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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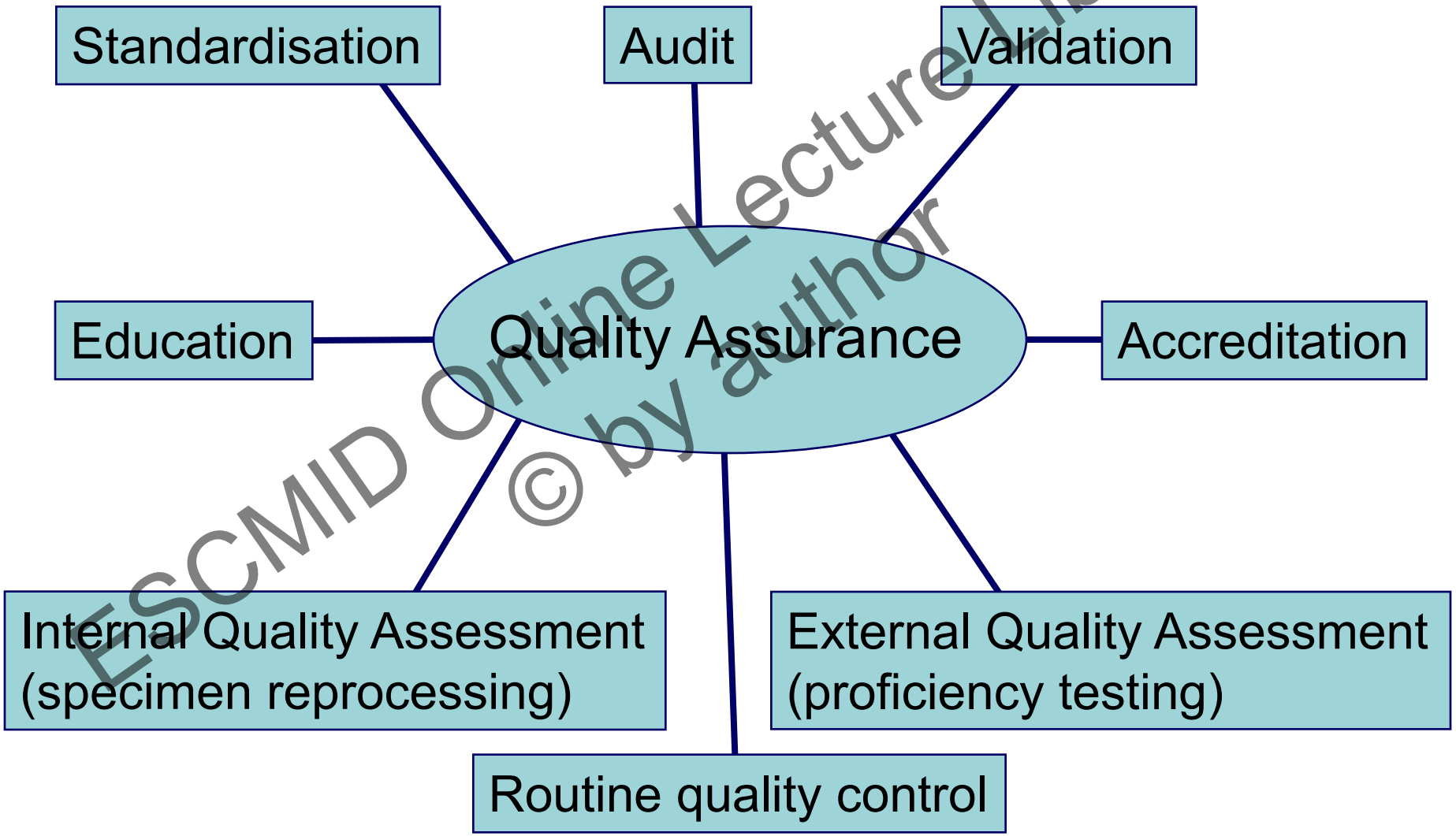
Quality Assurance in the clinical diagnostic laboratory

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

Components of quality assurance



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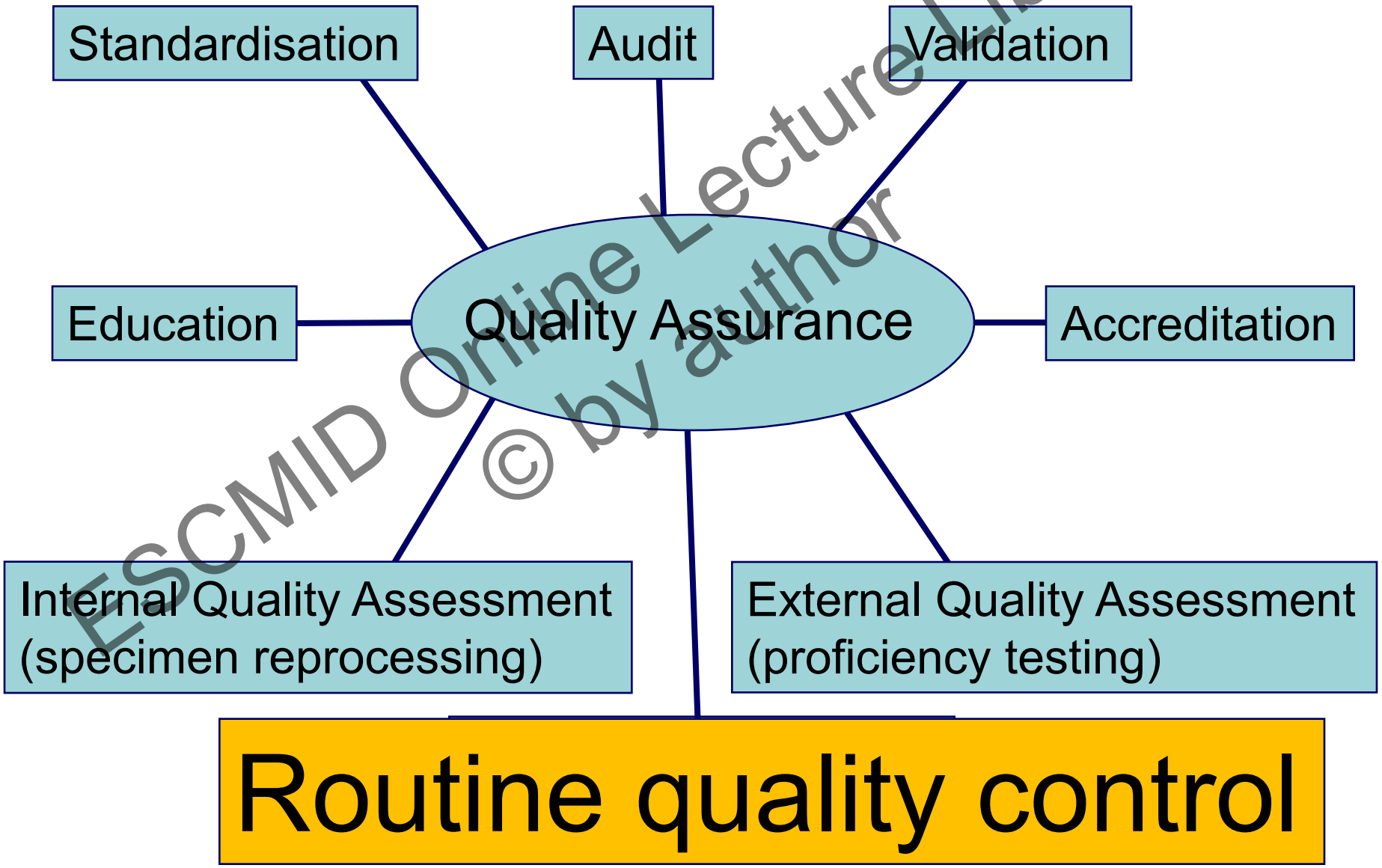


Routine quality control

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

Quality 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
E. coli	ATCC 25922; NCTC 12241; CIP 7624 DSM 1103; CCUG 17620, CECT 434	Susceptible, wild-type
P. aeruginosa	ATCC 27853; NCTC 12903; CIP 76110 DSM 1117; CCUG 17619; CECT 108	Susceptible, wild-type
S. aureus	ATCC 29213; NCTC 12973; CIP 103429 DSM 2569; CCUG 15915; CECT 794	Weak β-lactamase producer
E. faecalis	ATCC 29212; NCTC 12697; CIP 103214 DSM 2570; CCUG 9997; CECT 795	Susceptible, wild-type
S. pneumoniae	ATCC 49619; NCTC 12977; CIP 104340 DSM 11967; CCUG 33638	Penicillin intermediate
H. influenzae	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/
CECT. Colección Española de Cultivos Tipo. Universidad de Valencia. 46100. Burjassot. Valencia. Spain. http://www.cect.org

Staphylococcus aureus ATCC 29213*

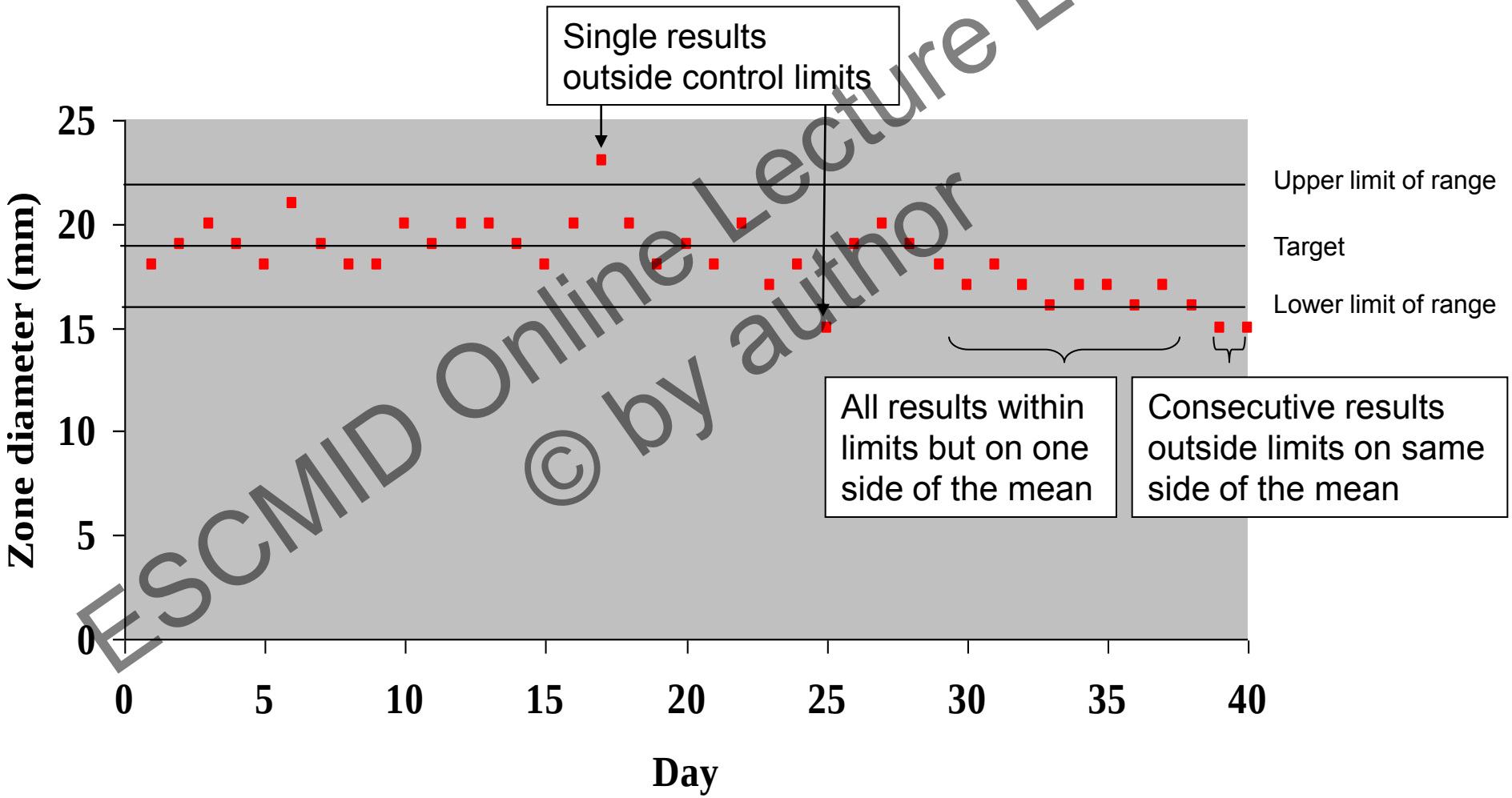
(NCTC 12973, CIP 103429, DSM 2569, CCUG 15915, CECT 794)

* β-lactamase-producing strain (weak)

Mueller-Hinton agar, McFarland 0.5, air, 35±1ºC, 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 (µg)	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
Chloramphenicol	4-8	2-16	30	24	20-28
Ciprofloxacin	0.25	0.12-0.5	5	24	21-27

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.

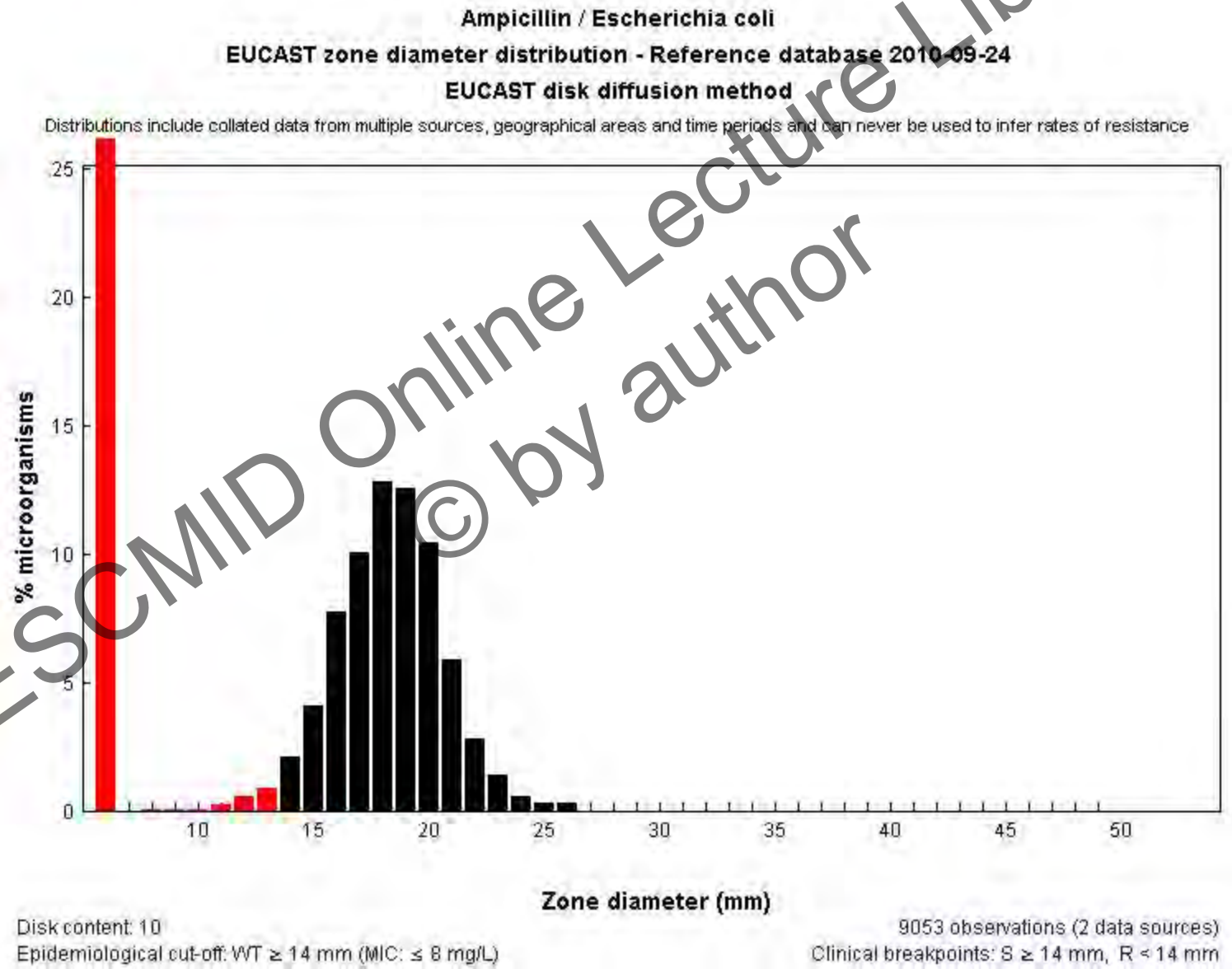
EUCAST strains for detection of resistance mechanisms (in progress)

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

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



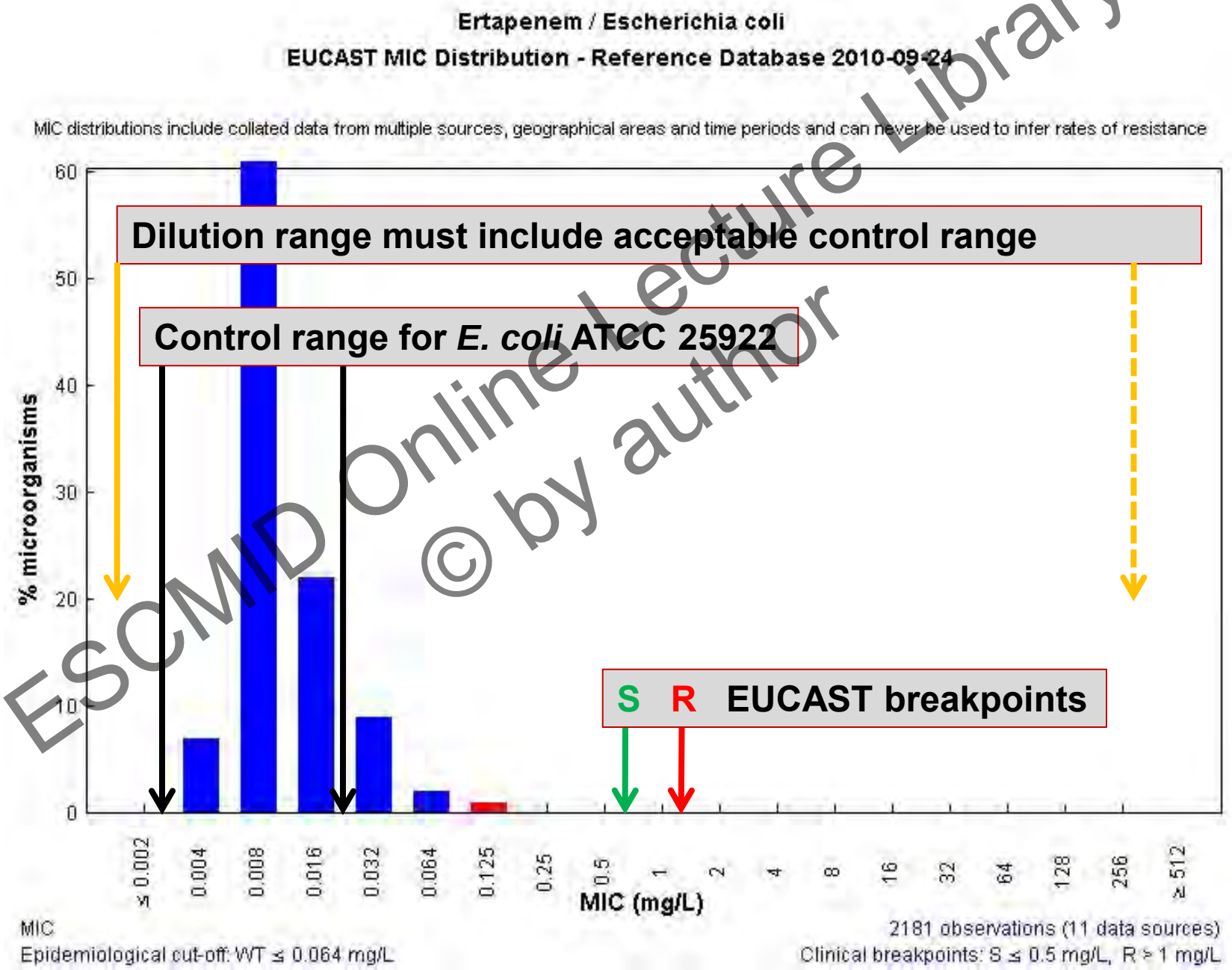
Sources of error in disk diffusion

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 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.

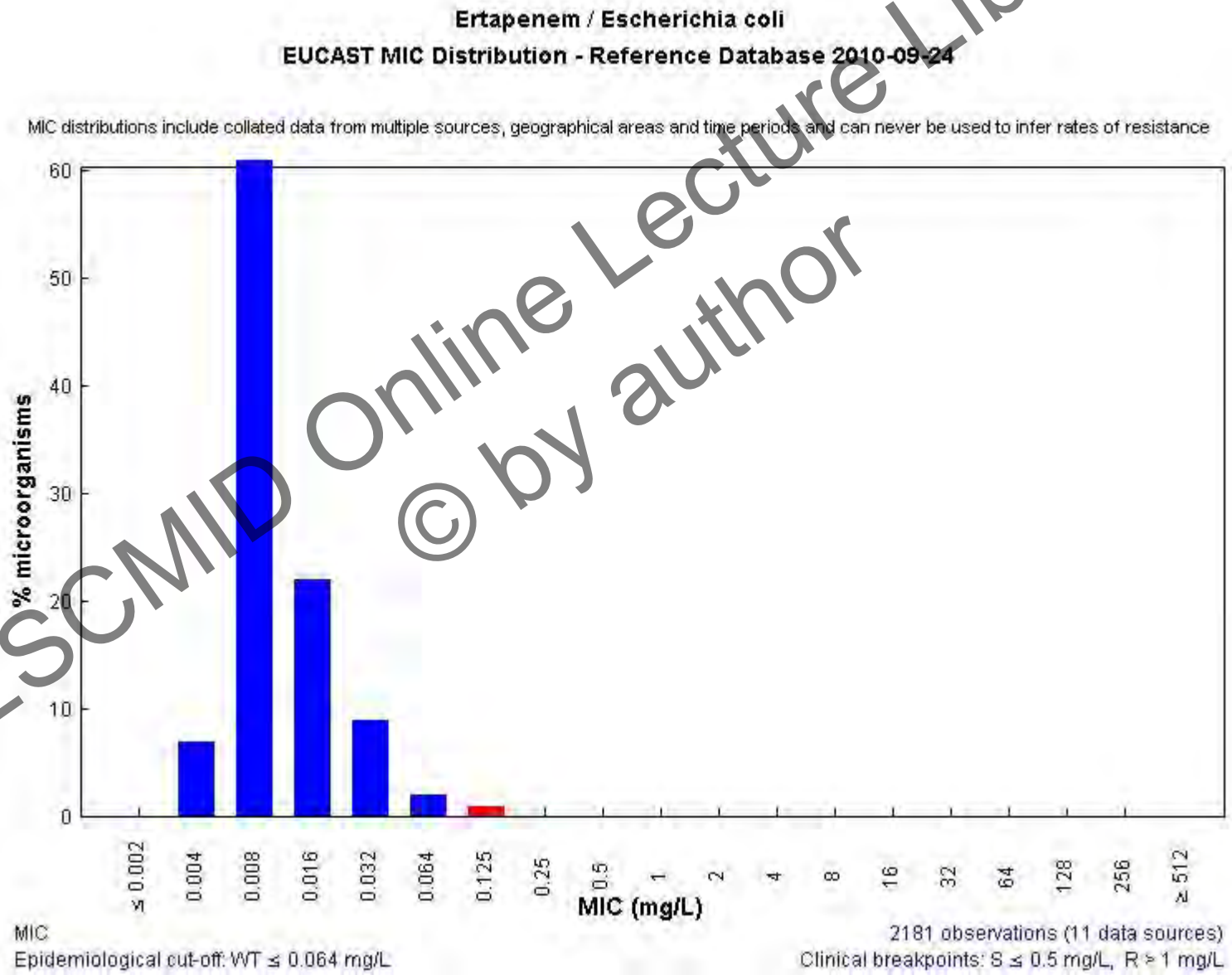
Quality control of MIC testing



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.

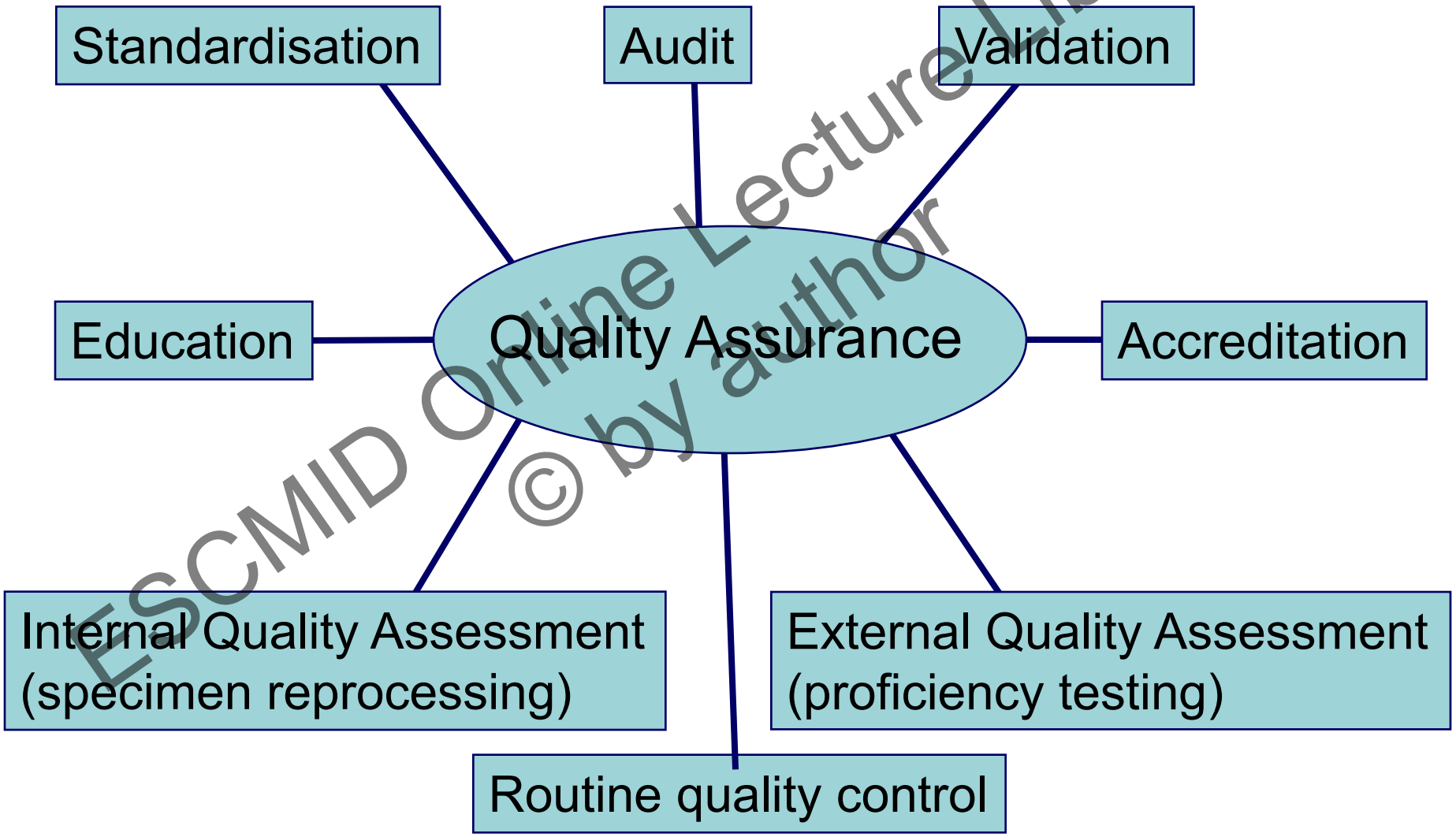
Quality control by comparison of wild type with reference distributions from EUCAST website



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



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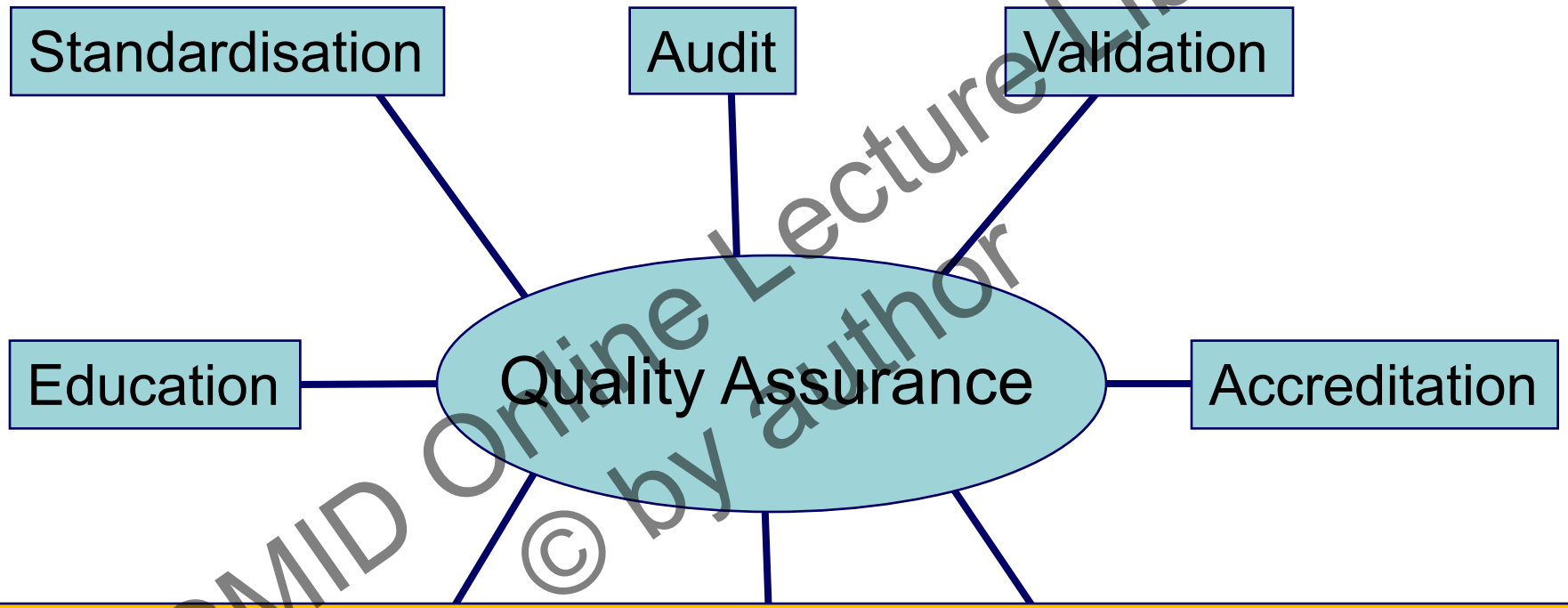

External Quality Assessment (proficiency testing)

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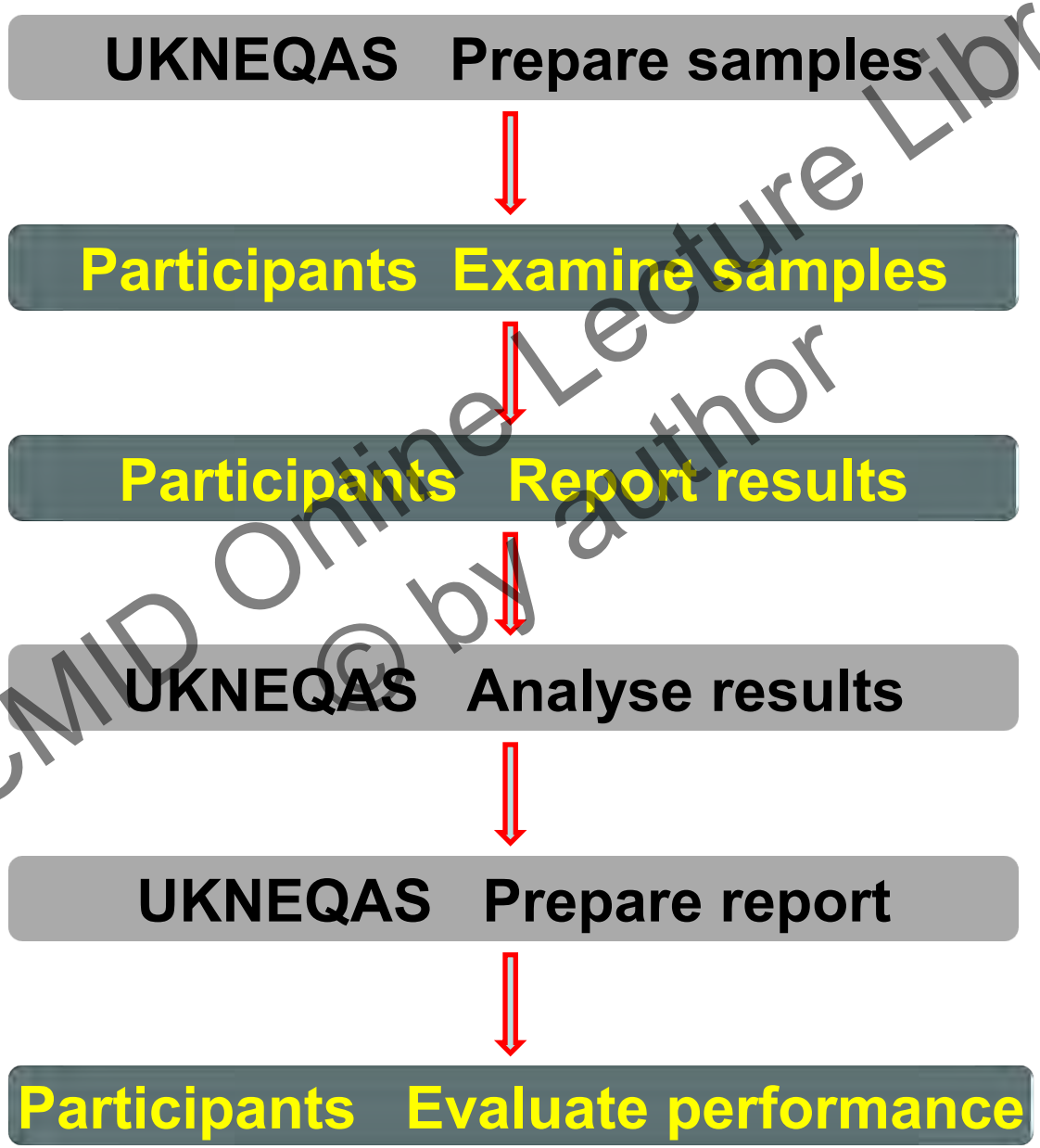
The challenge of laboratory procedures with specimens of known but undisclosed content

The EQA process (UKNEQAS)



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

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?

Enterococcus faecium (EARS-Net 0273)

Vancomycin (MIC 8-16 mg/L, VanB)

- EUCAST resistant, CLSI intermediate

- Reports from participants

  8.0% susceptible, 8.7% intermediate, 83.3% resistant

- Differences between guidelines

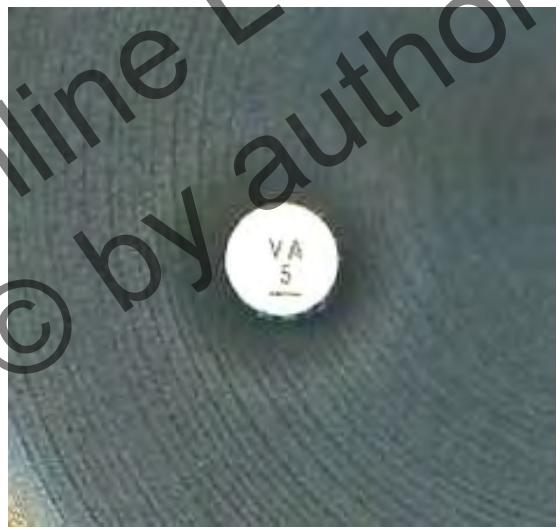
	S (%)	I (%)	R (%)
– EUCAST (n=395)	7.9	4.8	87.3
– CLSI (n=378)	8.7	14.8	76.5

- Differences between methods

	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

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

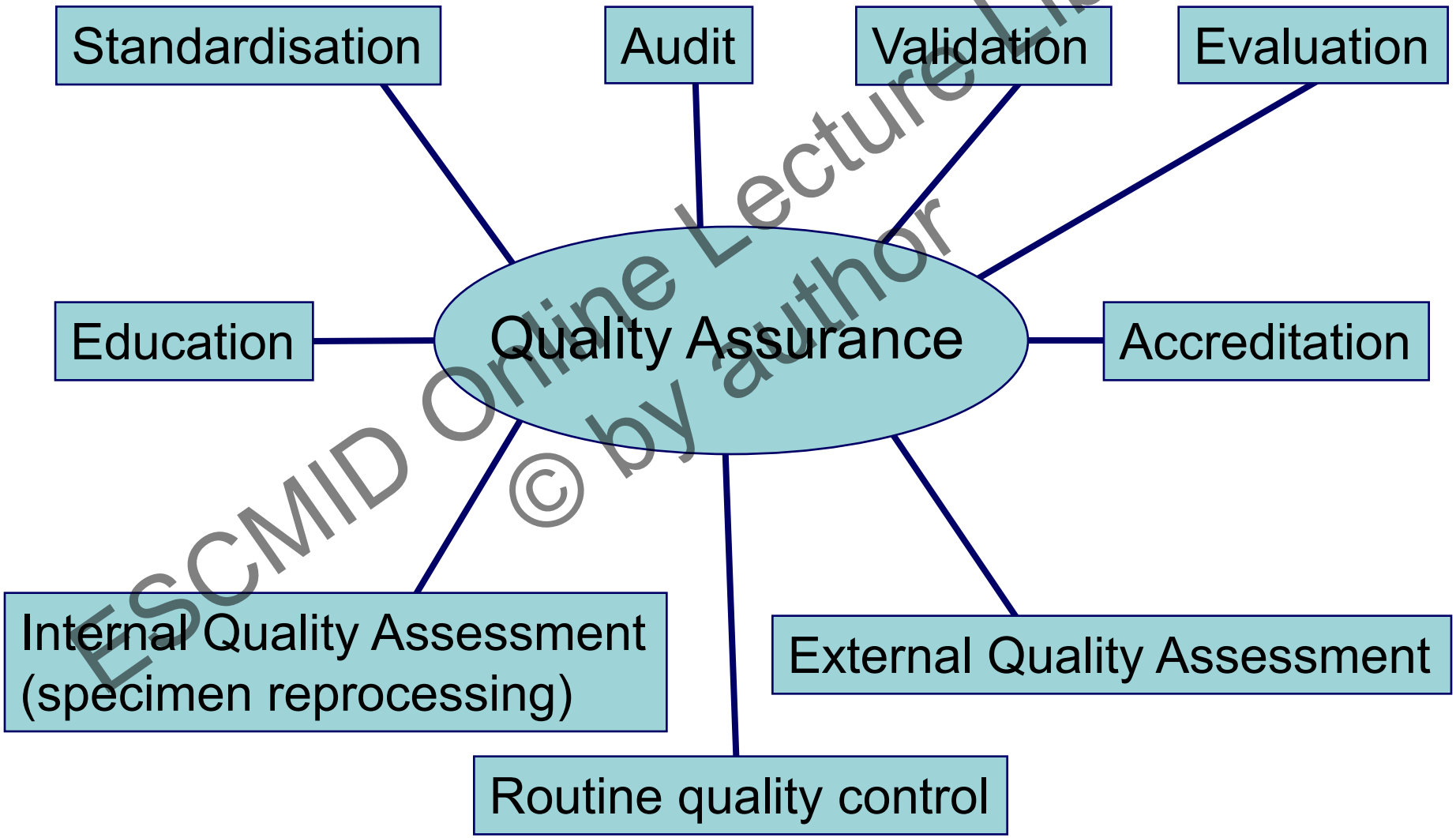
Benefits of EQA in antimicrobial susceptibility testing

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



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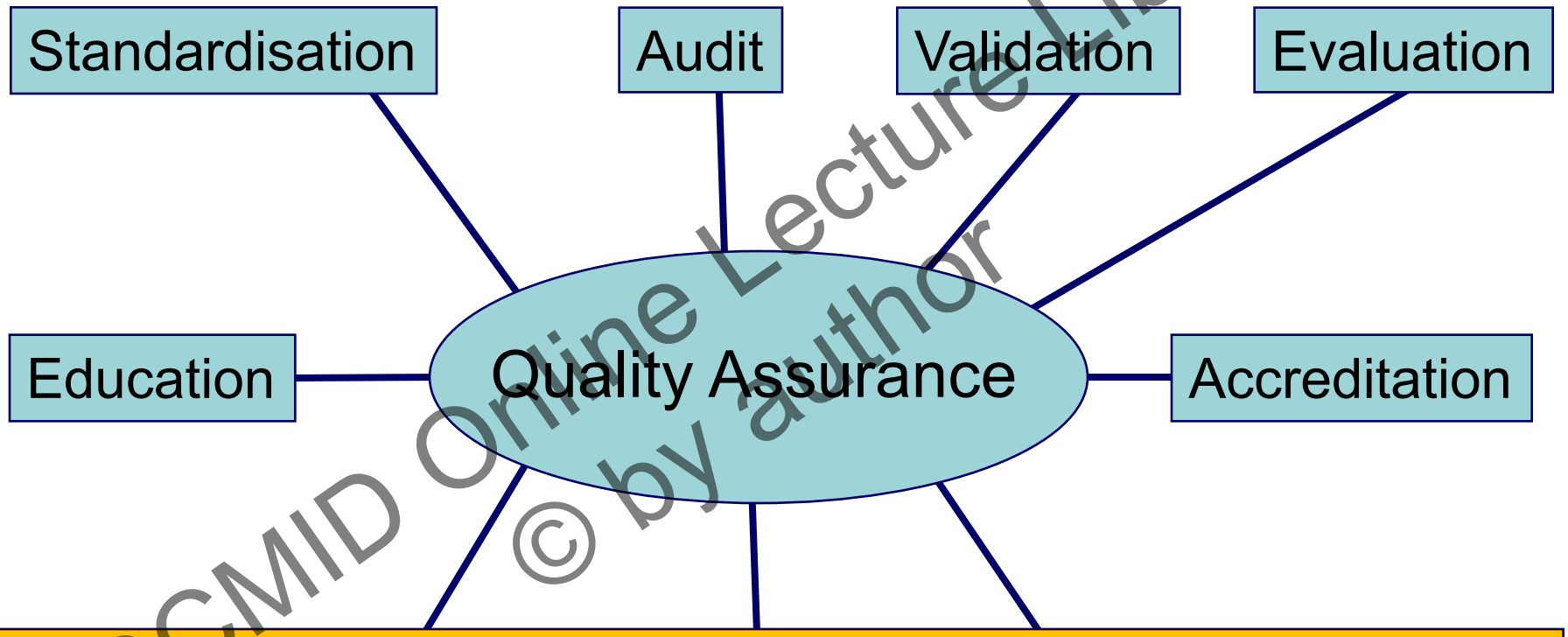

Internal Quality Assessment (specimen reprocessing)

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
  - Oxacillin 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

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