# HADEA SERVICE CONTRACT 20197409

Provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in *Salmonella* species and *Campylobacter* species in human samples

FWD AMR·
RefLabCap

# INTRODUCTION TO QUALITY MANAGEMENT SYSTEMS AND QUALITY ASSURANCE

## AND INTERNATIONAL STANDARDS RELEVANT FOR PERFORMING ANTIMICROBIAL SUSCEPTIBILITY TESTING

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FWD AMR· RefLabCap

# Agenda items

1. International standards organisations
2. International standards relevant for performing AST
3. Quality management systems
4. Quality assurance when performing AST

# My background

- Food engineer
- >20 years experience with quality assurance (HACCP, BRC, ILAC, ISO)
- >15 years at National Food Institute
- Employed at EURL for antimicrobial resistance
- Coordinator of proficiency tests
- QA officer in the research group
- Internal auditor at the institute

Area:

- Microbiology, documents, technical stuff

# Agenda items

1. **International standards organisations**
2. International standards relevant for performing AST
3. Quality management systems
4. Quality assurance when performing AST

# Standardization bodies

International organizations include:

- ISO
- CEN
- WHO
- Codex

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- CLSI
- EUCAST

# International Organization for Standardization

International Organization for Standardization

- World's largest developer and publisher of international standards
- Standards are applicable to many kinds of organizations including clinical and public health laboratories
- Uses consensus process in developing standards

- Upcoming:

ISO for Whole Genome Sequencing

(ISO 23418 Microbiology of the food chain — Whole genome sequencing for typing and genomic characterization of foodborne bacteria — General requirements and guidance)


DS/EN ISO/IEC 17025:2017

INTERNATIONAL STANDARD

ISO/IEC 17025

Third edition
2017-11-30

General requirements for the competence of testing and calibration laboratories

*Exigences générales concernant la compétence des laboratoires d'étalonnages et d'essais*

# European Committee for Standardization

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

- Founded by the national standards bodies in the European Economic Community and associated countries => work together to develop European Standards in a large number of sectors to help build the European internal market in goods and services, removing barriers to trade and strengthening Europe's position in the global economy
- General terms include openness and transparency, consensus, and integration
- A standard is a technical document designed to be used as a rule, guideline or definition. It is a consensus-built, repeatable way of doing something

# World Health Organization


- has developed several standards for disease-specific diagnostic laboratories, such as polio, tuberculosis, influenza, measles

# Codex Alimentarius

CODEX ALIMENTARIUS
INTERNATIONAL FOOD STANDARDS

Food and Agriculture Organization of the United Nations
World Health Organization

- Several standards on foods

http://www.fao.org/fao-who-codexalimentarius/codex-texts/list-standards/en/

| Reference | RU |
|---|---|
| CODEX STAN | ✓ |
| CODEX STAN | ⊘ |
| CODEX STAN | ✓ |
| CODEX STAN | ⊘ |
| CODEX STAN | ⊘ |
| CODEX STAN | ✓ |
| CODEX STAN | ✓ |
| CODEX STAN | ⊘ |
| CODEX STAN | ✓ |



**CODE OF PRACTICE TO MINIMIZE AND CONTAIN ANTIMICROBIAL RESISTANCE**

***CAC/RCP 61-2005***

# Self-developed standards

Many agencies, organizations, or regions develop
their own accreditation requirements (inspection criteria) rather than using internationally recognized standards.

- **Advantages:**
  - optimized for local use, recognized local strengths and weaknesses
  - can be developed in progressive steps
  - can lead to full international recognition
- **Weaknesses:**
  - may be narrow or biased
  - may not be recognized by other organizations

# Agenda items

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# Clinical and Laboratory Standards Institute

- Global, nonprofit, standards-developing organization
- Detailed; standards apply specifically to medical laboratories
- Promotes the development and use of voluntary consensus standards and guidelines
- Documents are developed by experts working on subcommittees or working groups (consensus process)

# EUCAST

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

- EUCAST is a standing committee jointly organized by ESCMID, ECDC and European national breakpoint committees
- Structure of consultations and decisions
- EUCAST subcommittees for specific issues or areas
- Harmonize breakpoints and technical aspects of phenotypic in vitro AST
- Presents standardized methods

# EUCAST

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

## MIC determination of non-fastidious and fastidious organisms

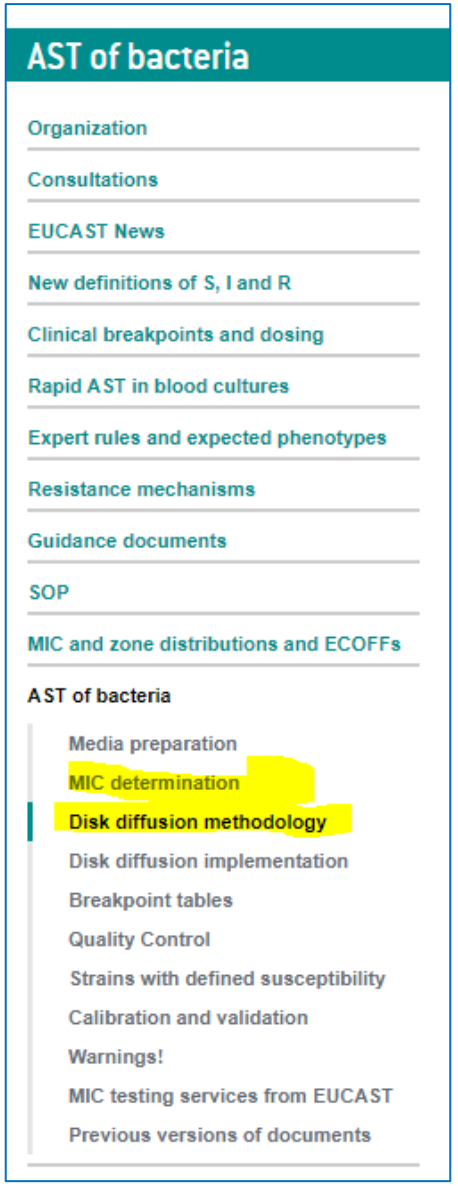
The EUCAST recommendations for MIC determination for non-fastidious organisms are in complete agreement with the recommendations from the International Standards Organisation (➔ ISO).

Reference to

**ISO 20776-1:2019**

**Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices — Part 1: Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases**

Snips from https://www.eucast.org/



# EUCAST

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

## EUCAST Disk Diffusion Test Meth

The EUCAST disk diffusion test is based on MH media
calibrated to EUCAST clinical breakpoints using broth
determination. Updates are published regularly.

See also EUCAST instruction videos.

- Disk diffusion - Manual v 10.0 (1 January, 2022)
- Disk diffusion - Slide show v 10.0 (1 January, 2022)
- Disk diffusion - Reading guide v 9.0 (1 January 202
- Anaerobic bacteria - disk diffusion methodology v 1
  recommendations.
  Disk diffusion breakpoints for anaerobic bacteria ar
  defibrinated horse blood as the only additive.
- Anaerobic bacteria - disk diffusion reading guide v 1
  Disk diffusion breakpoints for anaerobic bacteria ar
  defibrinated horse blood as the only additive.

For translations to other languages - contact National AST committees (NAC).

For previous versions of documents - see → Previous versions.

Snipped from the EUCAST DD method, version 10.0, January 2022

**1 Introduction**

Disk diffusion is one of the oldest approaches to antimicrobial susceptibility testing and remains one of the most widely used antimicrobial susceptibility testing methods in routine clinical laboratories. It is suitable for testing the majority of bacterial pathogens, including the more common fastidious bacteria, is versatile in the range of antimicrobial agents that can be tested and requires no special equipment.

In common with several other disk diffusion techniques, the EUCAST method is a standardised method based on the principles defined in the report of the International Collaborative Study of Antimicrobial Susceptibility Testing, 1972, and the experience of expert groups worldwide.

The zone diameter breakpoints in the EUCAST disk diffusion method are calibrated to the harmonised European MIC breakpoints that are published by EUCAST and are freely available from the EUCAST website (http://www.eucast.org).

As with all standardised methods, the described technique must be followed without modification in order to produce reliable results.

## AST of bacteria

- Organization
- Consultations
- EUCAST News
- New definitions of S, I and R
- Clinical breakpoints and dosing
- Rapid AST in blood cultures
- and expected phenotypes
- and ECOFFs
- ology
- entation
- usceptibility
- tion
- Warnings!
- MIC testing services from EUCAST
- Previous versions of documents

Snips from https://www.eucast.org/

# ISO and CLSI standards

**ISO 17025:2017** General requirements for the competence of testing and calibration laboratories

**ISO 15189:2012** Medical laboratories – Requirements for quality and competence

**ISO 20776-1:2019** Susceptibility testing of infectious agents…
… - Part 1: Broth micro-dilution reference method

**CLSI**

- M02-Ed13 – Performance standards for Disk Diffusion
- M07-Ed11 – Methods for Dilution AST
- M100-Ed32 – Performance standards for AST

# ISO and CLSI standards

**ISO 17025:2017** General requirements for the competence of testing and calibration laboratories

→ This European Standard was approved by CEN on 10 November 2017.

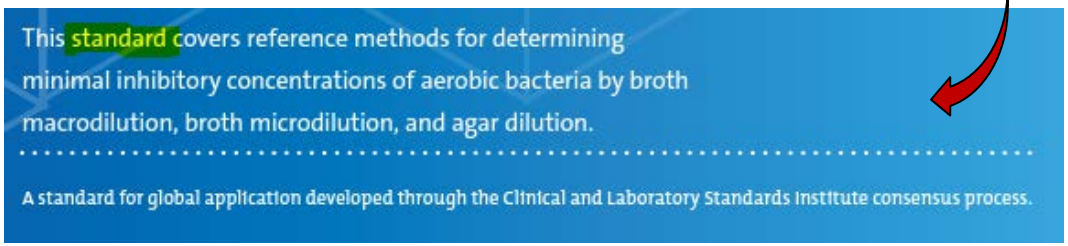
**ISO 15189:2012** Medical laboratories – Requirements for quality and competence → This European Standard was approved by CEN on 31 October 2012.

**ISO 20776-1:2019** Susceptibility testing of infectious agents…
… - Part 1: Broth micro-dilution reference method

→ This European Standard was approved by CEN on 22 April 2020.

**CLSI**

- M02-Ed13 – **Performance standards** for Disk Diffusion
- M07-Ed11 – Methods for Dilution AST → snip from front of doc
- M100-Ed32 – **Performance standards** for AST



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**CLSI**

- M02-Ed13 – **Performance standards** for Disk Diffusion
- M07-Ed11 – Methods for Dilution AST → snip from front of doc
- M100-Ed32 – **Performance standards** for AST

- QMS01-A5 – Quality Management System: A Model for Laboratory Services

# Concept of following standards

Adhere to the procedure described in the standard

If you have an accreditation: Your accreditation body will require validation reports for any introduced modification
– i.e. comparison of results from a representative set of analyses using both the unmodified standard and the modified standard

# Agenda items

1. International standards organisations
2. International standards relevant for performing AST
3. **Quality management systems**
4. Quality assurance when performing AST

# CLSI guideline

**QMS01-A5** Quality Management System: A Model for Laboratory Services

Snip from the document foreword:

## Foreword

Increased awareness of the costly personal and economic effects of medical errors has underscored the importance of managing quality in health care services. In the present environment of limited resources, quality cannot be taken for granted by those who fund, receive, or provide laboratory services. The historical perspective of quality control and quality assurance as defining quality needs to be superseded by a more comprehensive view of internationally accepted quality practices applied to a laboratory's entire scope of work.

This guideline is intended as a reliable, practical, and easily understood perspective that can be implemented in any laboratory.

QMS01 is a **guideline** that can help laboratories implement a QMS to achieve quality laboratory services and meet international standards and regulatory and accreditation requirements. **QMS01 is not a standard;** that is, this guideline **does not set requirements** for implementing a QMS. Rather, it **reorganizes existing requirements** for medical laboratories into a more understandable approach. It can be used along with other quality-related documents to design the foundation necessary to achieve an efficient, effective, and sustainable QMS.

**NOTE:**

QMS01 **is not a standard;** it simply reorganizes existing requirements in a more understandable way.

# Definition

A quality management system can be defined as:

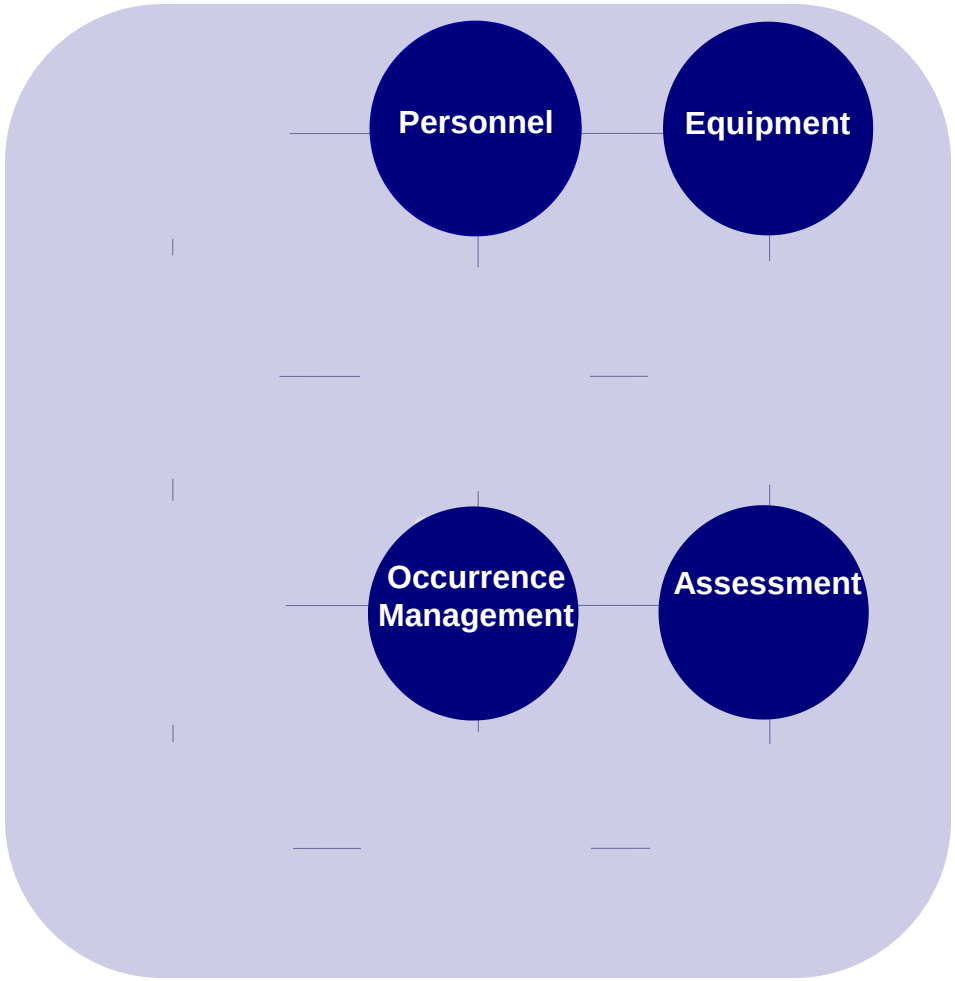
*Coordinated activities to direct and control an organization with regard to quality*

- ISO and CLSI defintion -


# Twelve Quality System Essentials

Quality management system model developed by CLSI (QMS01-A5)

(and is fully compatible with ISO standards)




# Twelve Quality System Essentials

Set of coordinated activities that function as building blocks for quality management

- Management commitment is crucial!

# To assure quality

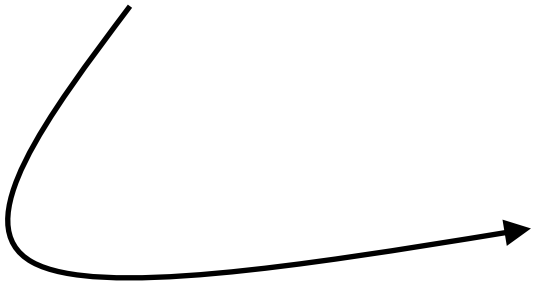
***Def.:*** *Coordinated activities to direct and control an organization with regard to quality*

- A quality management system includes all aspects of the laboratory operation, i.e.:
  - Organizational structure
  - Processes
  - Procedures

- The entire process of managing a sample must be considered:
  - sample collection
  - reporting and saving of results
  - all processes in between

# Standardization

Standardized and
quality assured methods



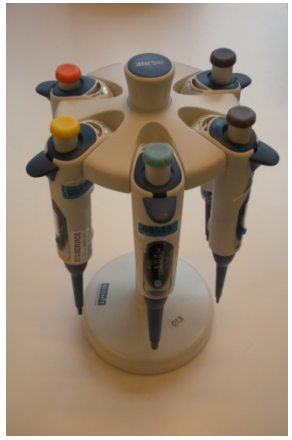
Reliable and
reproducible data

# Personnel

- human resources
- job qualifications
- job descriptions
- training
- competency assessment
- professional development
- continuing education
- documentation

# Equipment

- Monitor temperatures of:
  - incubators
  - refrigerators
  - freezers
- Perform function checks for:
  - pipettes
  - autoclaves
  - pH meter
  - weight and measures
- Management assures:
  - Responsibility assigned
  - Relevant training of personnel
- Maintain records



# Occurrence Management

- complaints
- mistakes and problems
- documentation
- trouble shooting
- root cause analysis
- immediate actions
- corrective actions
- preventive actions

# Laboratory Assessment

## Internal

Quality indicators

Audit program

Audit review

## External

Proficiency testing (EQA)

Inspections

Accreditations

# Agenda items

1. International standards organisations
2. International standards relevant for performing AST
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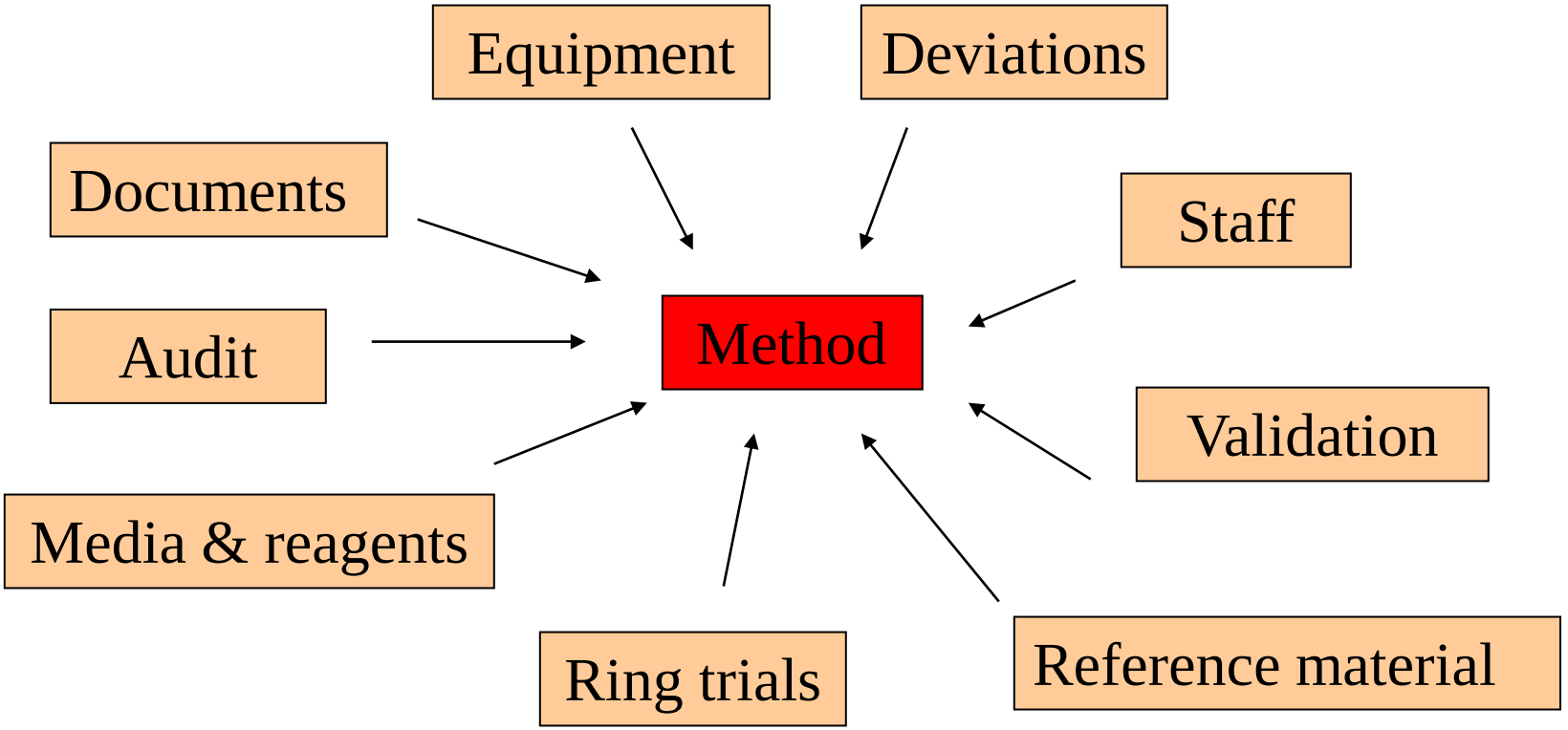
# **Standardization** – our world




All AST methods are extremely sensitive to variations

# Dilution methods

1. Two-fold dilutions of the antimicrobial →
2. Inoculate with the test bacterium → Incubate o/n →
3. Lowest concentration with no visible growth is the MIC-value

→ **MIC = Minimal Inhibitory Concentration (ug/ml or mg/L)**

Agar dilution (plate dilution)


Broth microdilution MIC

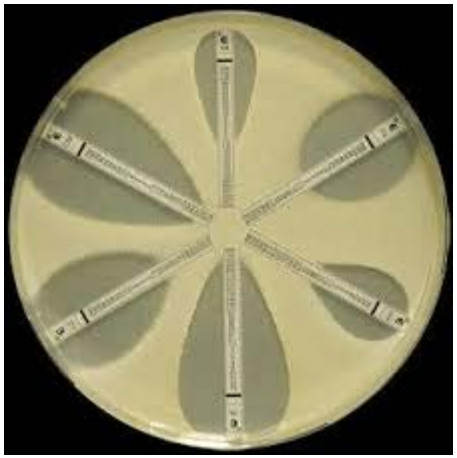

*Broth macrodilution (tubes) – 1 isolate per drug*

# Diffusion methods

1. An agarplate is inoculated with the testbacteria →
2. A paper disk or strip with antimicrobial is applied →
3. Incubation o/n (antimicrobial diffuses into the agar) →
4. The zone of growth inhibition is measured

Disk diffusion

Gradient strip test/E-test



*Note: Even if the gradient strip test provides the possibility to read an MIC-value, it is still a diffusion test….*

# **Standardization** – our world

All AST methods are extremely sensitive to variations

Factors that influence the result of AST:

- Size of inoculum
- Contents and acidity (pH) of the broth or agar
- Incubation time and temperature
- Reading procedures

Moreover, for the *diffusion methods*:

- Diffusion rate of the antimicrobial into the agar
- Depth of the agar
- Dryness of the agar
- Growth rate of the bacteria

Reliable and reproducible data: Standardized methods and daily/regular method control using QC strains tested in parallel AND participation in proficiency testing

# EUCAST standardized methods

Describes all steps in the method in detail

E.g.
- MH (*Salmonella*) or MH-F (*Campylobacter*) as agar/broth
- MH and MH-F acidity 7.2-7.4
- agar depth 4 +/- 0.5 mm

QC reference strains and acceptable QC ranges

Breakpoints for interpretation of the result for
- MIC-values
- Zone diameters

# EUCAST standardized methods

Snipped from the EUCAST DD method, version 10.0, January 2022

## 9 Quality control

9.1 Use the quality control (QC) strains specified in **Table 4** to monitor the pe
of the test. Principal recommended control strains are typical susceptible
resistant strains can also be used to confirm that the method will detect r
mediated by known resistance mechanisms (Extended QC, **Table 5**). QC
may be purchased from culture collections or from commercial sources.

9.1.1 To control the inhibitor component of β-lactam-inhibitor combinati
specific β-lactamase-producing strains are recommended (**Table**
should be part of the routine QC. The active component is checke
susceptible QC strain.

9.2 Store control strains under conditions that will maintain viability and orga
characteristics. Storage on beads at -70°C in glycerol broth (or commerc
equivalent) is a convenient method. Two vials of each control strain shou
stored, one as an in-use supply and the other as an archive.

9.3 Each week, subculture a bead from the in-use vial onto appropriate non-
media and check for purity. From this pure culture, prepare one subcultu
day of the week. For fastidious organisms that will not survive on plates f
subculture the strain serially from day to day. QC strains may be subcultu
maximum of six days, then discard plates and prepare a new purity plate
frozen in-use vial. When the in-use vial is depleted, subculture from the archive vial
and prepare another in-use vial from the subculture.

When subculturing a control strain, use several colonies to avoid selecting a mutant.

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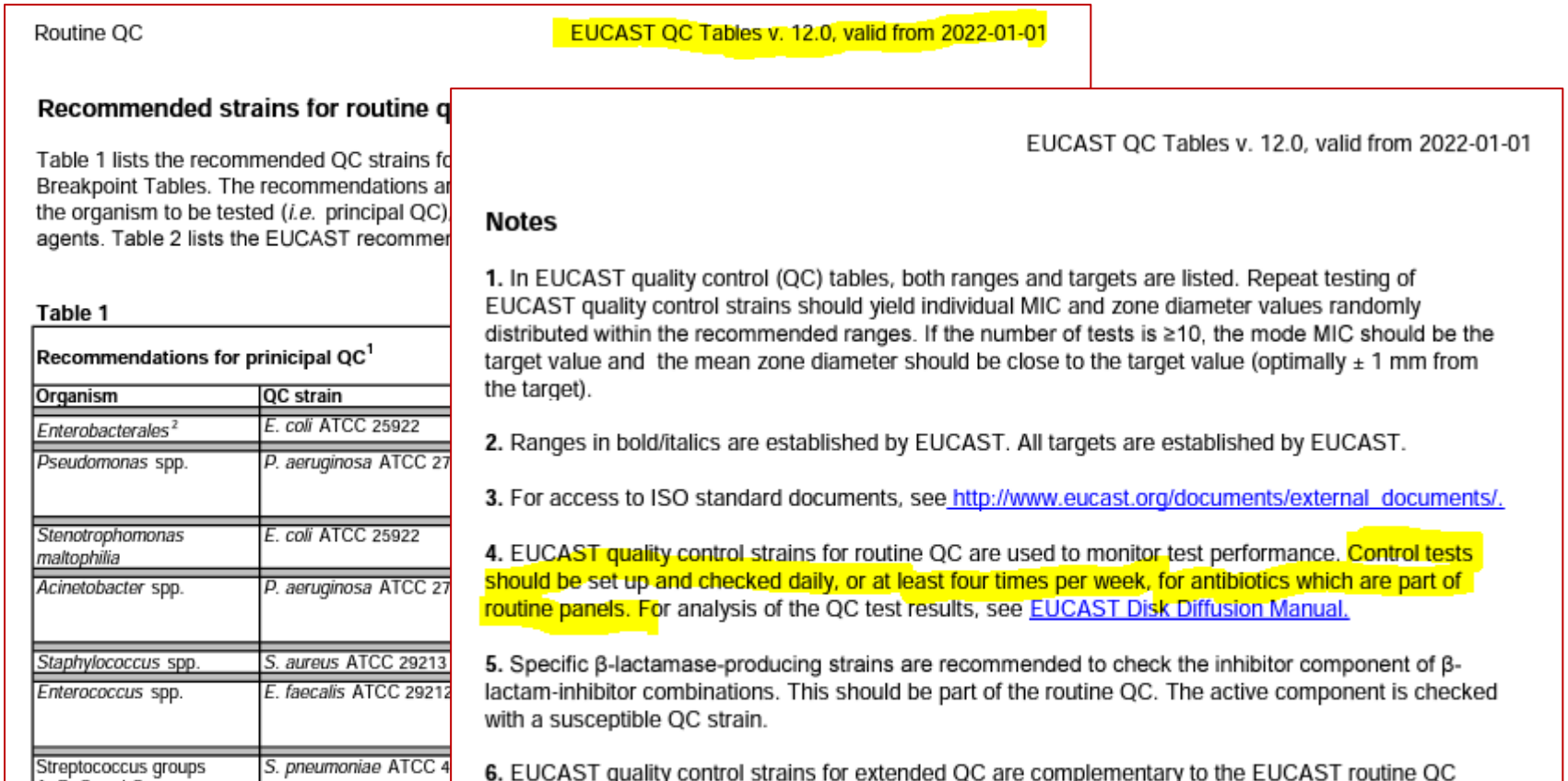
# Reading guide

## EUCAST disk diffusion method for antimicrobial susceptibility testing

Version 9.0
January 2022

# QC testing in the lab

- Each new lot of agar/broth
- Each new lot of MIC-panels/disks
- If acceptable ranges for QC strains are not defined, in-house reference values can be used



Routine QC

EUCAST QC Tables v. 12.0, valid from 2022-01-01

**Recommended strains for routine q**

Table 1 lists the recommended QC strains fo
Breakpoint Tables. The recommendations ar
the organism to be tested (*i.e.* principal QC)
agents. Table 2 lists the EUCAST recommer

**Table 1**

| Recommendations for prinicipal QC[1] | |
|---|---|
| **Organism** | **QC strain** |
| *Enterobacterales*[2] | *E. coli* ATCC 25922 |
| *Pseudomonas* spp. | *P. aeruginosa* ATCC 27 |
| *Stenotrophomonas maltophilia* | *E. coli* ATCC 25922 |
| *Acinetobacter* spp. | *P. aeruginosa* ATCC 27 |
| *Staphylococcus* spp. | *S. aureus* ATCC 29213 |
| *Enterococcus* spp. | *E. faecalis* ATCC 29212 |
| Streptococcus groups | *S. pneumoniae* ATCC 4 |

EUCAST QC Tables v. 12.0, valid from 2022-01-01

**Notes**

**1.** In EUCAST quality control (QC) tables, both ranges and targets are listed. Repeat testing of EUCAST quality control strains should yield individual MIC and zone diameter values randomly distributed within the recommended ranges. If the number of tests is ≥10, the mode MIC should be the target value and the mean zone diameter should be close to the target value (optimally ± 1 mm from the target).

**2.** Ranges in bold/italics are established by EUCAST. All targets are established by EUCAST.

**3.** For access to ISO standard documents, see http://www.eucast.org/documents/external_documents/.

**4.** EUCAST quality control strains for routine QC are used to monitor test performance. Control tests should be set up and checked daily, or at least four times per week, for antibiotics which are part of routine panels. For analysis of the QC test results, see EUCAST Disk Diffusion Manual.

**5.** Specific β-lactamase-producing strains are recommended to check the inhibitor component of β-lactam-inhibitor combinations. This should be part of the routine QC. The active component is checked with a susceptible QC strain.

**6.** EUCAST quality control strains for extended QC are complementary to the EUCAST routine QC

# Action on deviations on the QC strain

9.5 Use the recommended routine QC strains to monitor test performance. Use an overnight culture of the QC strain and follow the same testing procedure as for clinical isolates.

Control tests should b
antimicrobial agents w
and evaluated before

9.5.1 Each day that
tests. Examine
below the targ

9.5.2 If two non-con
susceptibility t
investigation is

9.5.3 If two consecutive tests are out of range or if multiple disks are out of range on one day, investigate before reporting susceptibility test results for clinical isolates. The tests may have to be repeated.

9.5.4 If resistance in a resistant control strain is not recognised, then suppress susceptibility test results for clinical isolates, investigate and retest.

9.5.5 When investigating for possible sources of errors in disk diffusion, consider problems related to antimicrobial disks, media, test conditions and quality control strains.

# Know your bugs

## Unexpected resistance or profiles

=> EUCAST expert rules

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European Society of Clinical Microbiology and Infectious Diseases

**EUCAST Expert Rules v 3.2** **Campylobacter** **June 2019**

| Rule No. | Organism(s) | Indicator Agent | Agents Affected | Rule | Remarks | Grade | References |
|---|---|---|---|---|---|---|---|
| **Macrolides, lincosamides and streptogramins** | | | | | | | |
| 1 | *Campylobacter* spp. | erythromycin | clarithromycin<br>azithromycin | IF susceptible to erythromycin THEN report clarithromycin and azithromycin susceptible.<br><br>IF resistant to erythromycin THEN report clarithromycin and azithromycin resistant (there are no separate breakpoints for these agents), | | C | |

=> Make a 'local list'?

# What went wrong?

- The organism was misidentified
- A typo/clerical error was made
- Switch of strains/samples
- The wrong test was ordered
- The sample was not preserved properly
- Contamination
- Weak growth
- Inoculation
- Incubation
- Media (new lot? expired?)
- Etc.

# QA in brief

- Describe what you are doing!
- Document that you did what you described!
- Ensure traceability!
- Use a standard method (or validate your method)!
- Participate in ring trials!

# Media and reagents

- Use only described media
- Procedure for testing each new lot
- Acceptance criteria for each new lot
- Describe how to maintain and store media/reagents
- Ensure traceability!
  - which lot of media/reagent was used

# Proficiency tests

- Participate in relevant ring trials
- Define acceptance criteria for your performance
- Evaluate the results
- Document
- Document any corrective actions
- Ensure traceability!
  - ”who did what”

# Validation and referencematerial

- Need for validation depends on the method used
- AST by MIC/DD – use of international standards => validation already done for you!
- Reference material for susceptibility testing: The QC reference strains (ATCC strains)
- Procedure for maintaining the QC referencestrains

# Deviations and audit

- Procedures when deviations appear
- State if reported results are influenced
- Deviation reports with corrective actions
- Internal audit system
- External audit
- Ensure traceability!

# With a quality management system

…laboratories are not *guaranteed* success by delivering 100% reliable results

It is only one step along the quality journey

Continual improvement is necessary!

# Summary

- Standards form the basis for quality practices. They are (typically) developed by organizations
- A method standard is a method standard. If modified, the standard was not followed
- AST methods are extremely sensitive to variations => ensure you follow the method description
  – and document to ensure traceability

Implementing Quality Management does not guarantee an *ERROR-FREE* Laboratory

But it detects errors that may occur and prevents them from recurring

**Laboratories *not* implementing a quality management system guarantees UNDETECTED ERRORS**

Thanks to Anne Mette Seyfarth for valuable input

Ref.: – and further information, see:
www.who.int/ihr/training/laboratory_quality/en/


# Thanks for your attention!