Quality Assurance

when performing antimicrobial resistance testing

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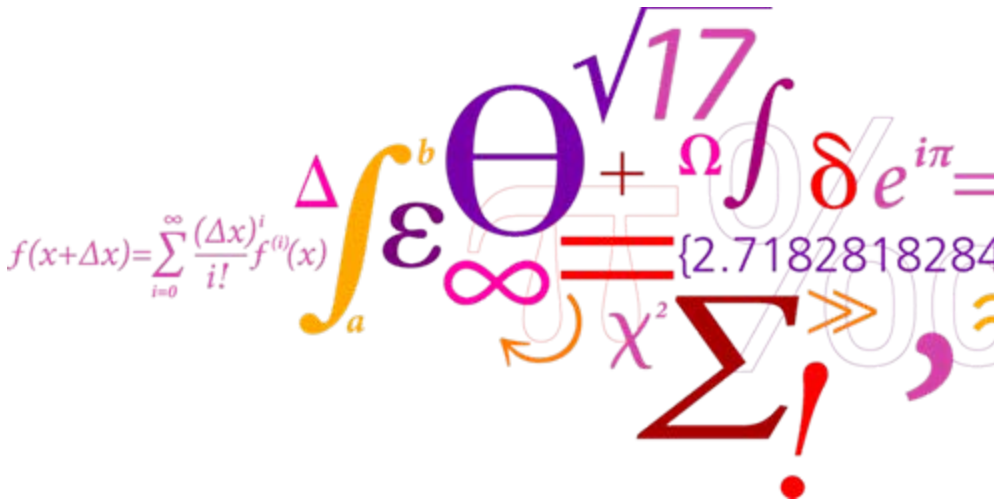

DTU Food
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Quality Management – definition

A quality management system can be defined as:

Coordinated activities to direct and control an organization with regard to quality

Definition is used by ISO and CLSI:
ISO (International Organization for Standardization)
CLSI (Clinical and Laboratory Standards Institute)

A quality management system includes all aspects of the laboratory operation, including:

- Organizational structure
- Processes
- Procedures

Quality Management – model

Quality management system model developed by CLSI (and fully compatible with ISO standards):

The 12 Quality System Essentials (QSE)

=>
Management commitment is crucial

Quality Assurance – intro

Laboratory quality can be defined as

- Accuracy
- Reliability
- Timeliness

Consequences of inaccurate results for clinical and public health settings:

- Unnecessary treatment; treatment complications;
- Failure to provide the proper treatment;
- Delay in correct diagnosis;
- Additional and unnecessary diagnostic testing;

Standardisation – our world

All AST methods are extremely sensitive to variations

Fact

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- Depth of the agar
- Dryness of the agar
- Growth rate of the bacteria

To obtain reliable and reproducible data => standardized methods and quality assurance are required

Guidelines

International standards describe the methods (DD and MIC) in detail: media, inoculum, incubation, etc.

- ISO (International Organization for Standardization)
- CLSI (Clinical and Laboratory Standards Institute)
- EUCAST (The European Committee on Antimicrobial Susceptibility Testing – www.eucast.org)

Quality Assurance – a bit of history

- In 1994, the decision to establish a QA system was taken
- In 1999, the first method was accreditated by DANAK according to EN/ISO 17045
- In 2002, ’MIC with Sensititre’ was accreditated
- Today, X methods in the area of microbiology are accreditated

Quality Assurance – at DTU Food

DS/EN ISO/IEC 17025

'Standard Operational Procedures' for everything

Institute: 'Head of QA' (The QA handbook)

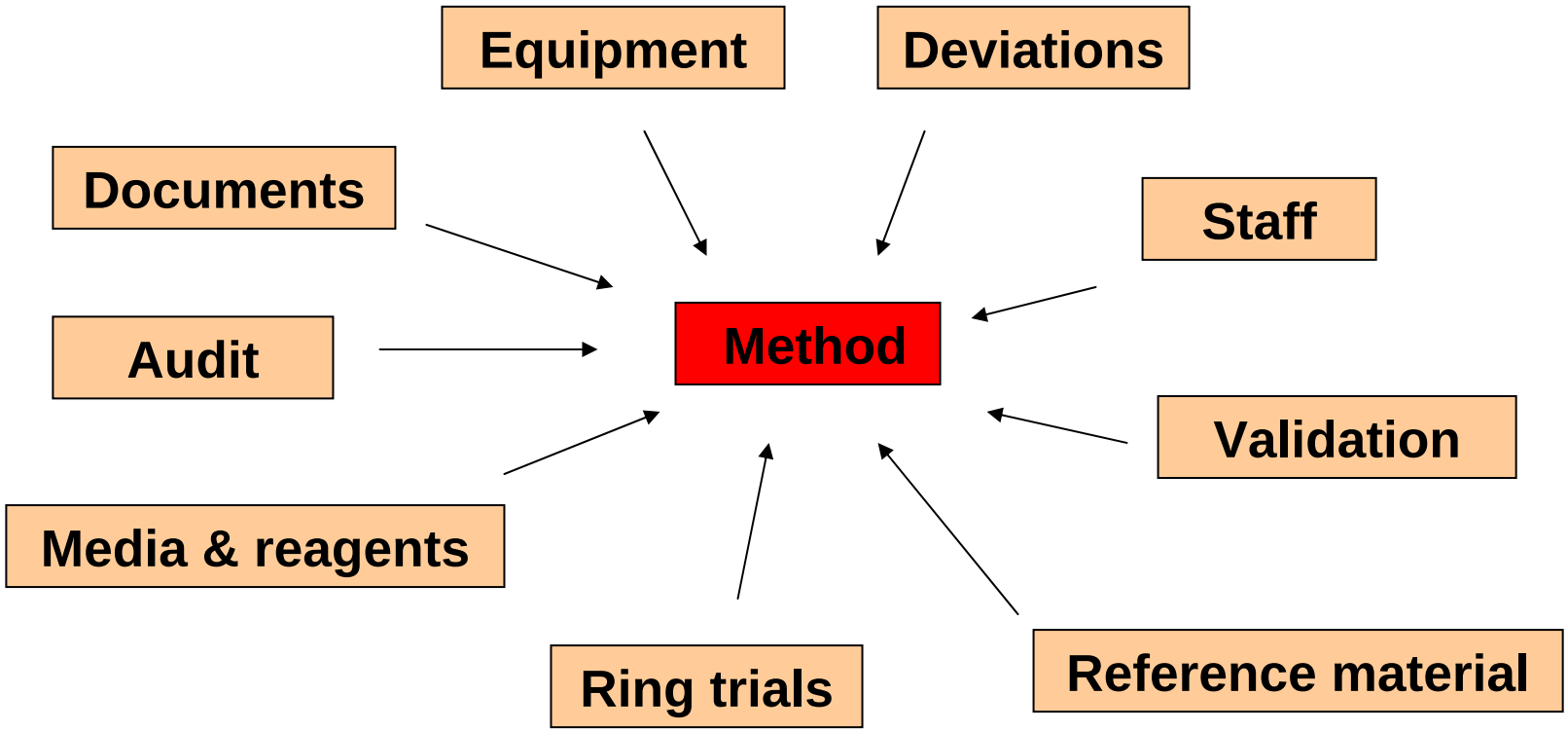
Department: 'Quality Co-ordinator' (dept. SOP collection)

Group: 'Quality Co-workers' – individual analysis

Quality Assurance – key words

- Describe what you are doing !
- Document that you did what you described !
- Keep traceability !
- Validate your method !
- Participate in ring trials !

Quality Assurance – overview



Quality Assurance – staff

- Training
- Documentation for the training
- CV and description of working areas
- Define areas of responsibility

Actually, the most important laboratory resource, is

competent, motivated staff!

Quality Assurance – documents

- Maintaining the documents (SOPs etc)
- Distribution of the documents (SOPs etc)
- Traceability (when was the SOP approved for use, etc.)

Quality Assurance – equipment

- Manual for use
- Test before use
- Procedures for maintaining and calibrating the equipment regularly
- Define acceptable deviations/deviation levels
- Software is also equipment!
- Traceability – 'who did what'
- Log-book (for use, repair etc.)
- And for incubators, freezers etc:
  - Daily check of the temperature (example)
  - Regular validation of the temperature over 24 hours and in different places inside the equipment

Quality Assurance – media and reagents

- Description of how to produce the media/reagent
- Procedure for testing each new batch (appendix 9)
- Accept criteria for each new batch
- Description of how to maintain and store media/reagent
- Traceability… again!
  - ’who did what’
  - which lot of media/reagent was used (appendix 6 and appendix 11)

Quality Assurance – ring trials

- Participate in relevant ring trials
- Define accept criteria for your performance in the ring trial
- Evaluate the obtained results
- Consider possible reasons for deviations
- Carry out corrective actions
- Document corrective actions
- Traceability all the way! – 'who did what'

Quality Assurance – deviations

- Procedures when deviations occur
- State if results are influenced by deviations
- Deviation reports with corrective actions stated
- Traceability all the way!

Example – appendix 3

If an atypical or rare resistance profile is observed, you may choose to:

- Check the reading
- Check purity control for contaminations and morphology
- Take material from well with high MIC and from control wells, check morphology next day before re-testing
- Verify ID before re-testing (microscope/API)
- Re-test (from top of 3-4 colonies)

Quality Assurance – validation and audit

- MIC = international standard = validation
- Need for validation depends on the method

Example – appendix 8

- Internal audit system – once a year
- External audit by DANAK – once a year

Quality Assurance – reference material I

Recommended reference material (CLSI)

Depending on the AST's performed in the lab:

- *E. coli* ATCC 25922
- *C. jejuni* ATCC 33560
- *E. faecalis* ATCC 29212 (relevant for revealing inappropriate antagonist content in the media – important when testing trimethoprim and sulphonamides)
- *Ps. aeruginosa* ATCC 27853 (relevant for revealing inappropriate cationic concentration in the media – if this is the case the MIC's for tetracycline and aminoglycosides will differ from the expected)
- QC ranges are not defined for all antimicrobials => in-house reference values may be used instead

Quality Assurance – reference material II

- See M100-S18 and M7-A7
- See 'subculture and maintenance of quality control strains' (http://crl-ar.eu/208-eqas.htm)
  - Do not use disc diffusion strains for MIC determination
  - Obtain QC strains from a reliable source such as ATCC
  - Perform QC either on the same day or weekly (weekly, if QC-testing in 30 days have not shown deviations)
  - Any changes in materials or procedure must be validated with QC before implemented
    - For example: Agar and broth methods may give different QC ranges for drugs such as glycopeptides, aminoglycosides and macrolides
  - Periodically perform colony counts to check the inoculum preparation procedure

Quality Assurance – inoculum check

According to ISO 20776-1:2006

- Inoculum should be adjusted to give a final cell number concentration of $5*10^5$ CFU/mL (range $2*10^5$ CFU/mL to $8*10^5$ CFU/mL)

Calculation example for micropanels containing dried antimicrobial:

- 4mL 0.9% NaCl adjusted to 0.5 McFarland => $1*10^8$ CFU/mL
- 50µL is added to 10mL broth (MH or MH+blood) => $5*10^5$ CFU/mL
- 50µL or 100µL is added to each well => $5*10^5$ CFU/mL

Quality Assurance - troubleshooting

If QC results are out of the acceptable range, consider:

- Switch of strains?
- Contamination?
- Growth weak or inoculation wrong?
- Incubation temperature and -time?
- New batches of broth or other media/reagents?
- Use of expired disks, broth, MIC-panel or other reagents?
- Etc.

Quality Assurance – deviations on QC strains

Ideally, test values should be in the middle of the acceptable range (see appendix 8)

When deviations on QC-strains show

- Re-testing should be performed (after sub-cultivation)
  - One dilution-step outside the acceptable QC range can be accepted, but….

    …if the same deviation appears over time or for other QC strains too, you should take action!
  - Graphing QC data points over time can help identify changes in data helpful for troubleshooting problems

Quality Assurance – QC at DTU Food

- Each new batch of broth
- Each new batch of MIC-panels
- Weekly QC in the lab with all the ATCC strains in use on the different panels (see appendix 8)
- Daily QC for *Campylobacter*

Sensititre System

- Commercially available microdilution MIC (microtitrewells) by Trek Diagnostics, UK
- Two fold dilution of dehydrated antimicrobials – just add the bacteria suspension
- The Sensititre system is standardized according to the CLSI and is validated by the FDA in the USA
- The system consists of:
  - MIC panels (pre-designed by Trek or custom designed)
  - nephelometer and MacFarland 0.5 standard
  - autoinoculator
  - tubes, dosing heads and broth
  - Sensitouch and software for reading the panels plus service visit once a year

Thanks to my colleagues,
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Rene S. Hendriksen

And **thanks** for your
attention!