Internal quality control

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EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

Internal quality control testing

- Tests which are routinely undertaken to monitor
  - the precision and accuracy of the test procedure,
  - the performance of reagents used in the test
  - the performance of the persons carrying out the tests.
- The basis of routine quality control is
  - inclusion of control strains to detect abnormal performance of the test,
- The internal quality control procedure should exactly mirror the performance of clinical susceptibility testing in the laboratory.

Use of QC strains are required for all susceptibility testing methods.

- Analysis of your data generated in your daily work

Characteristics of Quality control strains

- Stable during long-term storage.
  - Resistant strains are likely to be genetically less stable than susceptible strains and must be monitored for loss of resistance mechanisms.
    - Strains susceptible to antimicrobial agents are commonly used
    - Resistant strains are necessary
      - in tests for resistance mediated by inactivating enzymes
      - for effective control of breakpoint methods and automatic AST methods with often narrow dilution ranges.
- Grow well on the media used for susceptibility testing.
- Preferably gives clear zone edges in diffusion tests and/or sharp endpoints in MIC tests.
- Reproducible results when the test is performing correctly.

Culture collections

- 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, Sweden
- CECT. Colección Española de Cultivos Tipo. Universidad de Valencia. 46100. Burjassot. Valencia. Spain.

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
Campylobacter jejuni	ATCC 33560; NCTC 11351; CIP 702 DSM 4688; CCUG 11284	Susceptible, wild-type

Quality control strains

- EUCAST strains for detection of resistance mechanisms

Organism	Culture collection numbers	Characteristics
E. coli	ATCC 35218; NCTC 11954; CIP 102181; DSM 5564; CCUG 30600; CECT 943	TEM-1 β-lactamase producer
K. pneumoniae	ATCC 700603; NCTC 13368; CCUG 45421; CECT 7787	ESBL producer (SHV-18)
S. aureus	NCTC 12493	Oxacillin hetero-resistant, *mecA* positive
S. aureus	ATCC BAA-977	*ermA*-positive
E. faecalis	ATCC 51922; 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)

Maintenance of control strains

- Control strains are stored using
  - freezing at ≤70°C in small aliquots in 7-10% glycerol broth
    - glass beads or gelatin disks can be used
  - freeze drying

- NB: Long term storage on agar slopes is not desirable!
  - Risk of contamination and the least likely to preserve strain characteristics.

Working cultures

- **Working cultures** are made by fresh subcultures on appropriate (non-selective) media from long term storage
  - When subculturing from cultures stored at -70°C, do not allow the entire contents of the frozen vial to thaw! Remove a bead and return the remainder to the freezer without delay /
  - Alternatively - discard the tube
    - Repeated freezing and thawing may change the characteristics of the organism
- The **working culture**
  - is checked for purity and used for preparation of the inoculum for the QC test
  - is stored in the refrigerator
  - can be subcultured daily for 1 week and is then discarded
- A new working culture is made each week

Monitoring of QC results

- Each day that tests are set up, confirm that the results is within the acceptable range
  - If outside the QC range, examine the data for the last 20 consecutive tests.
    - A single control organism-antimicrobial combination out of range will not necessarily require action unless the result is far out of range or there is other indication of a problem
      - e.g. small control zone together with a higher frequency of resistant isolates than normal.
    - If two or more of 20 tests are out of range investigation is required

Results outside of QC range

- If two non-consecutive control values of 20 tests are outside the acceptable range
  - report susceptibility test results and investigate.
- If two consecutive values of 20 tests are outside the acceptable range
  - investigate before reporting susceptibility test results. The tests may have to be repeated.
- If multiple antibiotics (>2) are out of range on one day
  - investigate before reporting susceptibility test results. The tests may have to be repeated.
- If resistance in a resistant control strain is not recognised
  - suppress susceptibility test results, investigate and retest.

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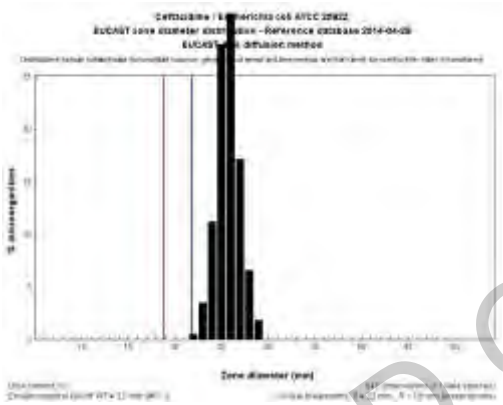
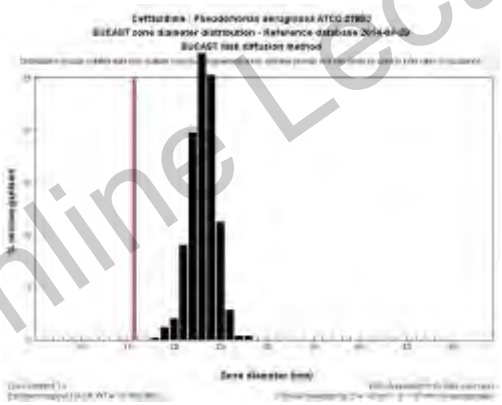
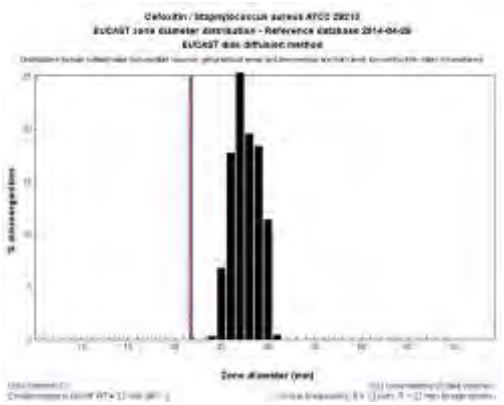
Monitoring of QC results

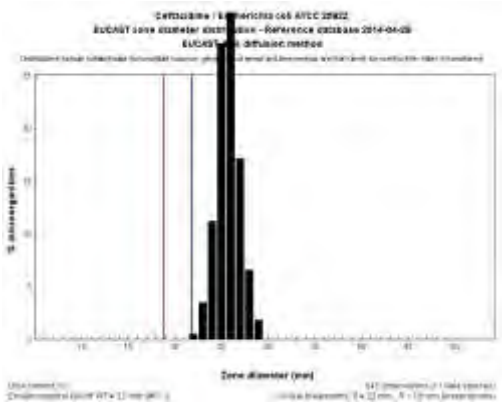
- Examine in-house zone diameter distributions and compare with EUCAST QC strain distributions on website.

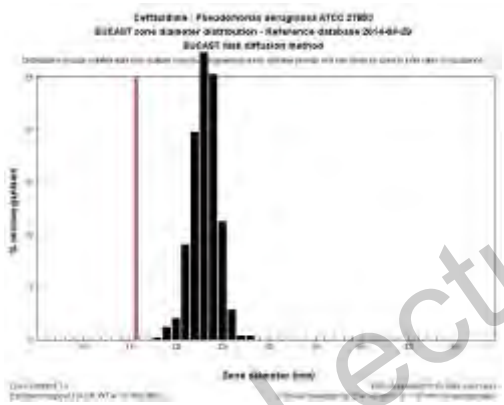


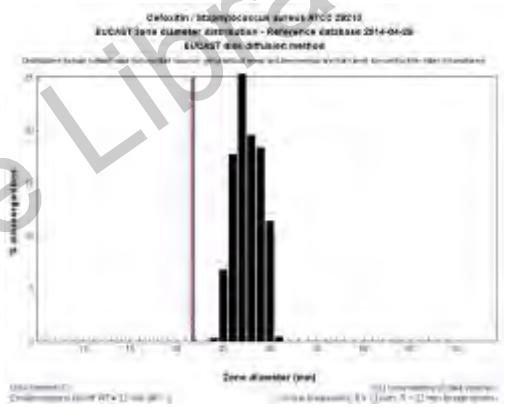




Monitoring of QC results







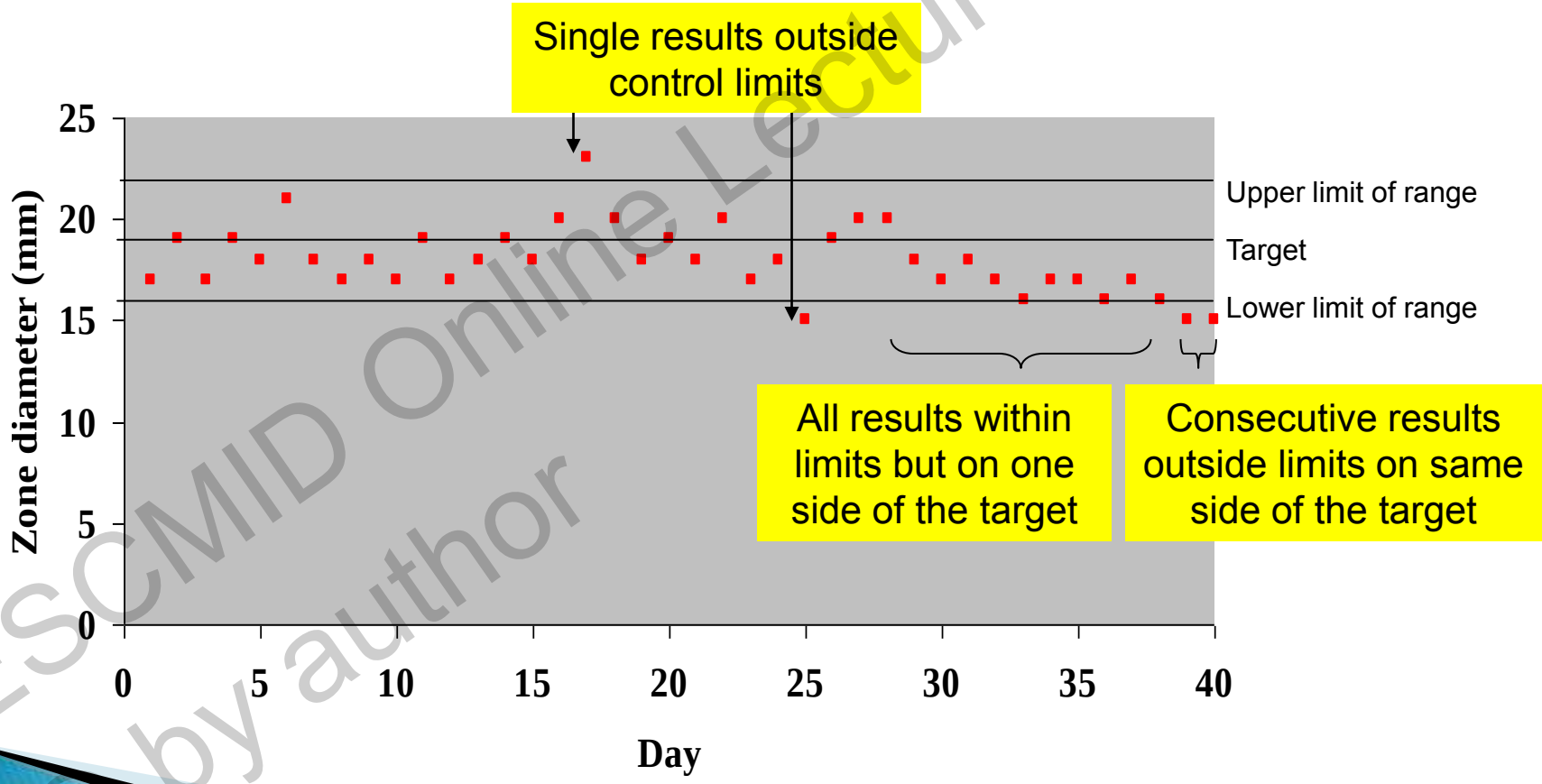
Acceptable performance

- In-house distribution is evenly Gauss-shaped
- The median is within +/-1 mm of the reference
- The width of the distribution corresponds to the EUCAST published distribution.

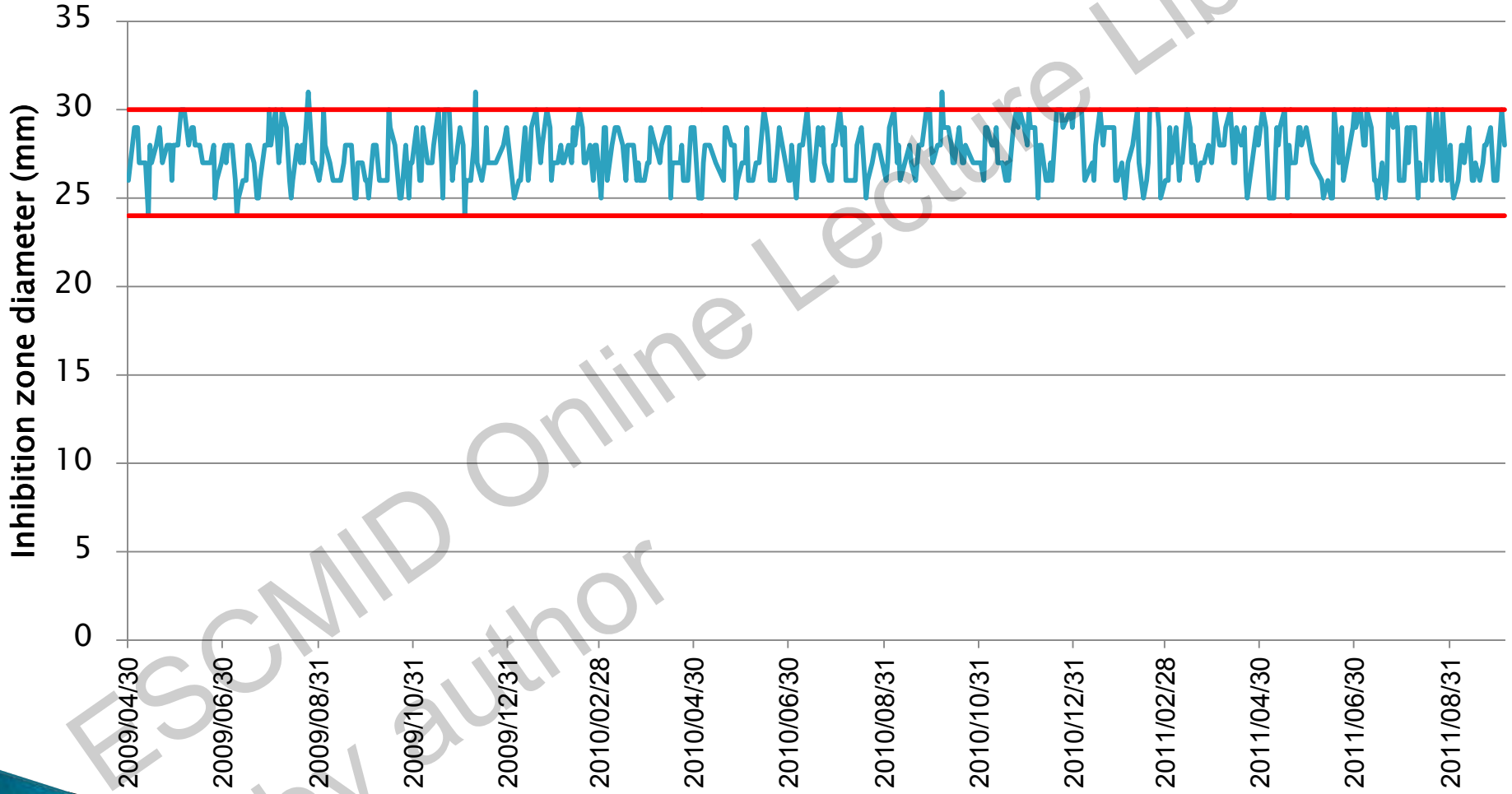
- If values are consistently either above or below the mean of the recommended target/range, there is a systematic error
  - methodology should be checked, including the depth of agar, time between inoculation and application of disks, time between application of disks and incubation, inoculum, incubation conditions.

Monitoring of test performance

- Examine results for trends in Shewart diagrams



S. aureus ATCC 29213 with cefoxitin 30 µg



Courtesy
E Matuschek, Växjö

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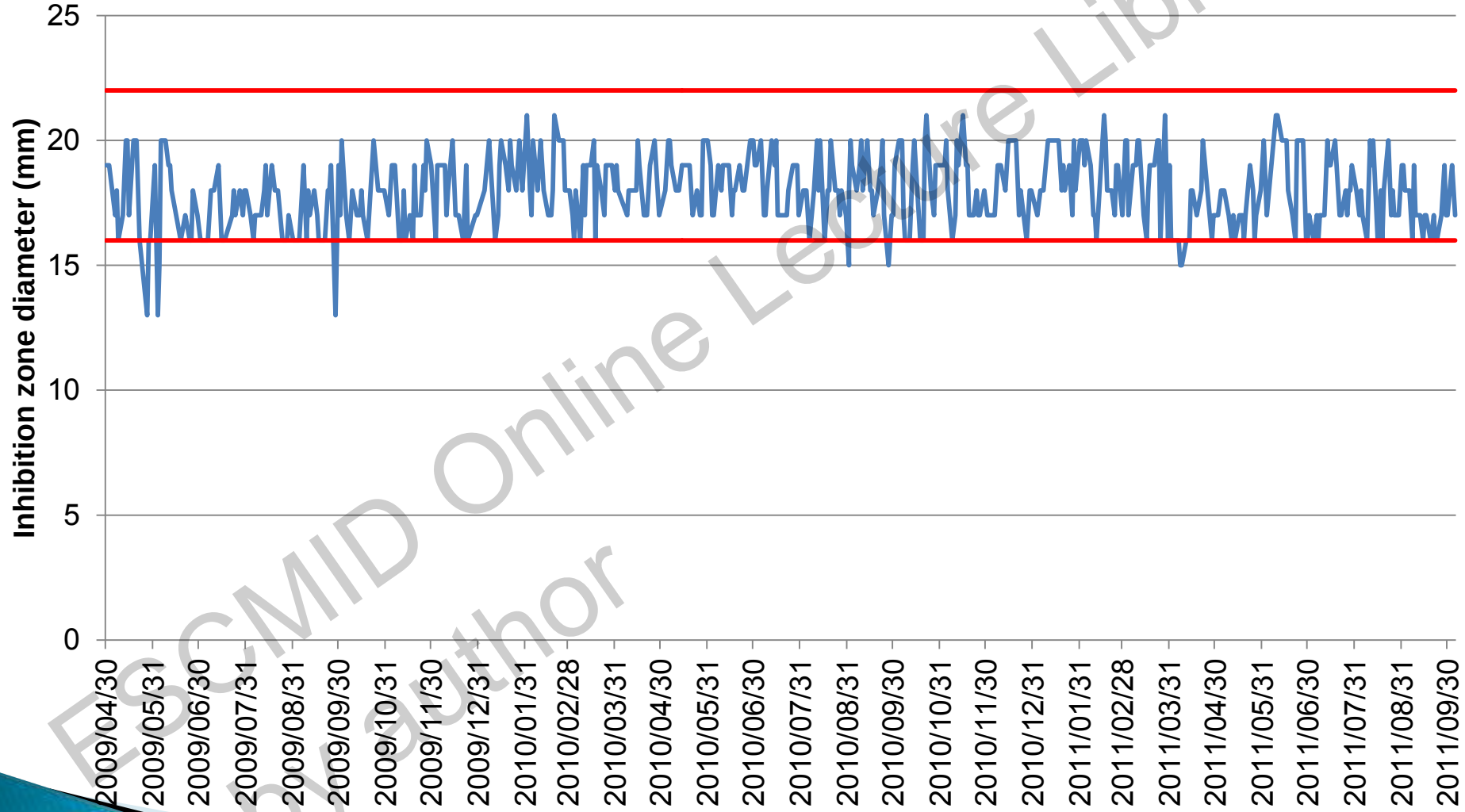
E. coli ATCC 25922 with ampicillin 10 µg



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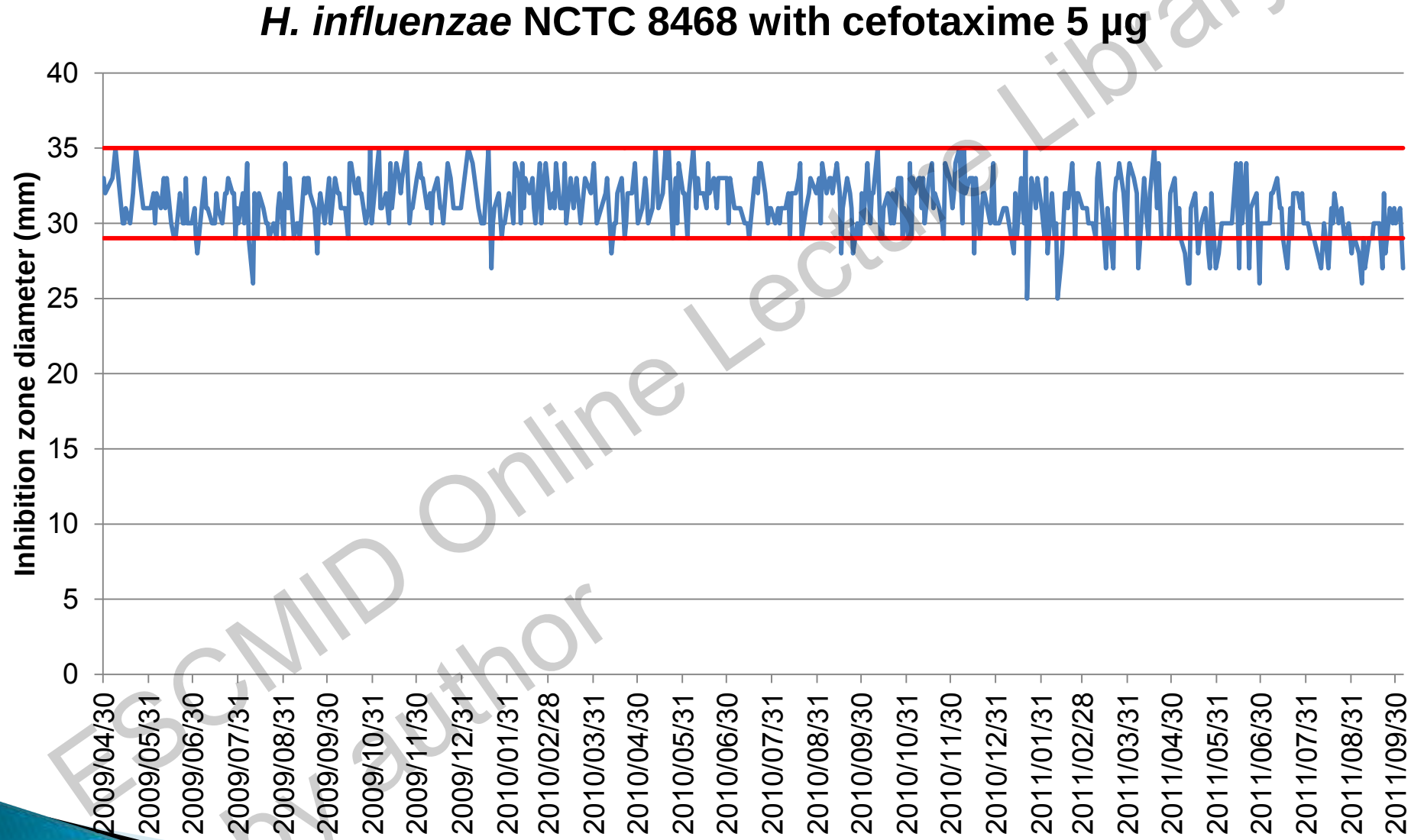

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Potential sources of error (1)

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 or expired)
	pH
	Agar depth/Agar volume
	Expiry date
Test conditions	“15-15-15”-rule not adhered to (suspension used within 15 min, disks applied within15 min, incubation within 15 min)
	Incubation (temperature, atmosphere and time)
	Incorrect inoculation (too light, too heavy or uneven)
	Reading conditions
	Reading zone edges

Potential sources of error (2)

Disks	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)
Control organisms	Incorrect QC strain
	Mutation
	Contamination
	Age of culture

Frequency Of QC tests

- EUCAST recommends that IQC is performed daily
- Include relevant QC strains as part of the everyday routine work.
  - For each lab department (Urine, Blood, Respiratory tract etc) choose one or two appropriate QC strains
  - Subject them to the standard panel of antibiotics used for that species in that department.
  - Start the day by registering zone diameters and before reading patient samples, sign off on the QC.

Frequency Of QC tests

- **Minimum criteria:**
  - When adequate performance is acceptable i.e. no more than 2 / 20 results are out of range, one can reduce frequency of testing to once weekly.
  - If the frequency of testing is reduced to once a week, any weekly control test out of range should result in corrective measures, including a return to daily testing if the reason for the error is not immediately obvious.
    - But the more frequent it is performed the more information is available!
- QC must always be performed when a new batch of media or reagents is used

QC of Media
and
breakpoint tests / any other methods with limited dilutions

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Quality control of media – MH agar

- Check that all Mueller-Hinton batches are within control limits for all bacteria-antimicrobial agent combinations.
  - Preferably include resistant isolates in addition to the standard QC strains (mostly fully susceptible)
  - Particular problems:
    - High or low concentrations of divalent cations may be indicated by inhibition zones for aminoglycosides with *P. aeruginosa* ATCC 27853 above/below quality control limits.
    - Excess thymidine may be indicated by inhibition zones for trimethoprim-sulfamethoxazole and *E. faecalis* ATCC 29212 below quality control limits.

Control of breakpoint test / any other methods with limited dilutions

- The one or two antibiotic concentrations tested in breakpoint method often differ considerably from the MICs of control strains
  - Hence the control strains used in the latter methods are of little value in breakpoint tests.
- Control strains for breakpoint tests should have MICs just above and just below each breakpoint,
  - but not so close to the breakpoint that the normal variation in the test results in day to day variation in results.
- Consequently a range of control strains will be necessary if several agents are being tested.
- A recommended set of strains with wide application is not yet available.

- Check the EUCAST website regularly for updates on methodology, QC ranges and breakpoints.

www.eucast.org

- Please send any comments and suggestions to the EUCAST AST Development Laboratory
  - erika.matuschek@ltkronoberg.se or to the EUCAST secretariat (see website).

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