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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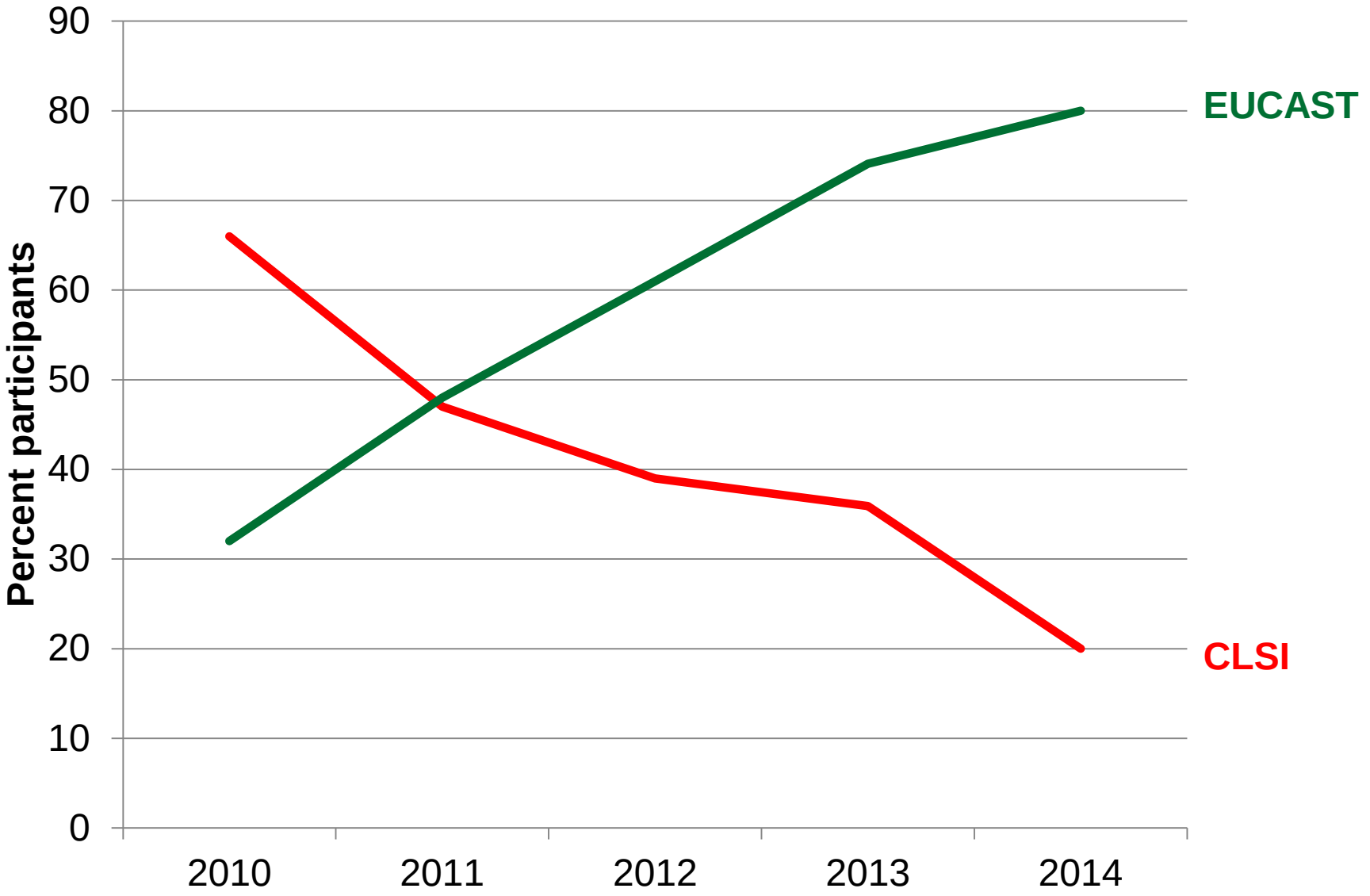
EUCAST Scientific Secretary

EUCAST Reloaded 5.0, Follow-up workshop, Vienna, 19 March 2015

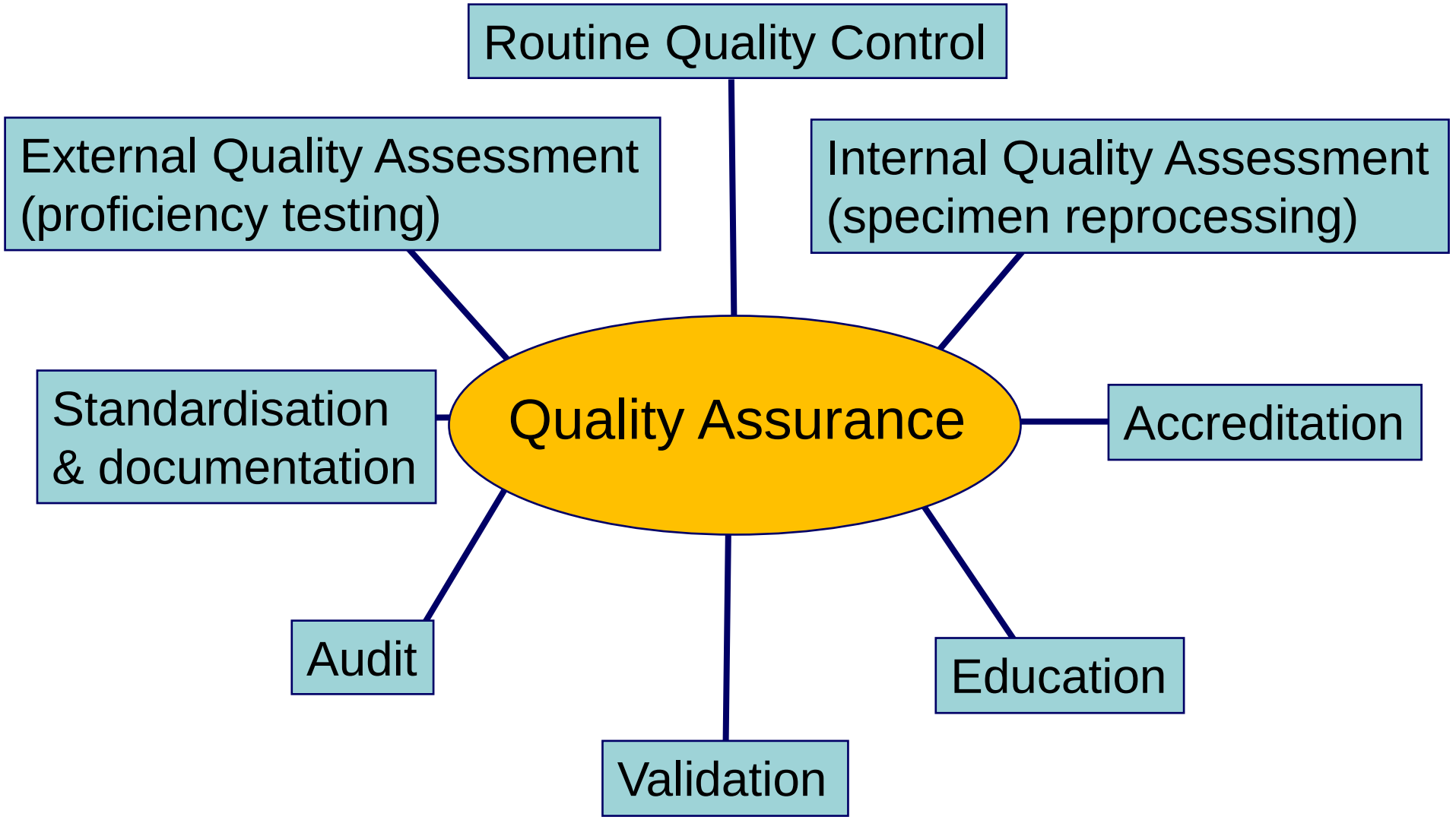
Trends in antimicrobial susceptibility testing guidelines (EARS-Net)



Quality Assurance

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

Components of quality assurance



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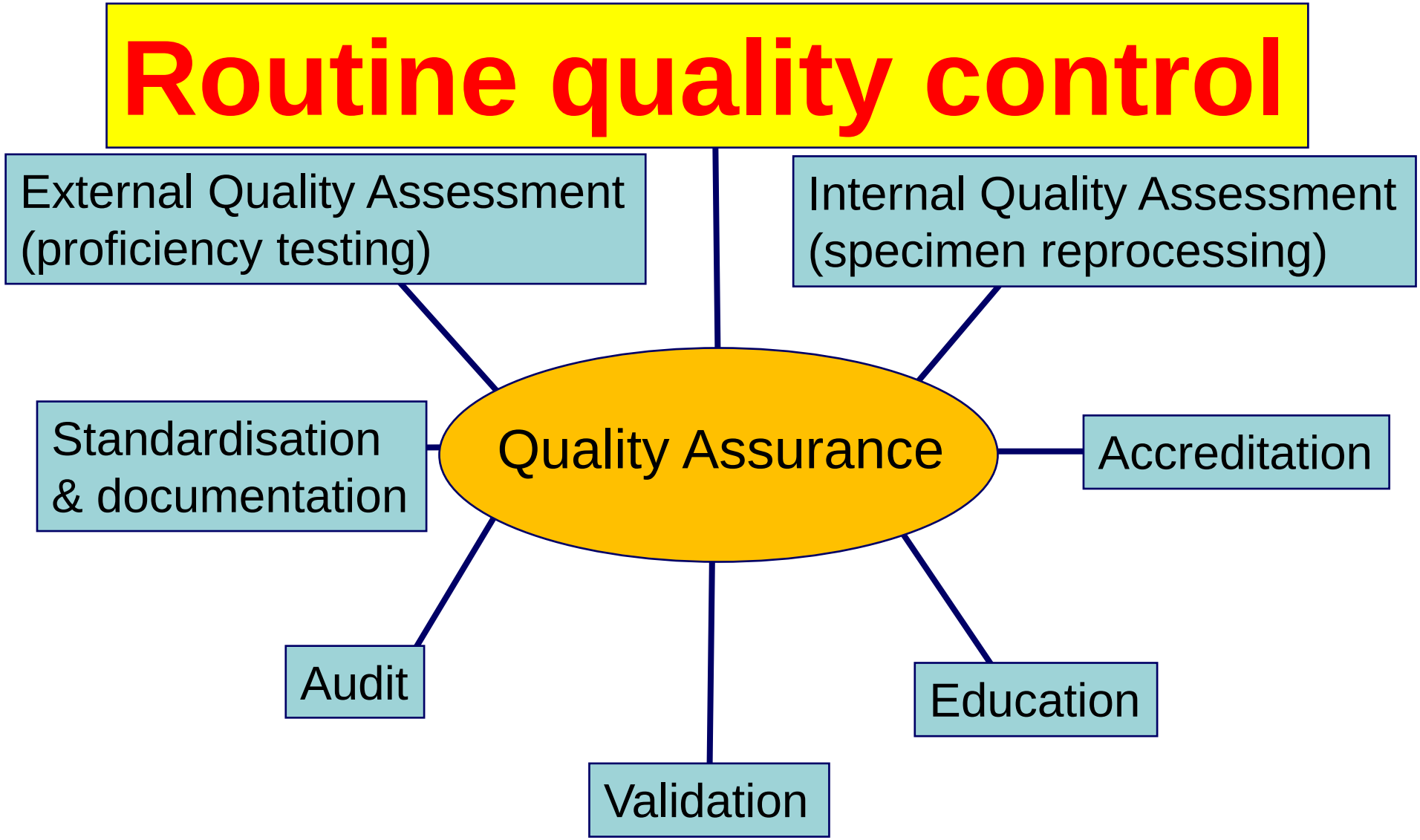
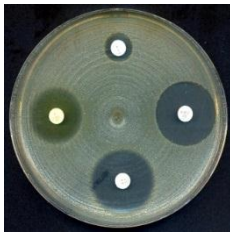


Routine quality control

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
E. coli	ATCC 35218; NCTC 11954, CIP 102181; DSM 5923, CCUG 30600; CECT 943	TEM-1 β-lactamase producer
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	ATCC 49766; NCTC 12975; CIP 103570; DSM 11970 CCUG 29539	Susceptible, wild-type
Campylobacter jejuni	ATCC 33560; NCTC 11351; CIP 702 DSM 4688; CCUG 11284	Susceptible, wild-type

EUCAST tables of target values and acceptable ranges for QC strains

http://www.eucast.org/ast_of_bacteria/qc_tables

Staphylococcus aureus ATCC 29213*

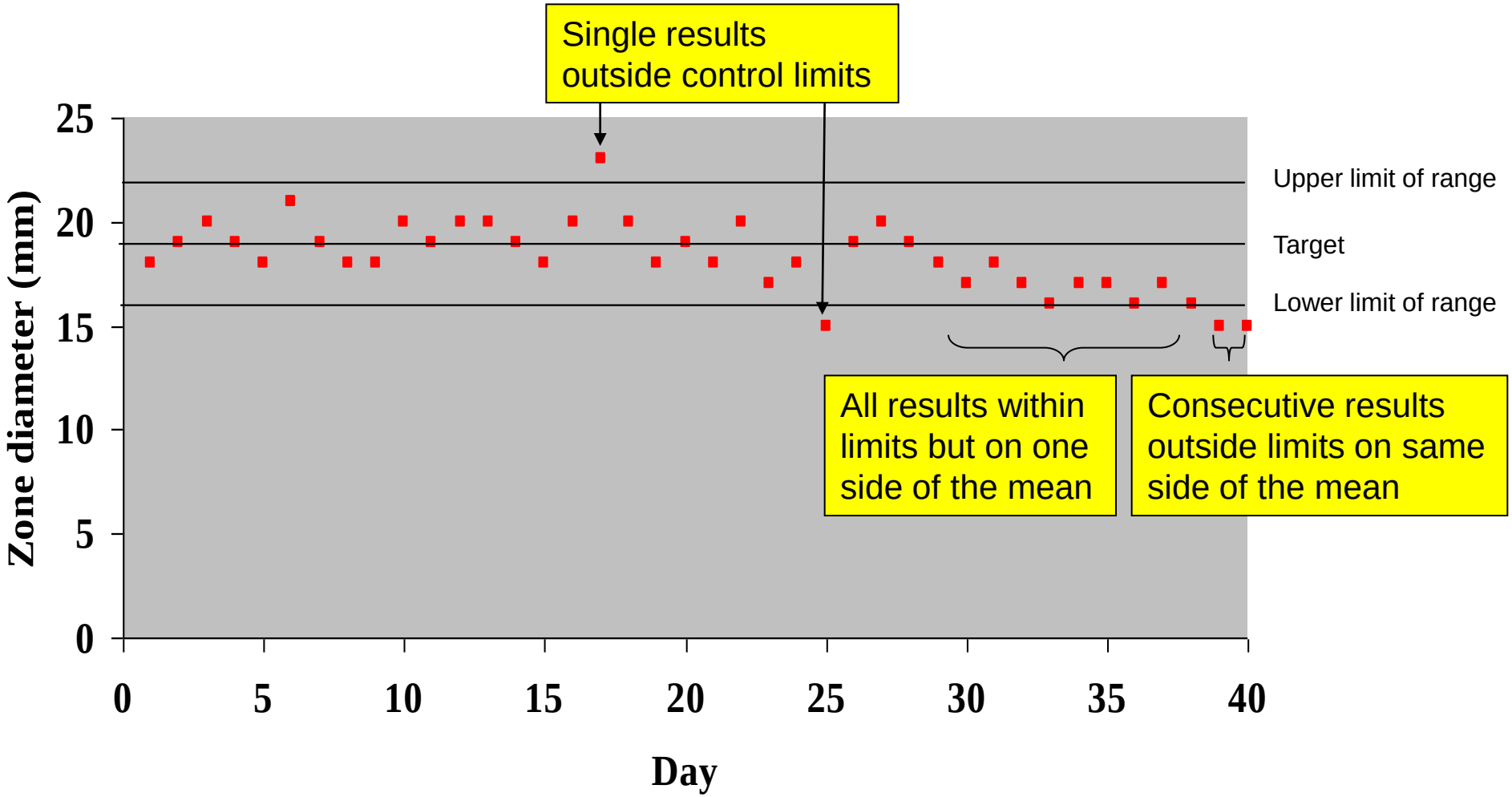
(NCTC 12973, CIP 103429, DSM 2569, CCUG 15915, CECT 794)

* 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
Ceftaroline	0.25	0.125-0.5[4]	5	***27***	***24-30***
Ceftobiprole	0.25-0.5	0.125-1[4]	IP	***IP***	***IP***

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 control strains for detection of resistance mechanisms

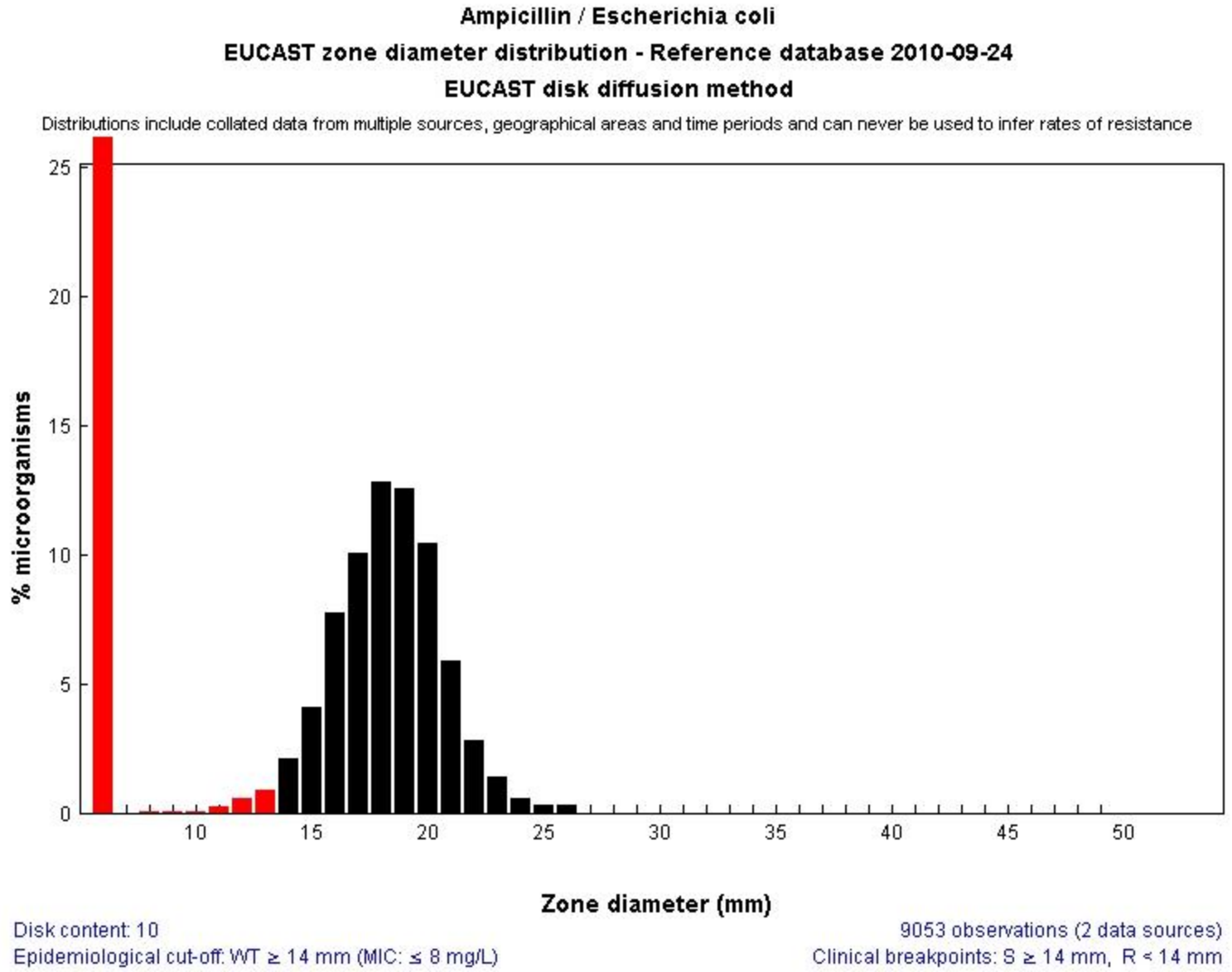
Quality control strains with defined resistance mechanisms may be used to confirm the ability to detect resistance. If resistance in a resistant control strain is not recognised suppress test results, retest and investigate.

Organism	Culture collection numbers	Characteristics
K. pneumoniae	ATCC 700603; NCTC 13368; CCUG 45421; CECT 7787	ESBL producer (SHV-18)
S. aureus	NCTC 12493	Oxacillin hetero-resistant, *mecA* positive
E. faecalis	ATCC 51299; NCTC 13379; CIP 104676; DSM 12956; CCUG 34289	High-level aminoglycoside resistant (HLAR) and vancomycin resistant (*vanB* positive)
H. influenzae	ATCC 49247; NCTC 12699; CIP 104604; DSM 9999; CCUG 26214	β-lactamase negative, ampicillin resistant (BLNAR)

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

Organism	Characteristics
S. pneumoniae	Penicillin resistant (MIC 4 mg/L)
E. coli	Different ESBL phenotypes
E. coli	OXA-48
E. coli	Plasmid AmpC
E. coli	Carbapenemase producers
K. pneumoniae	KPC enzyme

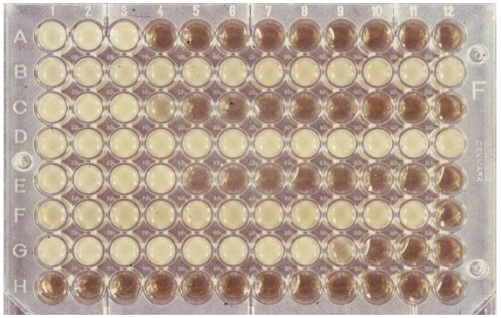
Quality control by comparison of wild type with reference distributions from EUCAST website

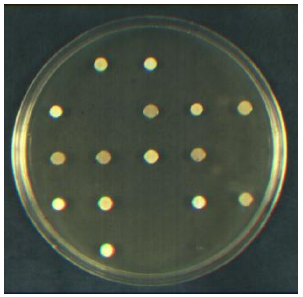


Sources of error in disk diffusion

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, disks not at room temperature when containers opened, labile agent, expiry date)
	Too many disks on plate (interference between agents)
Control organisms	Incorrect QC strain
	Mutation
	Contamination
	Age of culture

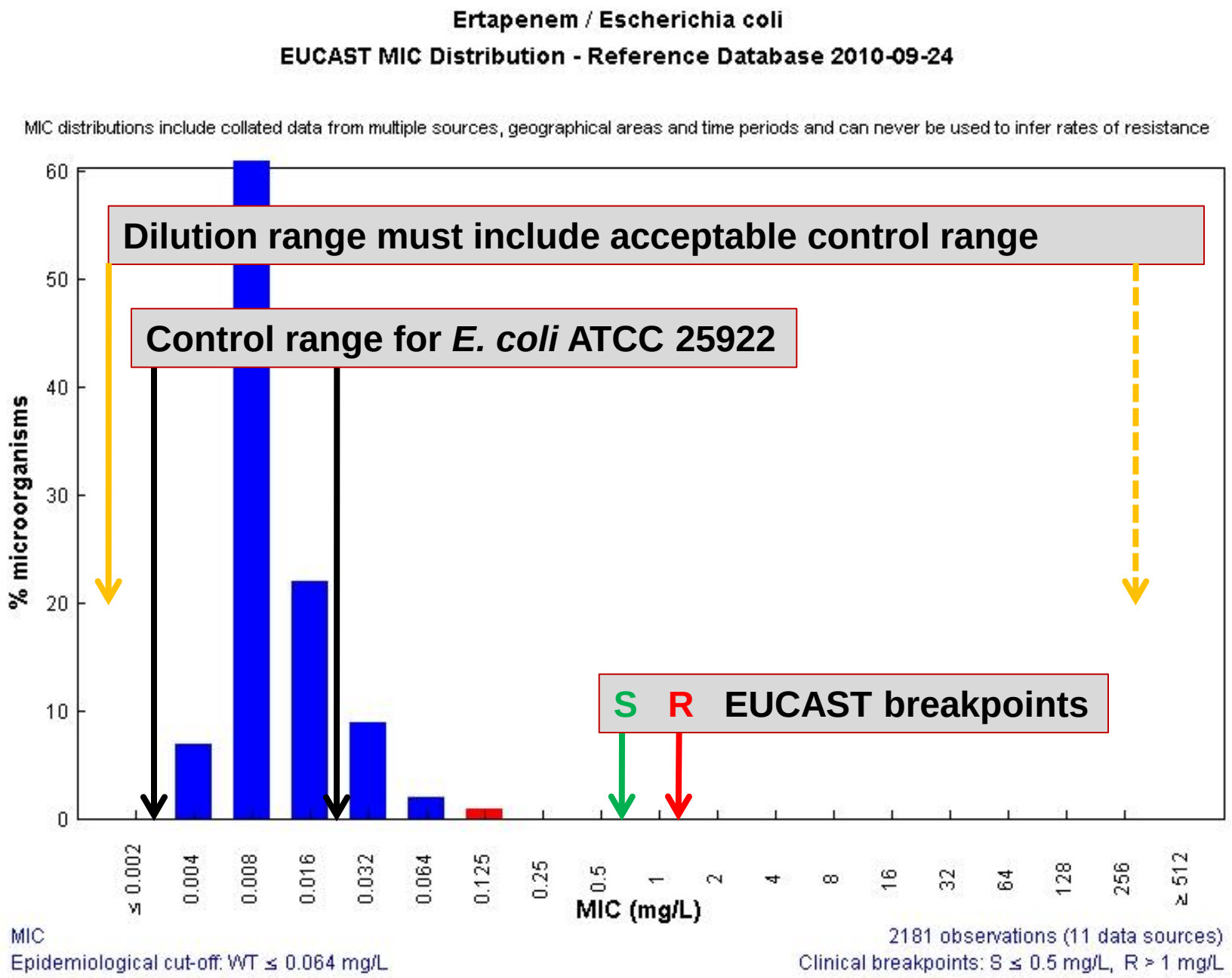
Quality control of MIC testing





- 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.

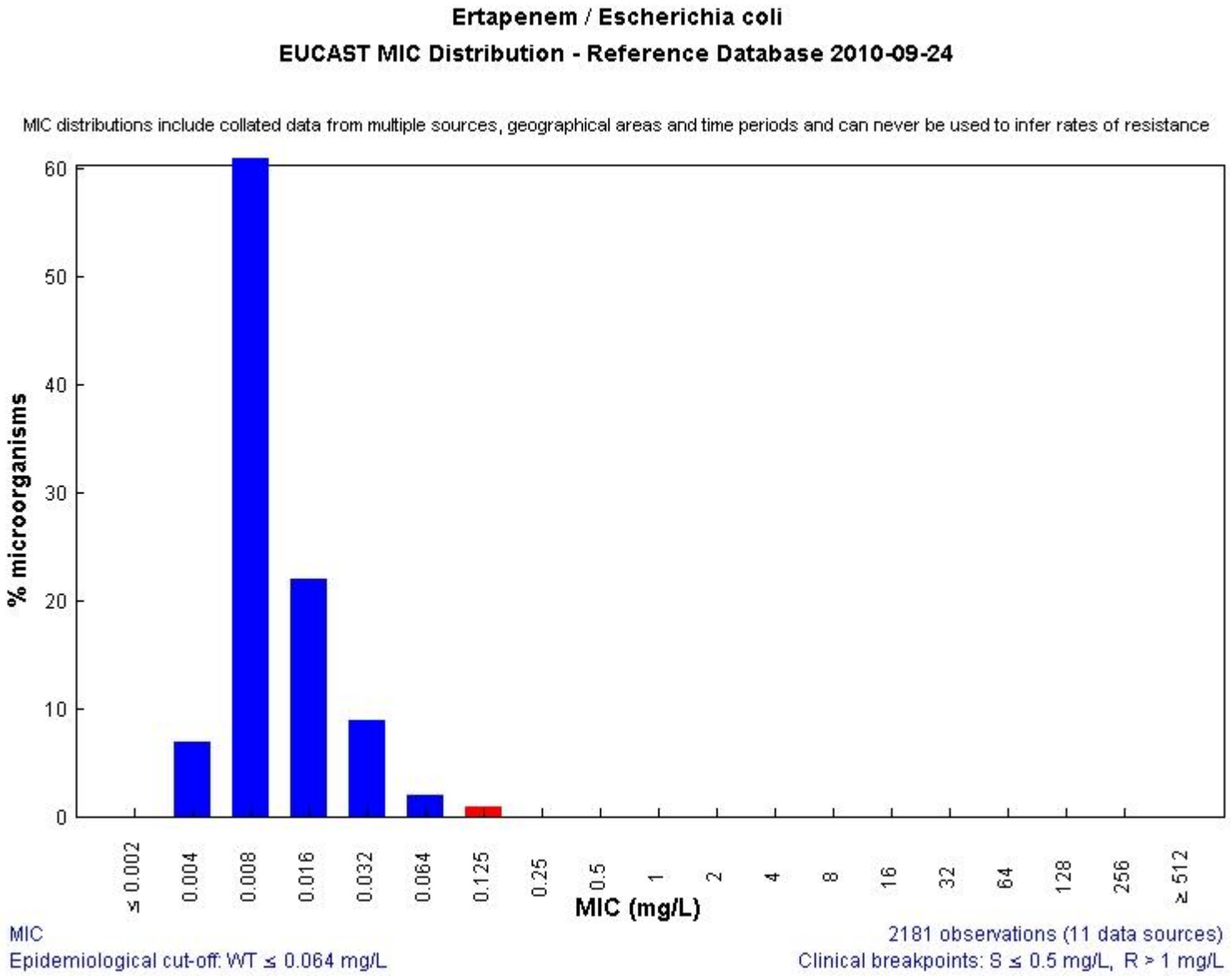
Quality control of MIC testing



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 antimicrobial agent to ensure that the test strain grows adequately.
- Test the purity of inoculum by culture on solid medium to obtain isolated colonies (broth microdilution).
- 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.

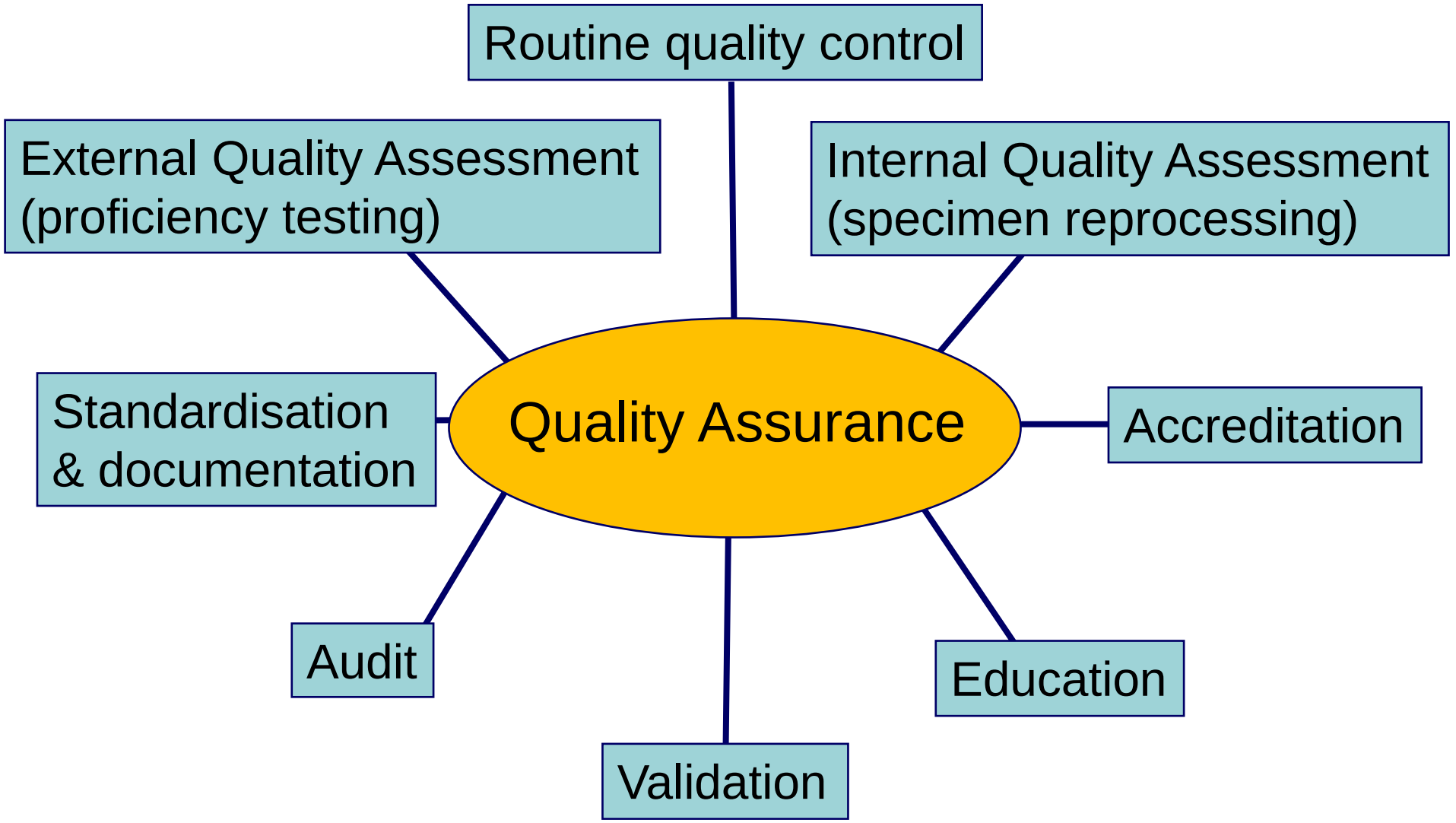
Quality control by comparison of wild type with reference distributions from EUCAST website



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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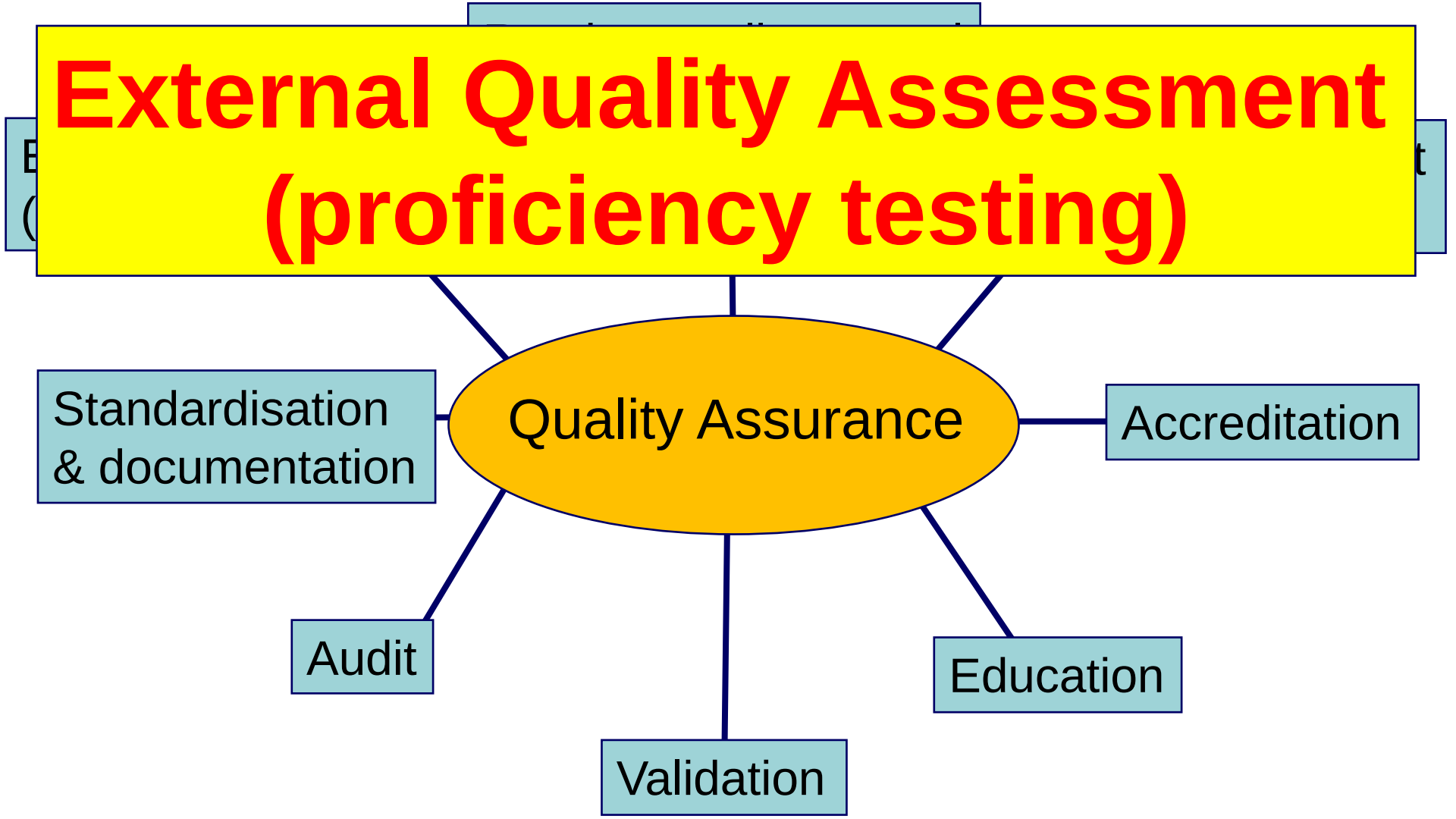
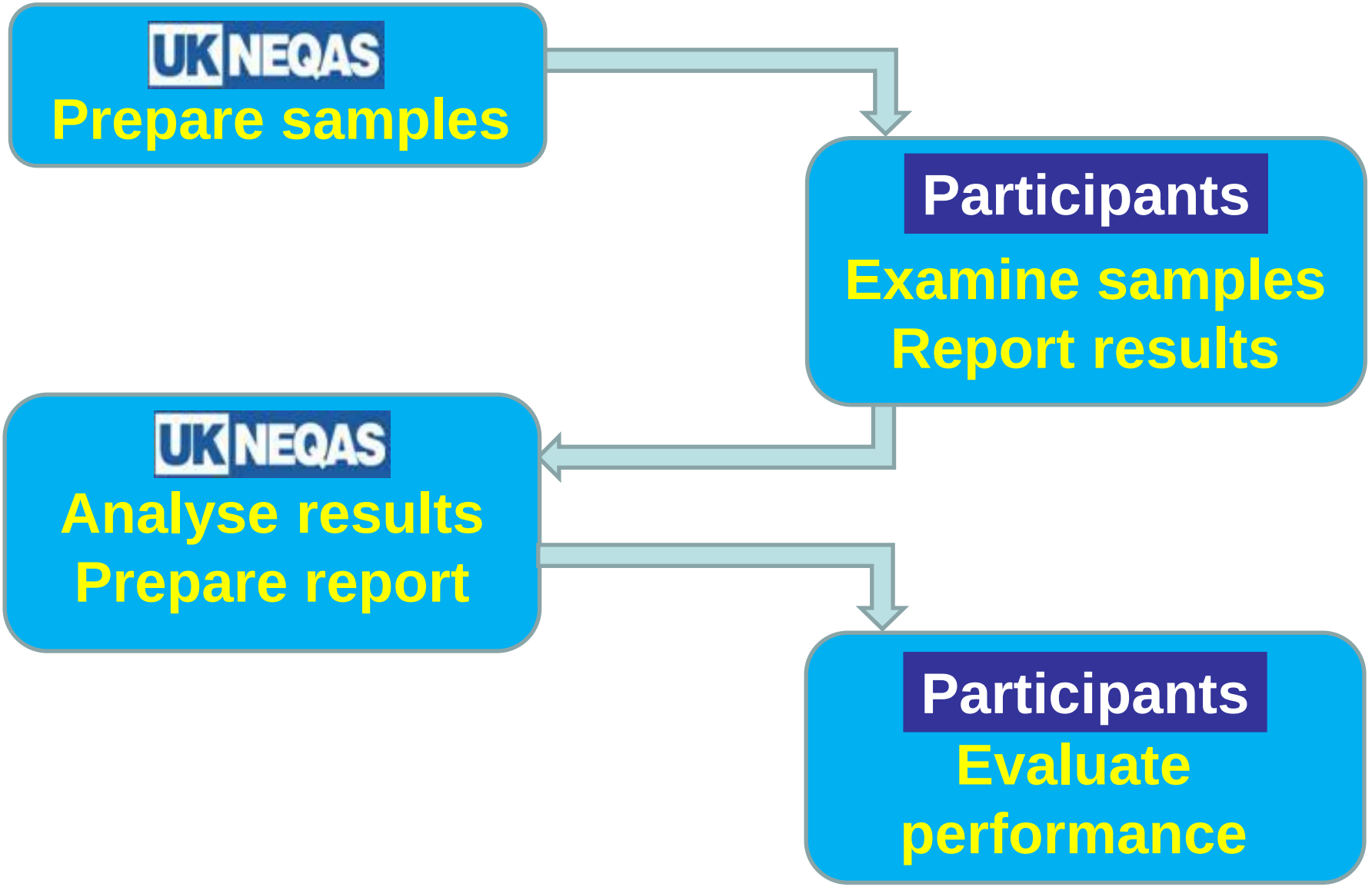


External Quality Assessment (EQA) (Proficiency testing)

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

The EQA process (UK NEQAS)



EQA report


UK NEQAS for Antimicrobial Susceptibility | Laboratory
Distribution : 1821 | Page 1 of 10
Dispatch date : 25-Oct-2004

Intended Result | Your Report | Your Score

Specimen 7240 *Staphylococcus aureus*

ciprofloxacin	susceptible	susceptible	2
clindamycin	susceptible	susceptible	2
erythromycin	susceptible	susceptible	2
cefoxitin	resistant	resistant	Not scored
fusidic acid	susceptible	susceptible	2
gentamicin	susceptible	susceptible	2
linezolid	susceptible	susceptible	2
mupirocin	susceptible	susceptible	2
oxacillin	resistant	resistant	2
penicillin	resistant	resistant	2
rifampicin	susceptible	susceptible	2
teicoplanin	susceptible	susceptible	2
tetracycline	susceptible	susceptible	2
vancomycin	susceptible	susceptible	2

Specimen 7241 *Escherichia coli*

ampicillin	resistant	resistant	2
ceftazidime	resistant	resistant	2
ciprofloxacin	susceptible	susceptible	2
cefotaxime	resistant	resistant	2
cefoxitin	resistant	resistant	2
ESBL	positive	positive	Not scored
gentamicin	susceptible	susceptible	2
meropenem	susceptible	susceptible	2
piperacillin/tazobactam	susceptible	susceptible	Not scored

Cumulative score information

Total number of specimens sent to you for UK NEQAS for Antimicrobial Susceptibility over the last 6 distributions was 12
Specimen numbers 7047 7048 7050 7085 7149 7150 7156 7157 7172 7175 7240 7241 have been analysed and scored

Your cumulative score for the specimen/test combinations that you reported was 208 out of a possible 208
The mean score for laboratories using the specimen/test combinations you examined was 198.76
with a standard error of 4.75
Your performance rating for UK NEQAS for Antimicrobial Susceptibility (i.e. the number of standard errors by which your cumulative score lies above or below the mean for UK laboratories) is 1.94

Cumulative scores may change if participants' results are amended.

Comments

The intended result for cefoxitin (specimen 7240) is based on the concensus result as this was not tested in the reference laboratories.

Please note where there is a difference in interpreted result between BSAC and NCCLS guidelines the organism/agent is not being scored. Additional tests used by laboratories for confirmation e.g. cefoxitin screen disc are not scored.

Users of the Stokes method are included in the 'not specified' group under guideline followed.

Enquiries
Repeat specimens can be obtained by fax +44 (0)20 8205 1488 or email organism@ukneqasmicro.org.uk
Please state your laboratory number, distribution number and type, and specimen number/s
Any technical enquiries related to this distribution, please contact Christine Walton using the email address above.

Digital images of the results obtained in QAL with this distribution are available on our website: www.ukneqasmicro.org.uk

Quality Assurance Laboratory
Health Protection Agency Centre for Infections
61 Colindale Avenue
London NW9 5HT
Phone ++ 44 (0)20 8905 9890 Fax +44 (0)20 8205 1488

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Participants must consult the scheme organiser before quoting data from the scheme.

Published at 07:58 on Tuesday 19 January 2005

- 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

EQA report


UK NEQAS for Antimicrobial Susceptibility | Laboratory
Distribution : 1821 | Page 1 of 10
Dispatch date : 25-Oct-2004

Intended Result | Your Report | Your Score

Specimen 7240 — *Staphylococcus aureus*

	Intended Result	Your Report	Your Score
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clindamycin	susceptible	susceptible	2
erythromycin	susceptible	susceptible	2
cefoxitin	resistant	resistant	Not scored
fusidic acid	susceptible	susceptible	2
gentamicin	susceptible	susceptible	2
linezolid	susceptible	susceptible	2
mupirocin	susceptible	susceptible	2
oxacillin	resistant	resistant	2
penicillin	resistant	resistant	2
rifampicin	susceptible	susceptible	2
teicoplanin	susceptible	susceptible	2
tetracycline	susceptible	susceptible	2
vancomycin	susceptible	susceptible	2

Specimen 7241 — *Escherichia coli*

ampicillin	resistant	resistant	2
ceftazidime	resistant	resistant	2
ciprofloxacin	susceptible	susceptible	2
cefotaxime	resistant	resistant	2
imipenem	susceptible	susceptible	2
ESBL	positive	positive	Not scored
gentamicin	susceptible	susceptible	2
meropenem	susceptible	susceptible	2
piperacillin/tazobactam	susceptible	susceptible	Not scored

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- Detailed results for laboratories using the same method as you
- Comments on particular problems

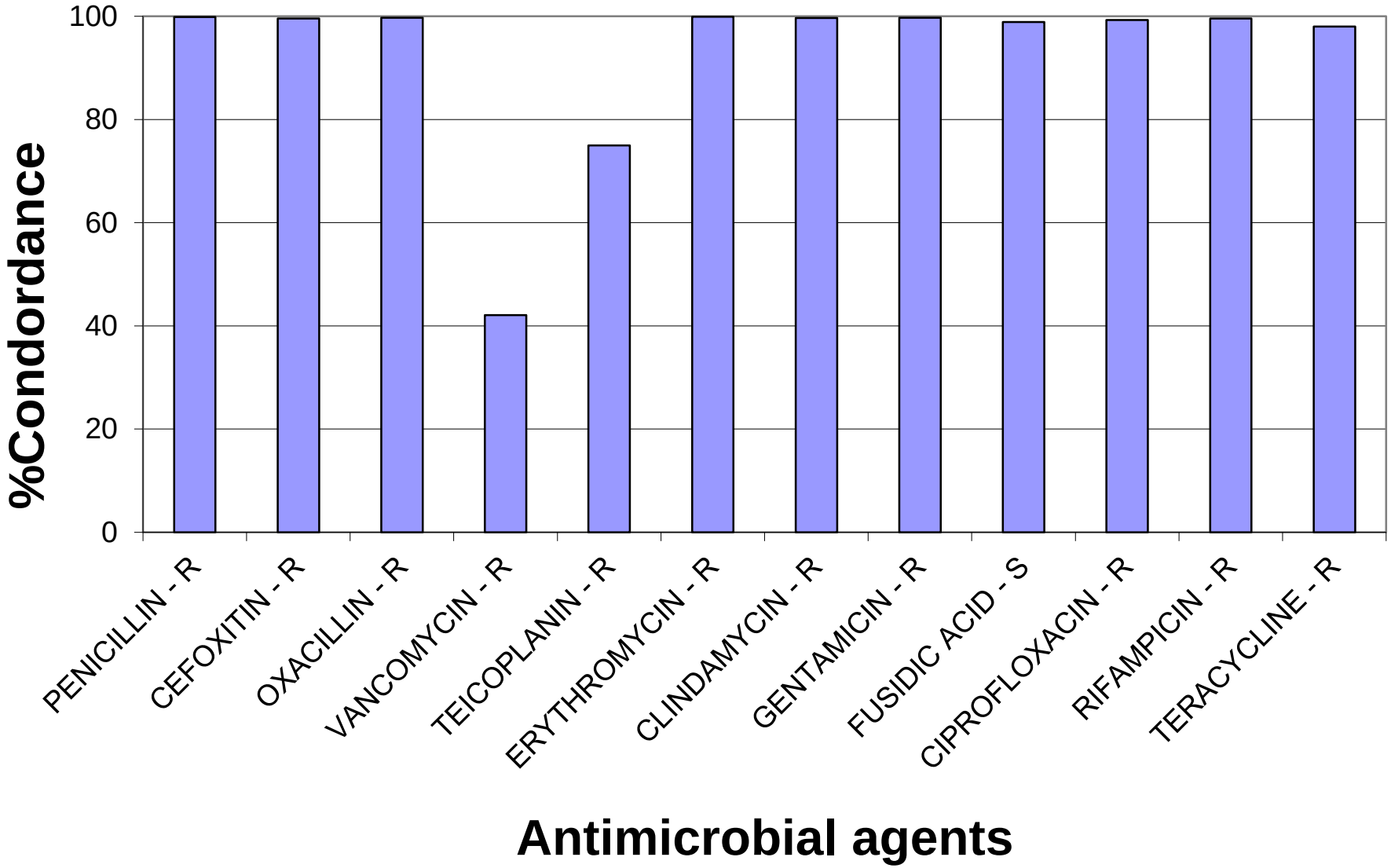
Evaluation

- 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?

Staphylococcus aureus
EARSNet specimen 2489 (VISA)

Antimicrobial agent	Reference MIC (mg/L)	EUCAST	CLSI
Penicillin	64	R	R
Cefoxitin	≥128	R	R
Oxacillin	≥128	R	R
Vancomycin	4	R	I
Teicoplanin	8-16	R	S/I
Erythromycin	≥128	R	R
Clindamycin	≥128	R	R
Gentamicin	128 – 256	R	R
Fusidic acid	0.06 - 0.12	S	-
Ciprofloxacin	16	R	R
Rifampicin	≥128	R	R
Tetracycline	64	R	R

Staphylococcus aureus specimen 2489



Staphylococcus aureus specimen 2489

Vancomycin resistance

MIC 4 mg/L

EUCAST resistant (breakpoints S ≤ 2, R > 2 mg/L)

CLSI intermediate (breakpoints S ≤ 2, R ≥ 16 mg/L)

- High error rate in reporting with all guidelines

Guideline	n	%S	%I	%R
EUCAST	655	45.8	2.4	51.8
CLSI	164	57.3	39.0	3.7

Staphylococcus aureus specimen 2489

Teicoplanin resistance

MIC 8-16 mg/L

EUCAST resistant (breakpoints S ≤ 2, R >2 mg/L)

CLSI susceptible/intermediate (breakpoints S ≤8, R ≥32 mg/L)

- Reporting in line with guidelines

Guideline	n	%S	%I	%R
EUCAST	573	7.2	1.0	91.8
CLSI	147	47.0	43.5	9.5

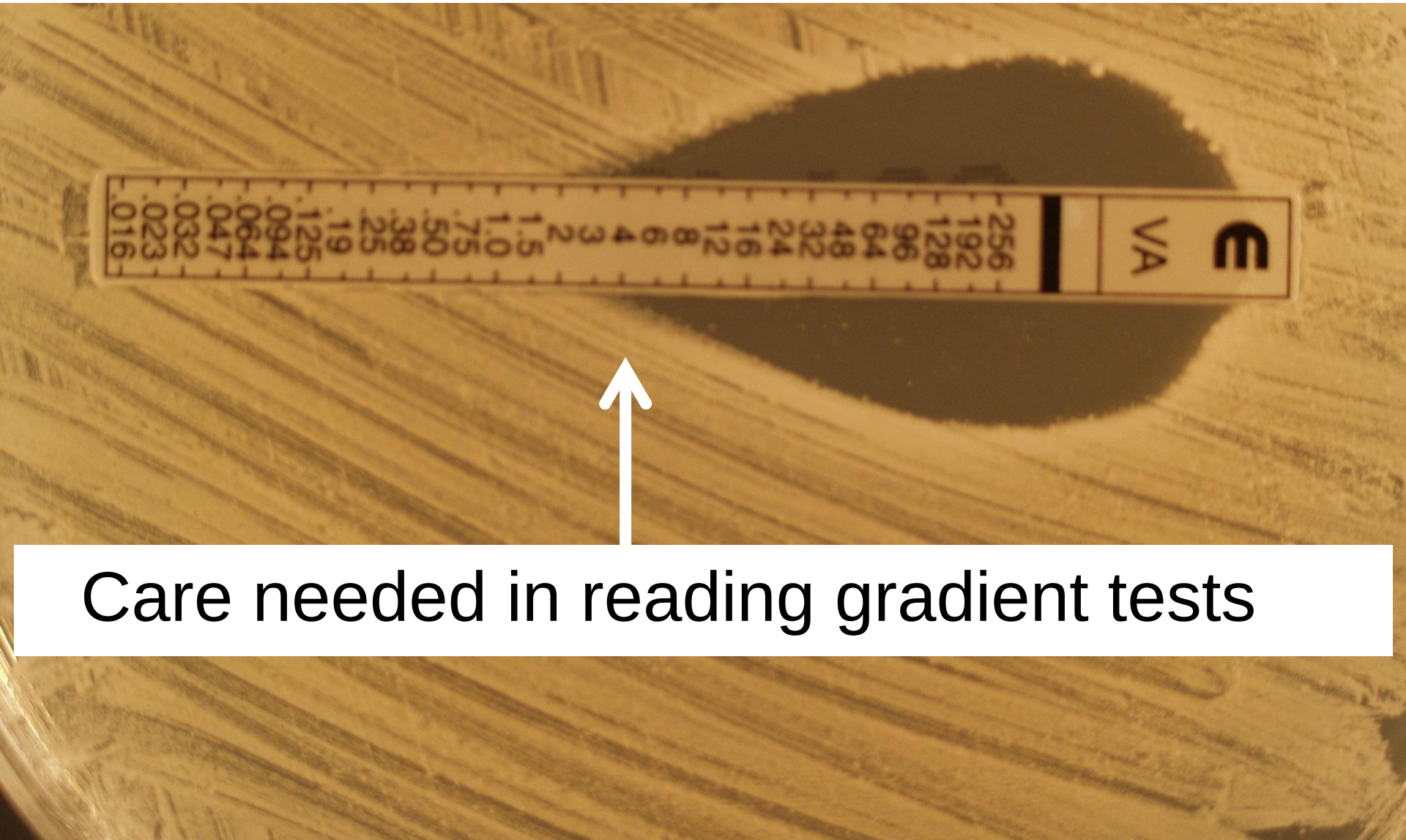
Staphylococcus aureus specimen 2489

Vancomycin resistance

- No testing method was reliable
- Disk diffusion should not be used

Method	n	% reporting S
MIC	370	41.4
Automated	430	50.5
Disk diffusion only	16	87.5

Staphylococcus aureus (VISA)
Gradient MIC test



Care needed in reading gradient tests

EQA conclusions

- Performance good for most organism-agent combinations
- Discrepancies more common when:
  - Susceptibility borderline
  - Critical differences between guidelines
  - Failure to follow guidelines
  - Specific technical issues
  - Guidelines permit variable reporting

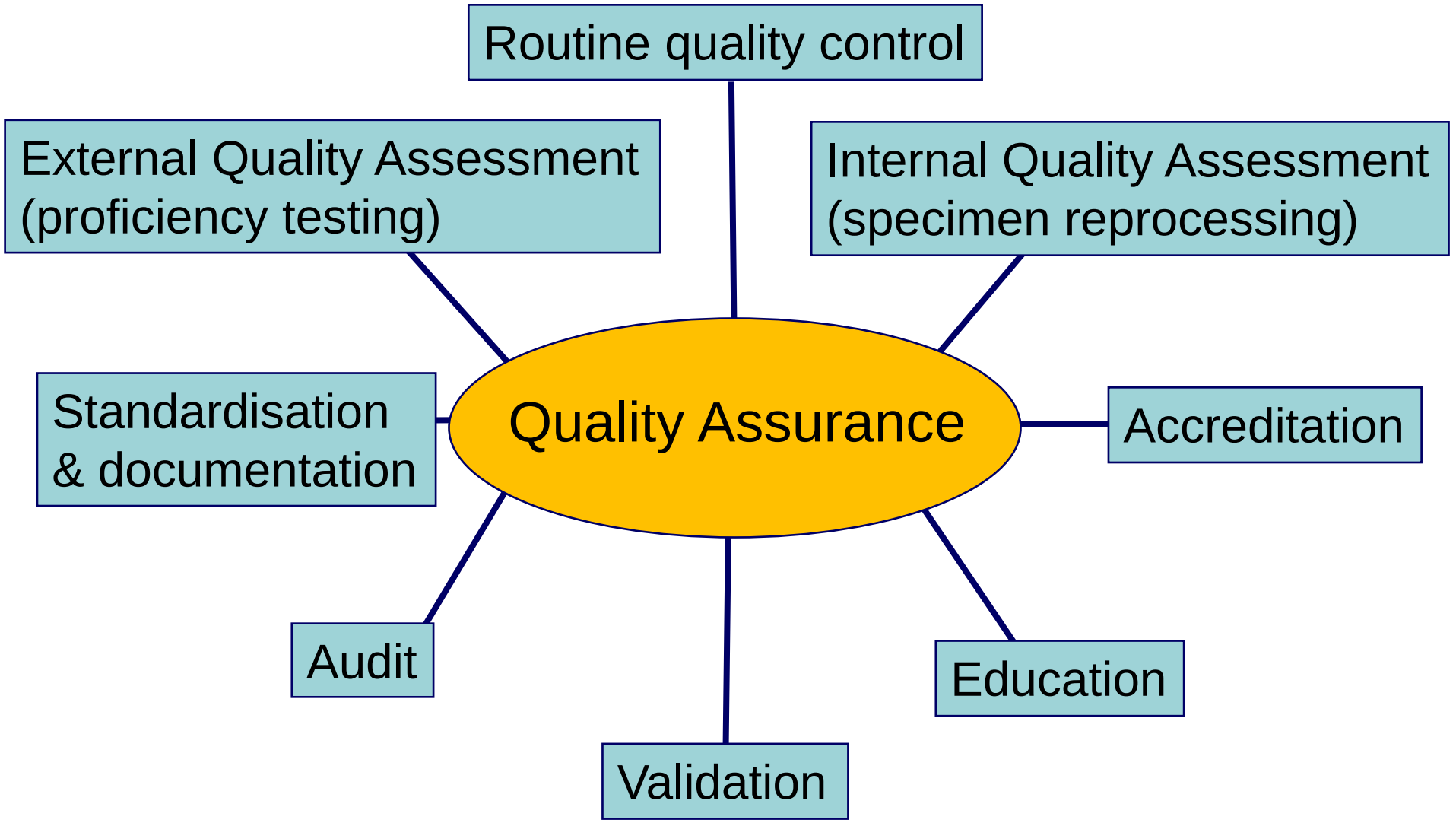
Benefits of EQA in antimicrobial susceptibility testing

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

Components of quality assurance



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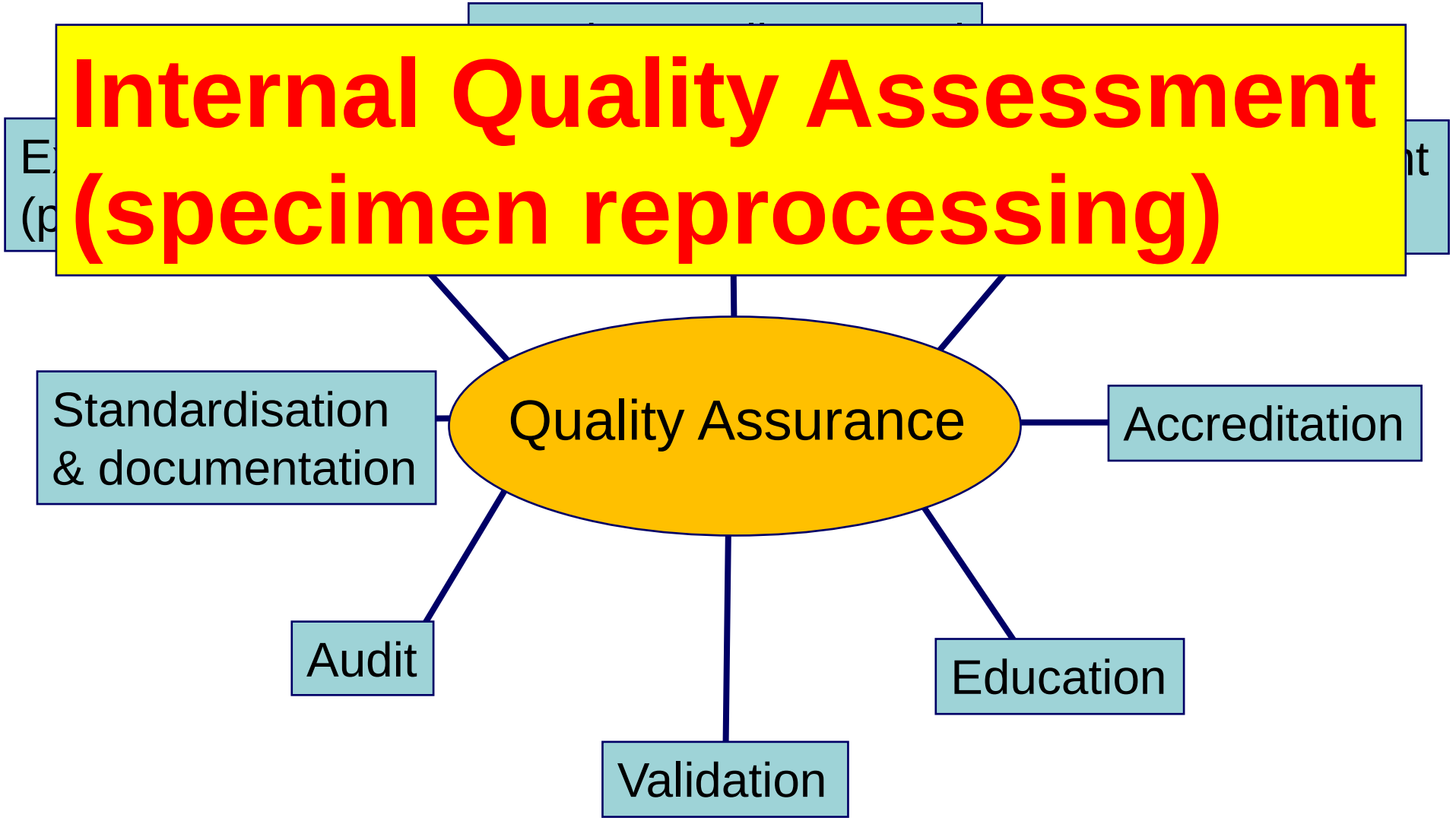

Internal Quality Assessment (specimen reprocessing)

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 the 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

Problems highlighted by IQA at Addenbrooke's Hospital, Cambridge

- 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

- Multiple components contribute to maintaining the quality of antimicrobial susceptibility testing

- Quality assurance is essential to ensure reliable results