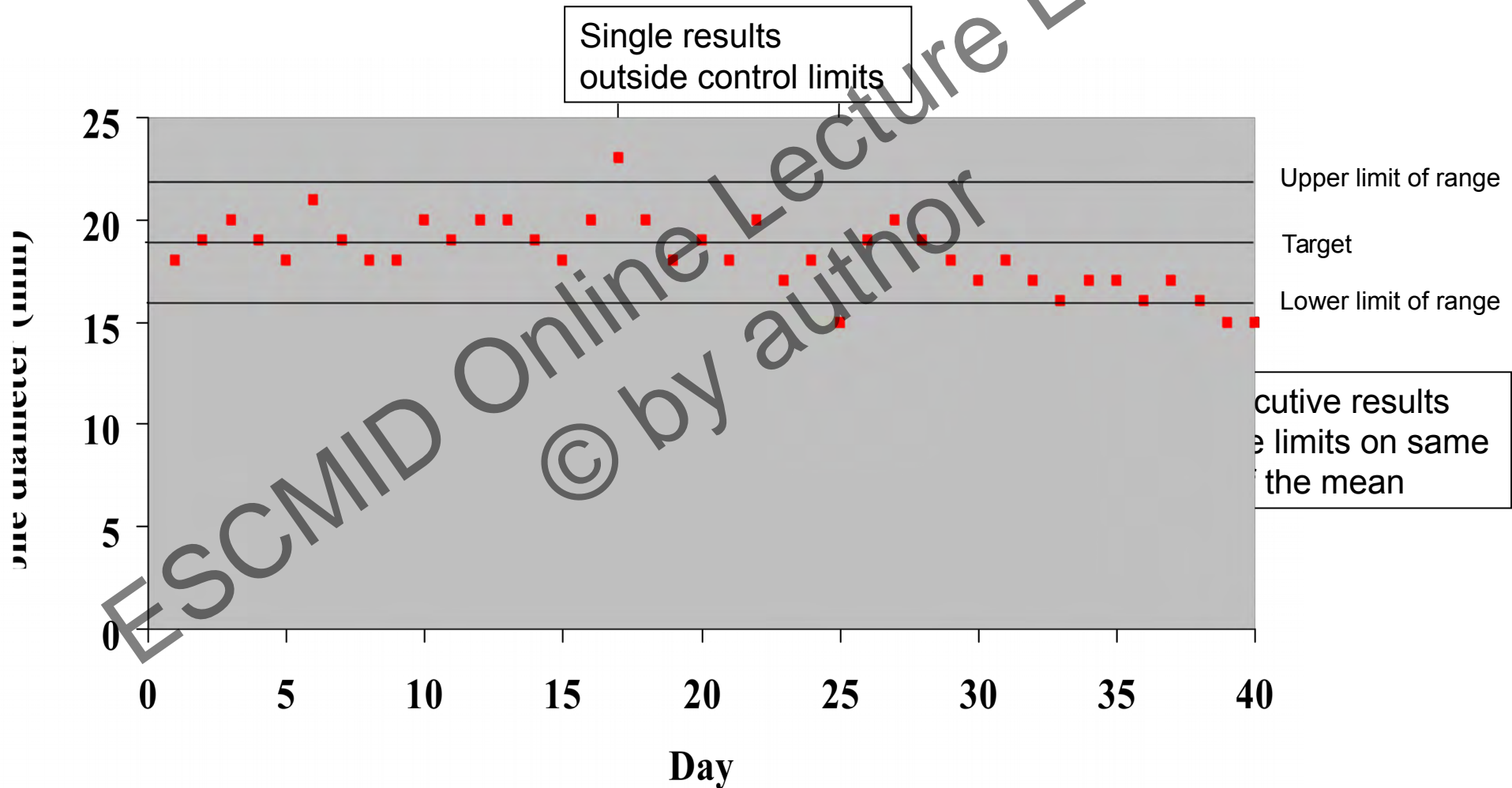
(NCTC 12973, CIP 103429, DSM 2569, CCUG 15915, CECT 794)

* β -lactamase-producing strain (weak)

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

Antimicrobial agent	MIC (mg/L)		Disk content (μ g)	Inhibition zone size (mm)	
	Target ¹	Range ²		Target ¹	Range ³
Amikacin	2	1-4	30	21	18-24
Ampicillin	-	-	2	18	15-21
Azithromycin	1	0.5-2	-	-	-
Benzylpenicillin	0.5-1	0.25-2	1 unit	15	12-18
Cefoxitin	2	1-4	30	27	24-30
Ceftaroline	0.25	0.125-0.5 ⁴	5	27	24-30
Ceftobiprole	0.25-0.5	0.125-1 ⁴	5	25	22-28

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.
- If two non-consecutive control zone diameters of 20 consecutive 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 agents (>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

Quality control strains with defined resistance mechanisms may be used to confirm the ability to detect resistance. If resistance in a resistant control strain is not recognised suppress test results, retest and investigate.

Organism	Culture collection numbers	Characteristics
<i>K. pneumoniae</i>	ATCC 700603; NCTC 13368; CCUG 45421; CECT 7787	ESBL producer (SHV-18)
<i>S. aureus</i>	NCTC 12493	Oxacillin hetero-resistant, <i>mecA</i> positive
<i>E. faecalis</i>	ATCC 51922; NCTC 13379; CIP 104676; DSM 12956; CCUG 34289	High-level aminoglycoside resistant (HLAR) and vancomycin resistant (<i>vanB</i> positive)
<i>H. influenzae</i>	ATCC 49247; NCTC 12699; CIP 104604; DSM 9999; CCUG 26214	β -lactamase negative, ampicillin-resistant (BLNAR)

EUCAST strains for detection of resistance mechanisms (possible additional strains)

Organism	Characteristics
<i>S. pneumoniae</i>	Penicillin resistant (MIC 4 mg/L)
<i>E. coli</i>	Different ESBL phenotypes
<i>E. coli</i>	Plasmid AmpC
<i>E. coli</i>	Carbapenemase producers
<i>K. pneumoniae</i>	KPC enzyme
<i>E. coli</i>	<i>mcr-1</i>

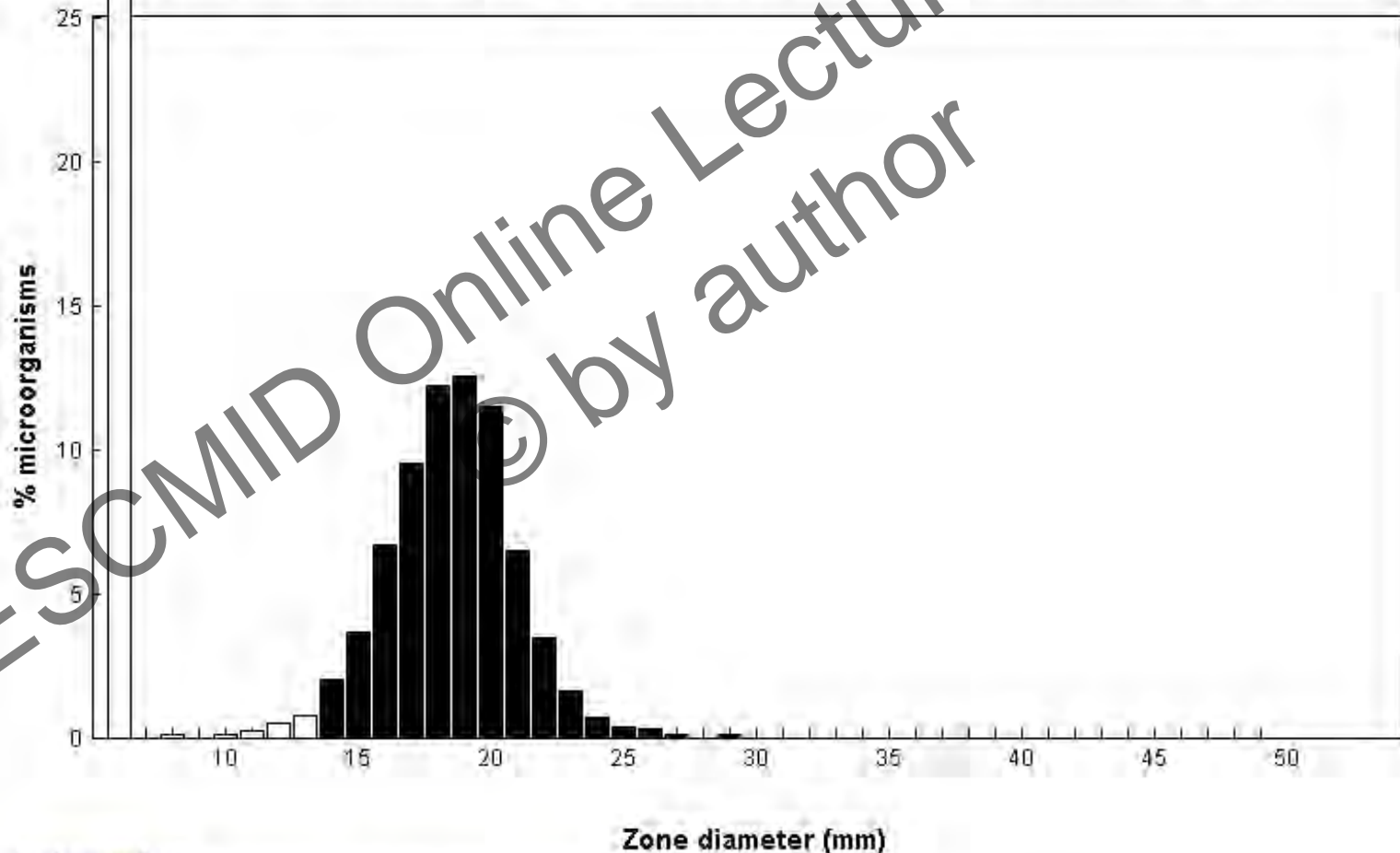
Quality control by comparison of wild type with reference distributions from EUCAST website

Ampicillin / Escherichia coli

International wild type zone diameter distribution - Reference database 2016-09-10

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 (ECOFF): 14 mm (MIC = 8 mg/L)

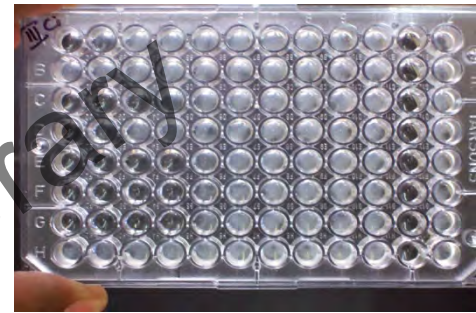
Wildtype (WT) organisms: ≥ 14 mm (MIC = 8 mg/L)

18972 observations (8 data sources)

Sources of error in disk diffusion

Medium	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
Test conditions	“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
Disks	Incorrect disk (wrong agent or wrong disk content)
	Disk potency (incorrect storage, labile agent, expiry date)
	Disks not at room temperature when containers opened
	Too many disks on plate (interference between agents)
Control organisms	Incorrect QC strain
	Mutation
	Contamination
	Age of culture

Quality control of MIC determination



- 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.

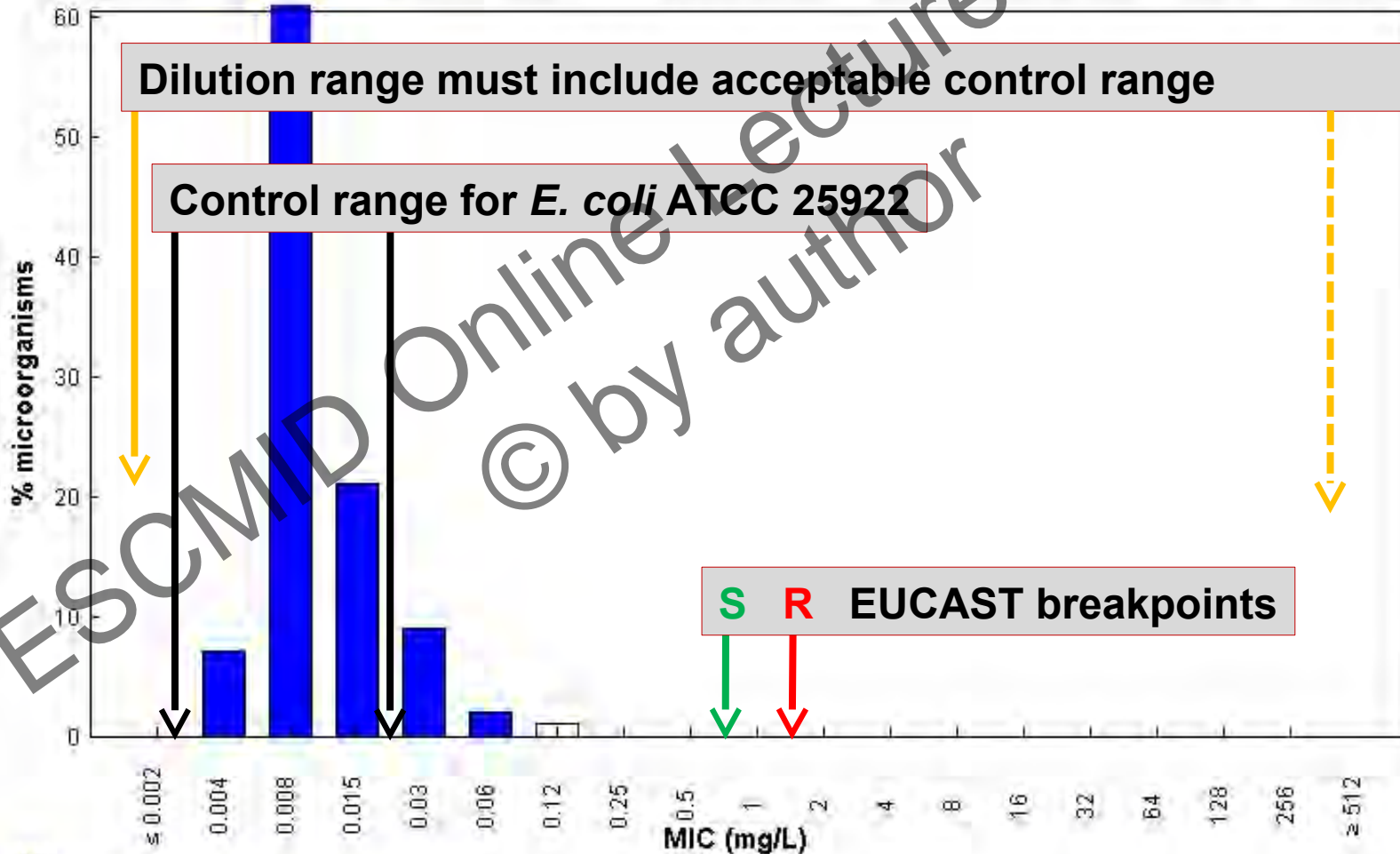
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Quality control of MIC testing

Ertapenem / *Escherichia coli*

International MIC Distribution - Reference Database 2016-09-10

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 (ECOFF): 0.064 mg/L

Wildtype (WT) organisms: ≤ 0.064 mg/L

2187 observations (12 data sources)

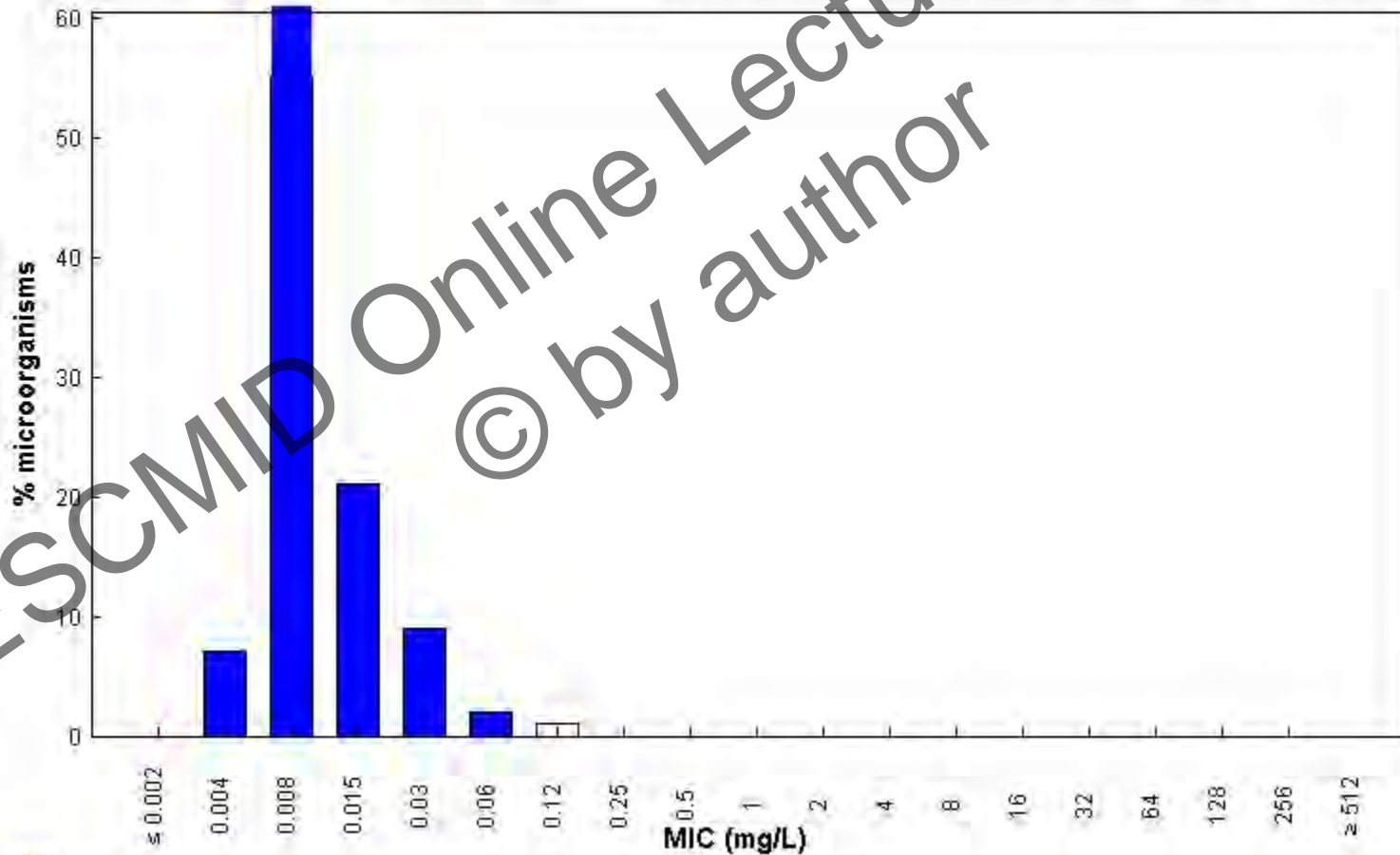
Quality control of MIC determination

- 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 antimicrobial agent 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
International MIC Distribution - Reference Database 2016-09-10

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



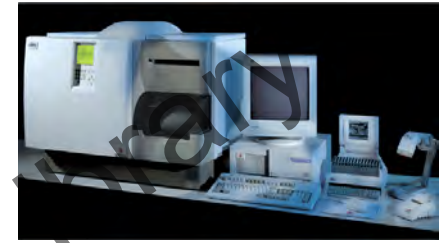
MIC

Epidemiological cut-off (ECOFF): 0.064 mg/L

Wildtype (WT) organisms: ≤ 0.064 mg/L

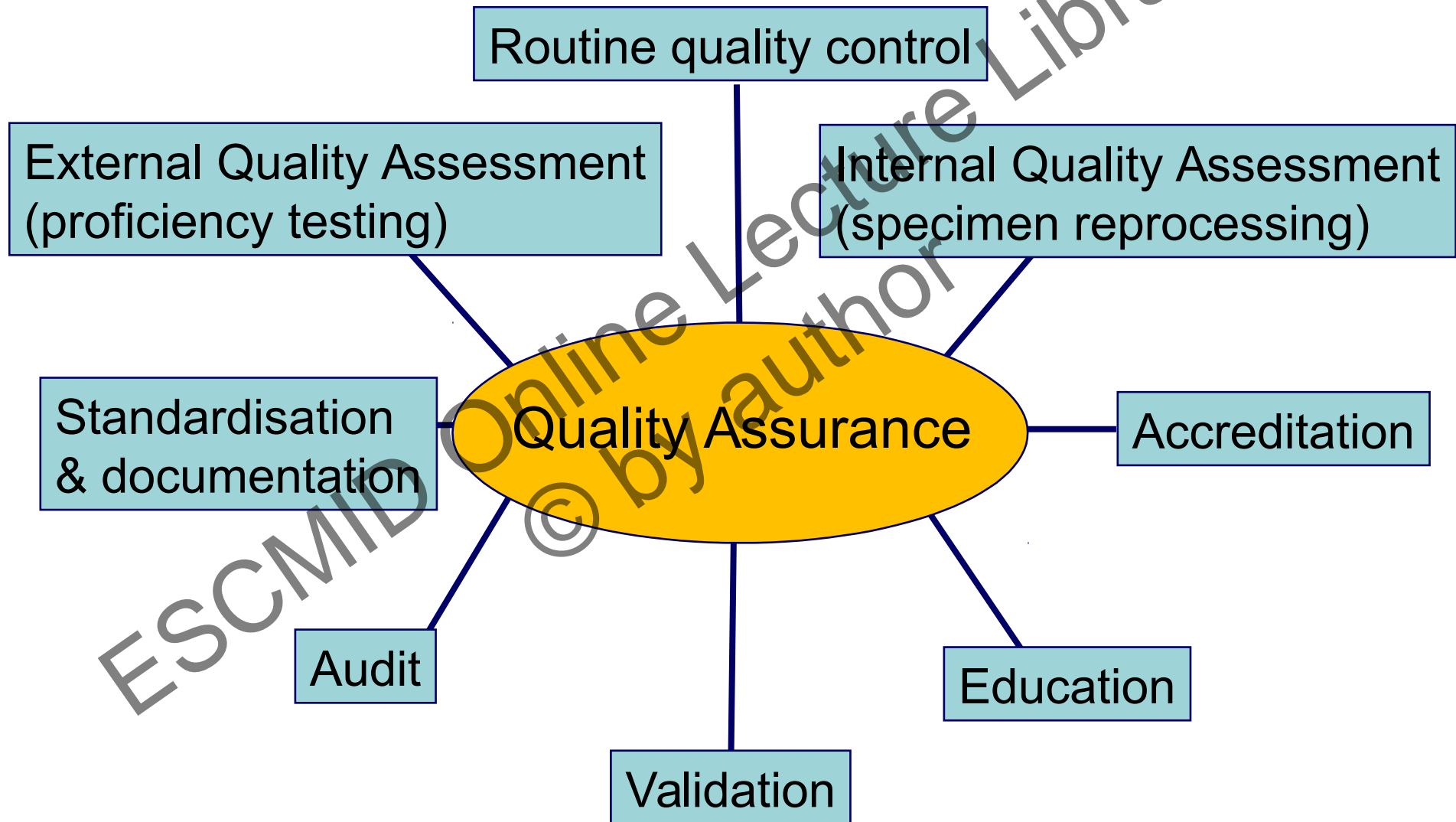
2187 observations (12 data sources)

Quality control of automated systems



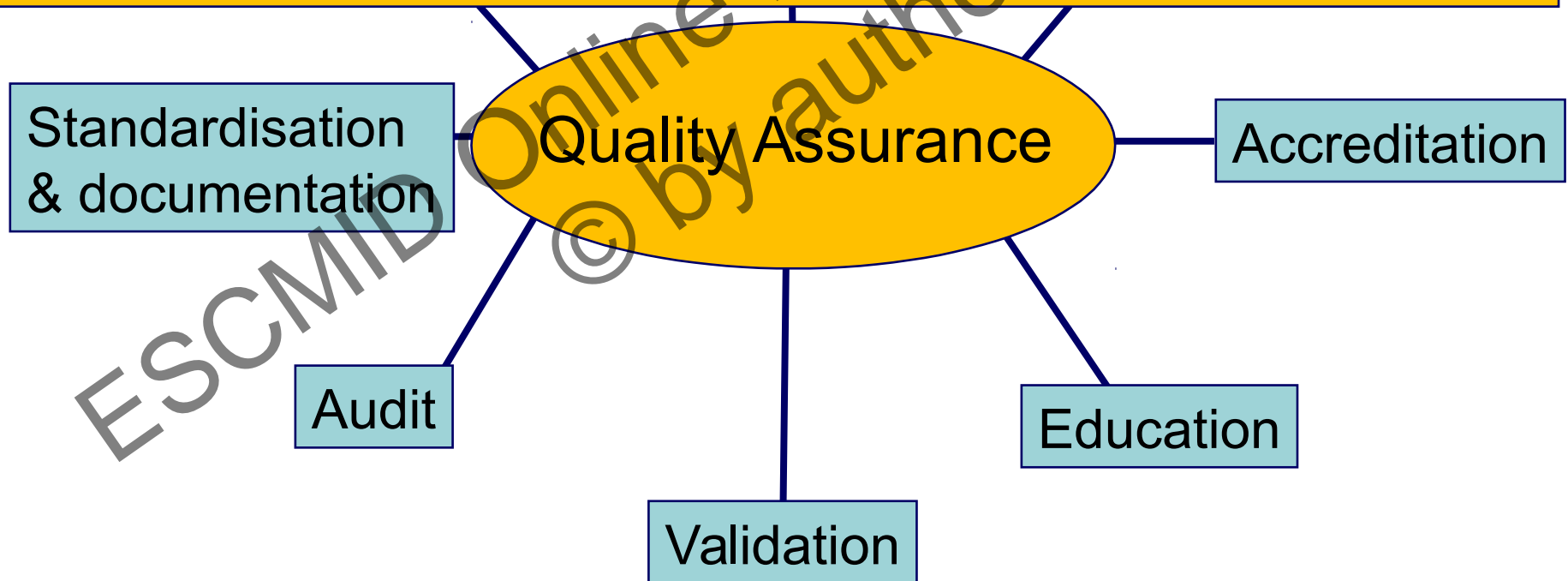
- Use the recommended routine quality control strains to monitor test performance (see manufacturer's instructions).
- Restricted range of test concentrations mean that the range may not include the MIC of the control strain.
- Purity of inoculum tested by culture on solid medium to obtain isolated colonies.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

Components of quality assurance



Components of quality assurance

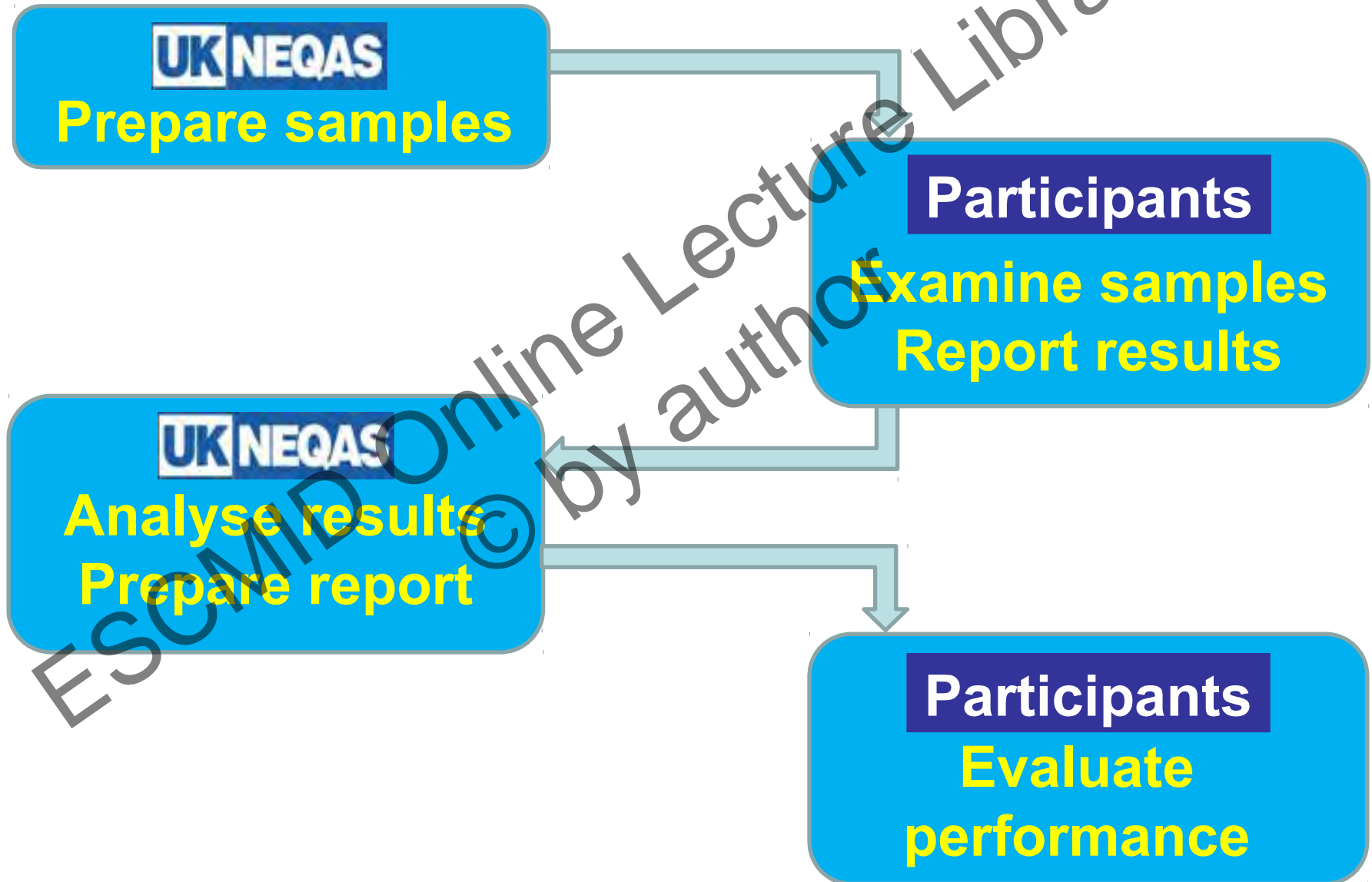
External Quality Assessment (proficiency testing)



External Quality Assessment (Proficiency testing)

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

The EQA process (UK NEQAS)



EQA report

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

UK NEQAS for Antimicrobial Susceptibility		Laboratory	
Distribution: 1821		Page 1 of 10	
Dispatch date: 25-Oct-2008			
Intended Result	Your Report	Your Score	
Openness 72%	Amoxicillin-sulbactam parenteral Amoxicillin Ampicillin Cefazolin Cefepime Cefotaxime Ceftriaxone Clindamycin Cloxacillin Colistin Gentamicin Meropenem Netilmicin Piperacillin-tazobactam Ticarcillin-clavulanate	Amoxicillin-sulbactam parenteral Amoxicillin Ampicillin Cefazolin Cefepime Cefotaxime Ceftriaxone Clindamycin Cloxacillin Colistin Gentamicin Meropenem Netilmicin Piperacillin-tazobactam Ticarcillin-clavulanate	100% 100% 100% 100% 100% 100% 100% 100% 100% 100% 100% 100% 100% 100% 100%
Cumulative score information	The number of specimens for which UK NEQAS for Antimicrobial Susceptibility (over the last 12 months) was 12. Specimens received: 1447 (141 (9.7%) 1106 (76.7%) 240 (16.6%) over the period and scored. Your cumulative score for the specimens submitted that you reported was 738 out of a possible 1308. The total score calculated from the reports received by UK laboratories using the open-access comparison was 10876. Your performance rating for UK NEQAS for Antimicrobial Susceptibility is (in the number of correct results) which your cumulative score has shown is based on the UK NEQAS mean of 130. Cumulative scores may change if participants' results are amended.		
Comments	The intended result for rifabutin (openness 72%) is based on the maximum score as this was not listed in the reference literature. Please note that there is a difference in reported results between BANC and NCCU guidelines for susceptibility to the beta-lactams. Additional scores will be awarded for the modifications, e.g. rifabutin which also are not scored. Status of the data entered are indicated in the test specified group score guidelines below: Excluded Please do not include any results to this +45 (20 020) 1985 or email enquiries@ukneqas.org.uk . Please do not include any results to this +45 (20 020) 1985 or email enquiries@ukneqas.org.uk . The national register (national data) is available on the website www.ukneqas.org.uk . Digital images of the results obtained at EQA, with this distribution, are available on the website www.ukneqas.org.uk .		
<small>Quality Assurance Laboratory An ISO 15189 Accredited Centre for Excellence 17, Leighton Road London, UK, E15 2JZ Phone: +44 (0)20 8205 1800 Fax: +44 (0)20 8205 1400</small>	<small>© Copyright. The data in UK NEQAS reports are confidential. Participants must consent for their name to appear on reports before data items are released.</small>	<small>Published on 27th Oct 2008 10:00:00</small>	

Evaluation

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

Enterococcus faecium (EARS-Net 3082)

Vancomycin MIC 8 mg/L (VanB)

EUCAST R (S \leq 4, R >4 mg/L), CLSI I (S \leq 4, R \geq 32 mg/L)

Method	n (%) of participants reporting		
	S	I	R
EUCAST			
Disk diffusion	11 (7.4)	4 (2.7)	133 (89.9)
Automated	5 (1.8)	0	278 (98.2)
MIC	15 (4.9)	8 (2.7)	281 (92.4)
Multi/other	1	0	14
Total	32 (4.3)	12 (1.6)	706 (94.1)
CLSI			
Disk diffusion	6 (20.7)	9 (31.0)	14 (48.3)
Automated	2 (2.8)	21 (29.6)	48 (67.6)
MIC	5 (11.9)	17 (40.5)	20 (47.6)
Multi/other	1	0	1
Total	14 (9.8)	47 (32.6)	83 (57.6)

Enterococci and vancomycin

- Examine with transmitted light (plate held up to light).
 - Fuzzy zone edges and colonies within zone indicate vancomycin resistance and should be investigated further.



E. faecalis
non-VRE



E. faecium
VRE

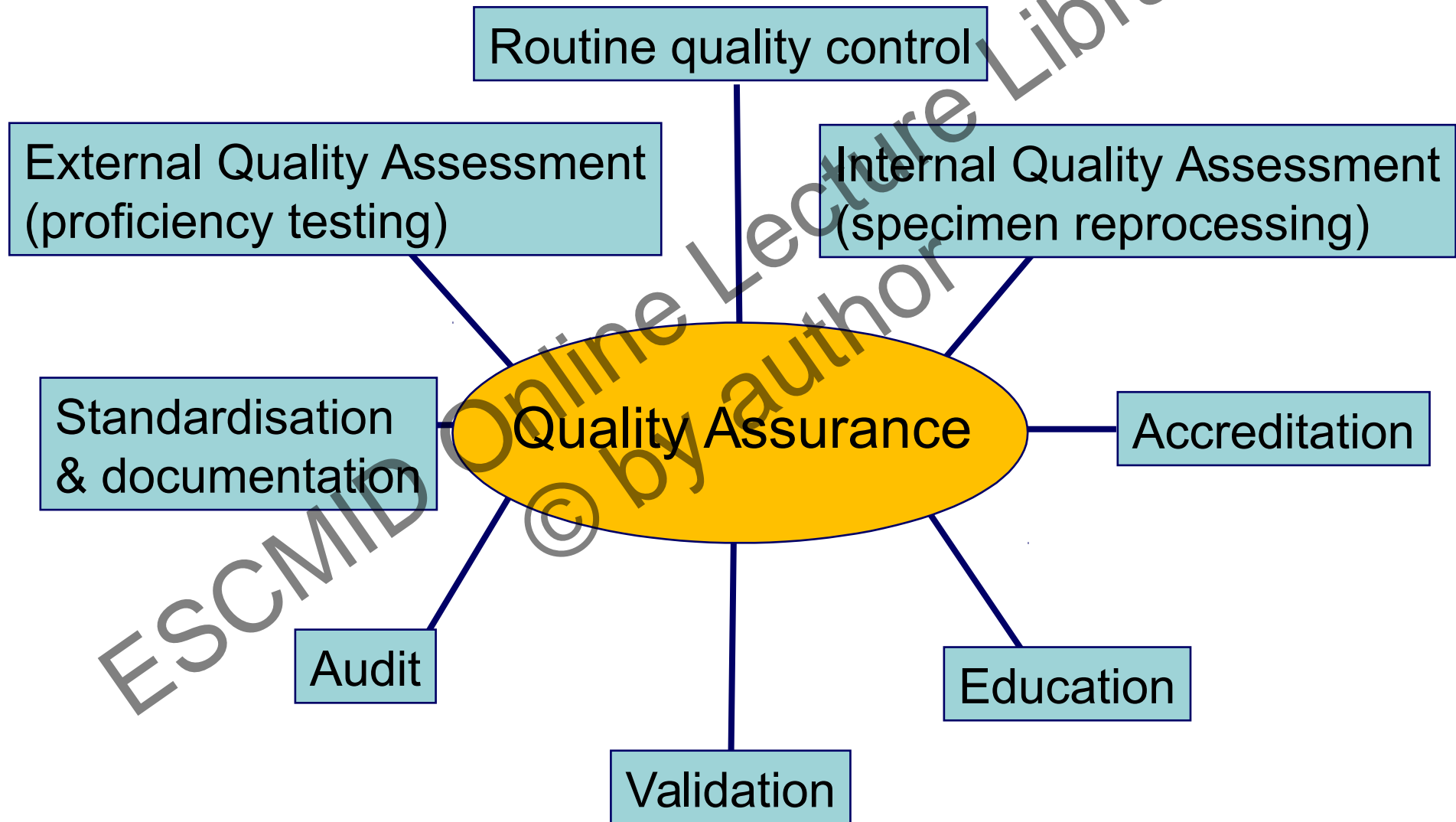
Benefits of EQA in antimicrobial susceptibility testing

- Independent assessment of performance
- Assessment of performance over time
- Comparison with other laboratories
- Performance indicator for accreditation
- Highlights problem areas
- Performance related to methods
- Differences in guidelines highlighted
- Education

“Limitations” of EQA in antimicrobial susceptibility testing

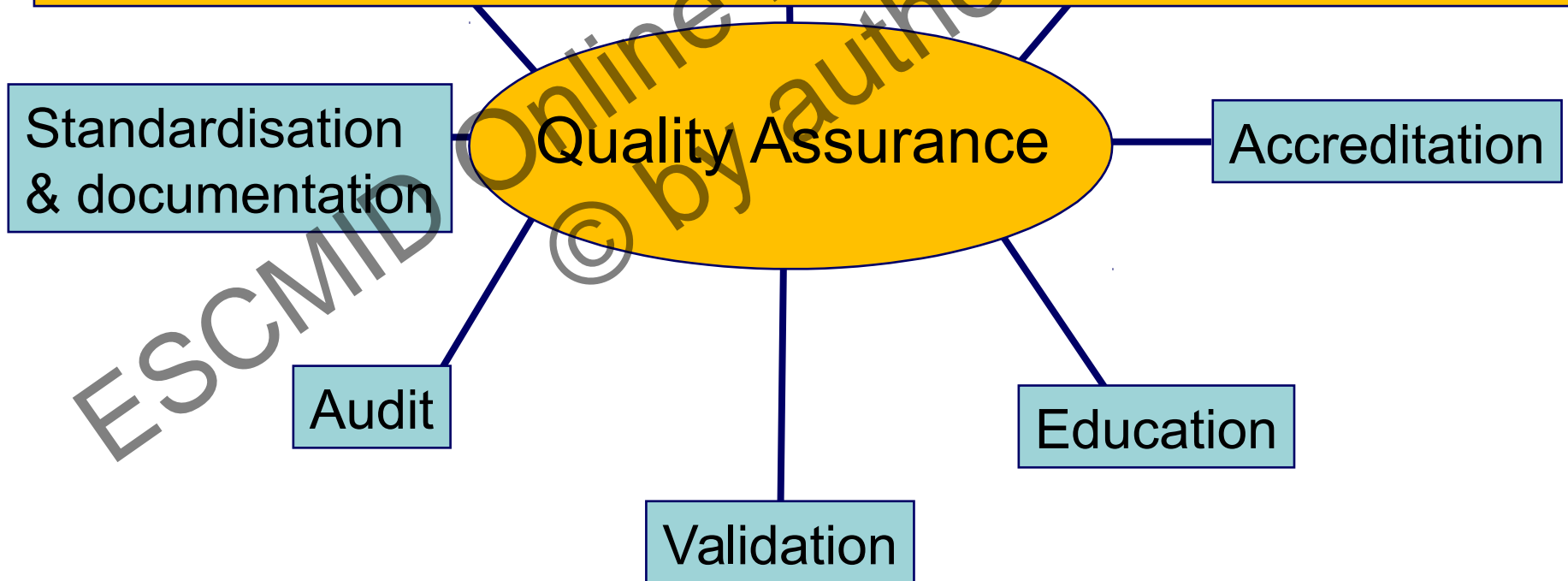
- Number of specimens distributed is small
- May be considered inappropriate to send some organisms
- Specimens do not reflect routine isolates
- Laboratories may not treat specimens as routine

Components of quality assurance



Components of quality assurance

Internal Quality Assessment (specimen reprocessing)



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 meetings

Antimicrobial susceptibility testing problems highlighted by IQA

- Variable susceptibility because different organisms picked from mixture on primary plates
- Wrong disk contents used e.g.
 - Ampicillin 10 µg instead of 2 µg for *H. influenzae*
- Borderline susceptibility leads to variable results 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
 - Cefoxitin with hetero-resistant MRSA
 - Vancomycin with VanB enterococcus
- Typographical errors

Benefits of IQA for 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

Limitations of IQA for antimicrobial susceptibility testing

- Discrepancies may not be related to susceptibility testing
- No reference results so the correct answer is unknown - both results could be wrong
- Cost

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Quality assurance of antimicrobial susceptibility testing

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