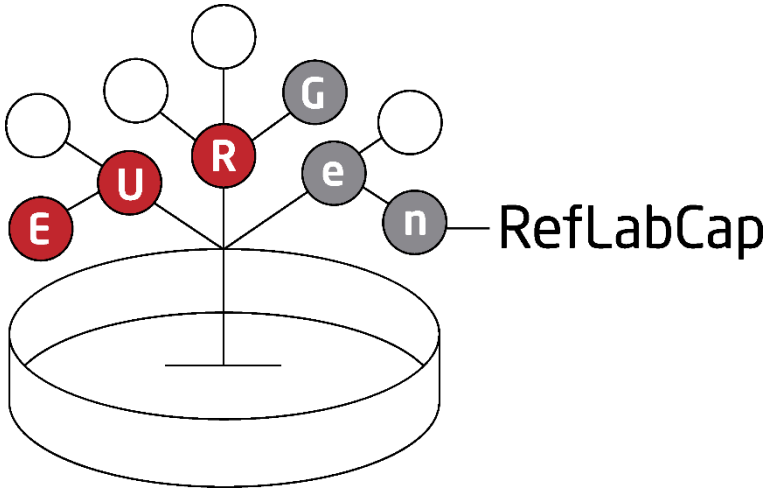
EURGen-RefLabCap Webinar

Guidance document on internal quality control schemes for clinical and reference laboratory antimicrobial susceptibility testing and molecular detection of antimicrobial resistance – WS2 pathogens



28 April 2023

14:00-15:00 CET

Ana Rita Rebelo (anrire@food.dtu.dk)

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Virtual Housekeeping

Please **turn off your cameras and microphones** unless you're speaking – this will help with bandwidth and maximise audibility.

Do frequently **use the chat function** to share your views, comments and challenges. Keep the chat constructive, respectful and on topic!

If you wish to make a comment for e.g. the discussion, please use the **'Raise hand'** function.

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Meeting agenda

1. Presentation of the guidance document

- Background and aim
- IQC in general
- IQC for AST

2. Discussion

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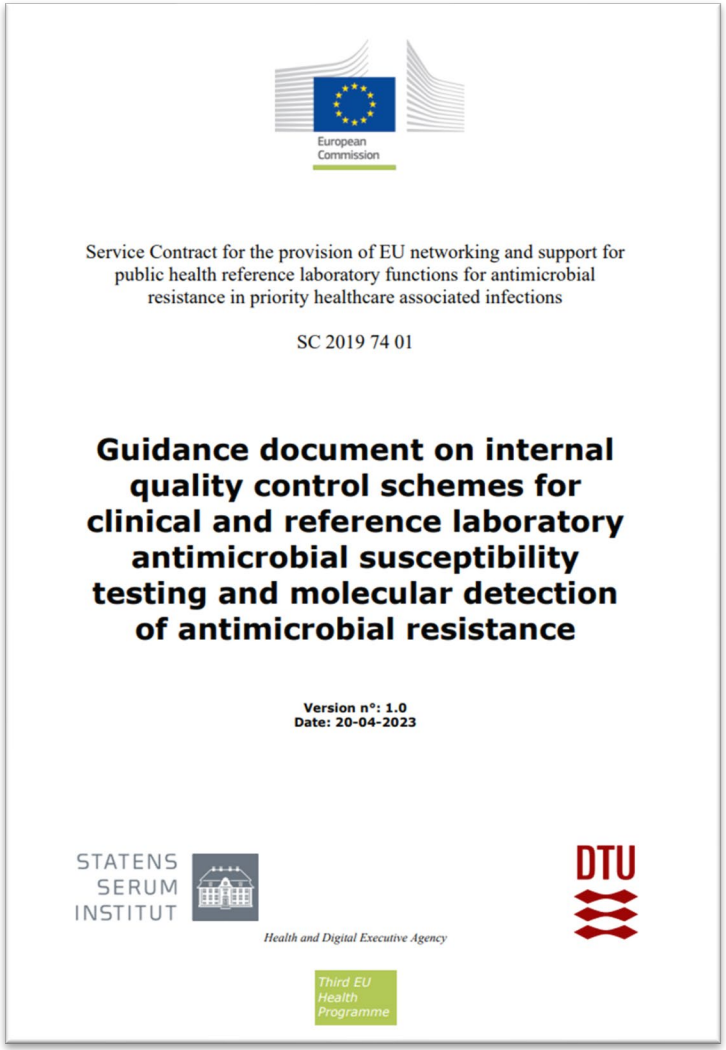
BACKGROUND AND AIM



European Commission

Service Contract for the provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in priority healthcare associated infections

SC 2019 74 01

Guidance document on internal quality control schemes for clinical and reference laboratory antimicrobial susceptibility testing and molecular detection of antimicrobial resistance

Version n°: 1.0
Date: 20-04-2023

STATENS SERUM INSTITUT

DTU

Health and Digital Executive Agency

Third EU Health Programme

Workstream 2 pathogens

Pseudomonas aeruginosa

Acinetobacter baumannii

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Background and aim

- Provide explanation and concrete examples of techniques for Internal Quality Control
- Collect the most recently available information from different regulatory agencies and other sources
- Aid the NRLs in optimizing their current methods and implementing molecular methods
- Allow the NRLs to easily provide guidance or training to local national laboratories
- Describe the standardized / recommended methods for antimicrobial susceptibility testing

***Reliable and accurate results for diagnostics and surveillance of* C/CRAb *and* C/CRPa**

Data that are comparable within Europe for surveillance purposes

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BACKGROUND AND AIM

TABLE OF CONTENTS

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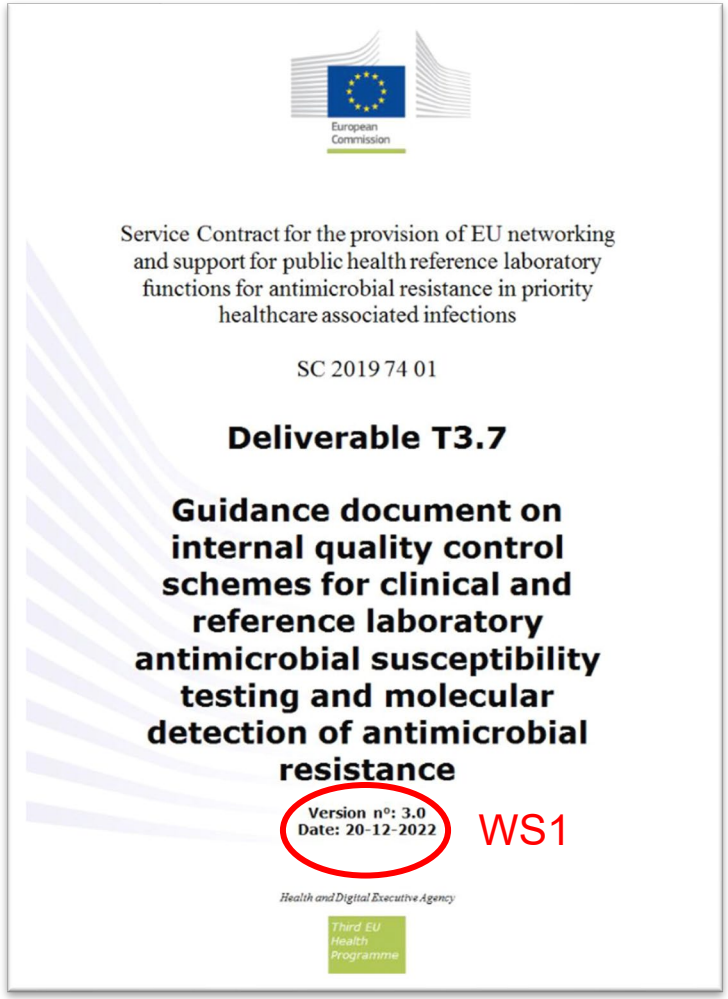
Background and aim


European Commission

Service Contract for the provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in priority healthcare associated infections

SC 2019 74 01

Deliverable T3.7

Guidance document on internal quality control schemes for clinical and reference laboratory antimicrobial susceptibility testing and molecular detection of antimicrobial resistance

Version n°: 3.0
Date: 20-12-2022

WS1

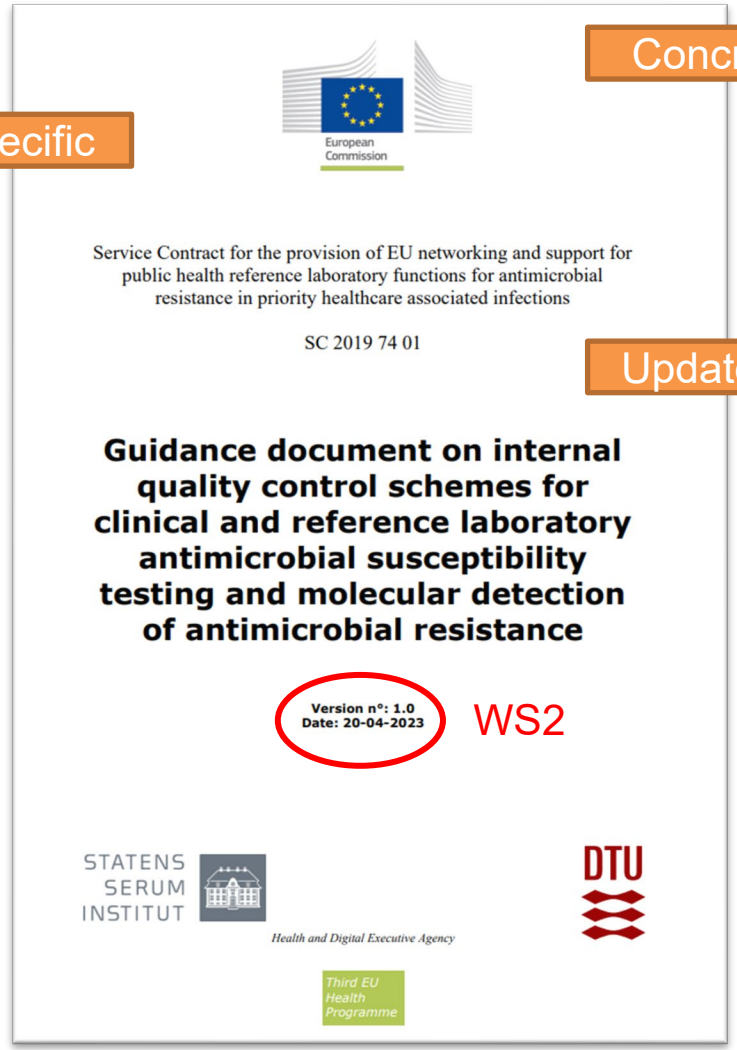
Health and Digital Executive Agency

Third EU Health Programme

Species specific

Concrete examples

Updated versions


European Commission

Service Contract for the provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in priority healthcare associated infections

SC 2019 74 01

Guidance document on internal quality control schemes for clinical and reference laboratory antimicrobial susceptibility testing and molecular detection of antimicrobial resistance

Version n°: 1.0
Date: 20-04-2023

WS2

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO standards

- ISO 15189
- ISO/IEC 17025

⇨

- Promote internal quality and competence
- Facilitate internal and external comparibility of results

EQA and accreditation

- External quality assessment exercises
- Strategy for accreditation

⇨

- Increase confidence regarding accuracy of results

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO 15189

ISO standards

- **ISO 15189**
- ISO/IEC 17025

EQA and accreditation

- External quality assessment exercises
- Strategy for accreditation

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO 15189

ISO 15189:2022

"Medical laboratories - Requirements for quality and competence"

Deep re-organization of the document compared with :2012 version

:2012	:2022
1. Scope 2. Normative references 3. Terms and definitions 4. Management requirements 5. Technical requirements	1. Scope 2. Normative references 3. Terms and definitions 4. General requirements 5. Structural and governance requirements 6. Ressource requirements 7. Process requirements 8. Management system requirements

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO 15189

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO 15189

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO 15189

Example: 6) Resource requirements; 6.5) Equipment calibration and metrological traceability

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO 15189

Example: 7) Process requirements; 7.3) Examination processes

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO 15189

Example: 8) Management system requirements; 8.4) Control of records

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO/IEC 17025

ISO standards

- ISO 15189 ✓
- **ISO/IEC 17025**

EQA and accreditation

- External quality assessment exercises
- Strategy for accreditation

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO/IEC 17025

ISO/IEC 17025:2017

"General requirements for the competence of testing and calibration laboratories"

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO/IEC 17025

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO/IEC 17025

Now more similar to ISO 15189:2022

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INTERNAL QUALITY CONTROL – IN GENERAL

EXTERNAL QUALITY ASSESSMENT EXERCISES

ISO standards

- ISO 15189 ✓
- ISO/IEC 17025 ✓

EQA and accreditation

- **External quality assessment exercises**
- Strategy for accreditation

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INTERNAL QUALITY CONTROL – IN GENERAL

EXTERNAL QUALITY ASSESSMENT EXERCISES

Examples:

EARS-Net EQA / UK NEQAS / ESfEQA / Labquality / Oneworld Accuracy

Recommended within the ISO standards

ISO 15189

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INTERNAL QUALITY CONTROL – IN GENERAL

STRATEGY FOR ACCREDITATION

ISO standards

- ISO 15189 ✓
- ISO/IEC 17025 ✓

EQA and accreditation

- External quality assessment exercises ✓
- **Strategy for accreditation**

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INTERNAL QUALITY CONTROL – IN GENERAL

STRATEGY FOR ACCREDITATION

International Laboratory Accreditation Cooperation (ILAC)

└ Specifically designated body - national institution

└ Evaluation and accreditation of reference/local laboratories

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO standards

- ISO 15189 ✓
- ISO/IEC 17025 ✓

EQA and accreditation

- External quality assessment exercises ✓
- Strategy for accreditation ✓

Internal quality control for AST

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INTERNAL QUALITY CONTROL – FOR AST

European guidance on AST methods:

European Committee on Antimicrobial Susceptibility Testing (EUCAST)

Recommendations:

- Broth microdilution or disk diffusion for AST
- Other methods (agar dilution / gradient strips) are not recommended due to lack of harmonisation and high variability
- Adhering to the recommended protocols for each method
- Regularly confirming warnings and new breakpoint tables
- Using the presentations for confirming reading procedures and special situations

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INTERNAL QUALITY CONTROL – FOR AST

Phenotypic antimicrobial susceptibility testing

- Broth microdilution
- Disk diffusion

Molecular detection of antimicrobial resistance

- PCR protocols
- Whole-genome sequencing

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – BROTH MICRODILUTION

Phenotypic antimicrobial susceptibility testing

- **Broth microdilution**
- Disk diffusion

Molecular detection of antimicrobial resistance

- PCR protocols
- Whole-genome sequencing

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – BROTH MICRODILUTION

Standard protocol – 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"*

EUCAST documents

- Clinical breakpoint tables
- Warnings page
- Visual guides (e.g. how to determine MIC endpoints)

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – BROTH MICRODILUTION

ISO 20776-1:2019

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – BROTH MICRODILUTION

ISO 20776-1:2019

- How to prepare stock and working solutions of antimicrobial agents, the broth medium and the microdilution trays
- Two methods for obtaining the bacterial inoculum: the broth culture method and the direct colony suspension method
  - Final concentration of 5 x 10^5 CFU/ml
- How to inoculate, incubate and read the minimum inhibitory concentrations (MIC) on the microdilution trays
- Lists of situations that require special attention, including the adjustment of medium composition or incubation conditions for certain bacterial species and for certain antimicrobials

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – BROTH MICRODILUTION

Examples of special situations (ISO 20776-1 + EUCAST)

- For *Acinetobacter* spp. and *Pseudomonas* spp.
  - Do not add surfactants to the medium when testing colistin
  - Adjust the zinc concentration of the broth medium for testing of carbapenems
  - Turbidity, even without a visible pellet, should be considered as bacterial growth
  - The breakpoint for colistin in ***P. aeruginosa***, from 2 mg/L (on v11.0 from January 2021) to 4 mg/L (on v12.0 from January 2022)
  - Testing of penicillins for ***A. baumannii*** yields unreliable results

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – BROTH MICRODILUTION

- Use of control strains

  - List from the Clinical Laboratory Standards Institute (CLSI) (available on the document CLSI M100 "Performance Standards for Antimicrobial Susceptibility Testing")

  - List from EUCAST (available on the document "Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST")

Always: *P. aeruginosa* ATCC 27853

Colistin: *mcr-1*-positive *E. coli* NCTC 13846

β-lactams+ β-lactamase inhibitors: *E. coli* ATCC 35218, others

Co-trimoxazole for *A. baumannii*: *E. coli* ATCC 25922

every day that AST is being performed

AND

every time a new batch or lot of materials is employed

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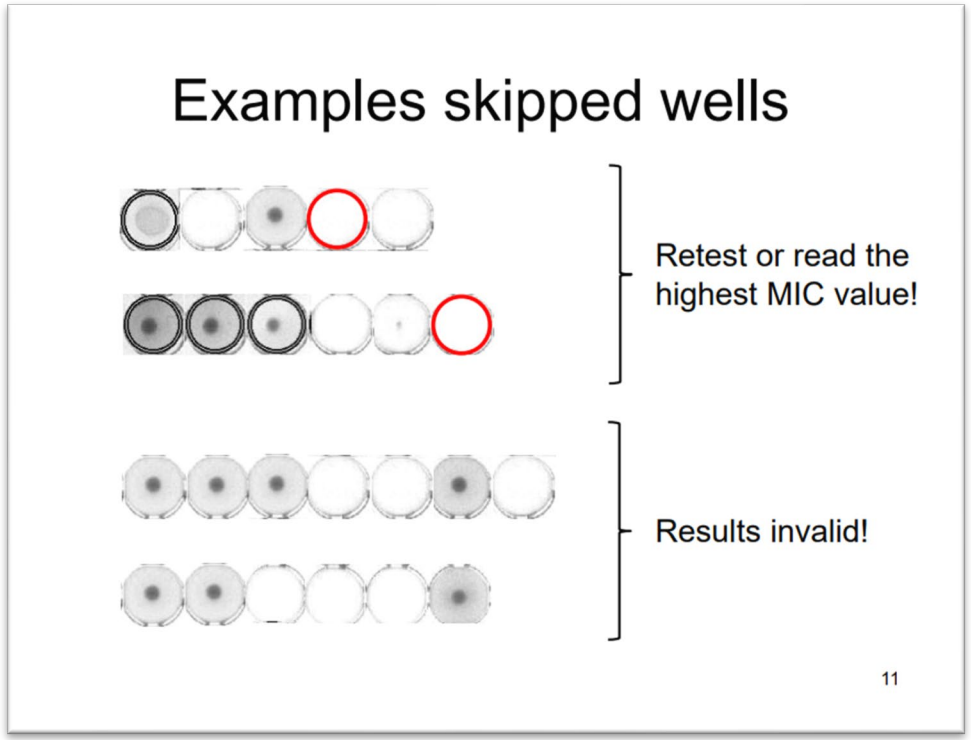
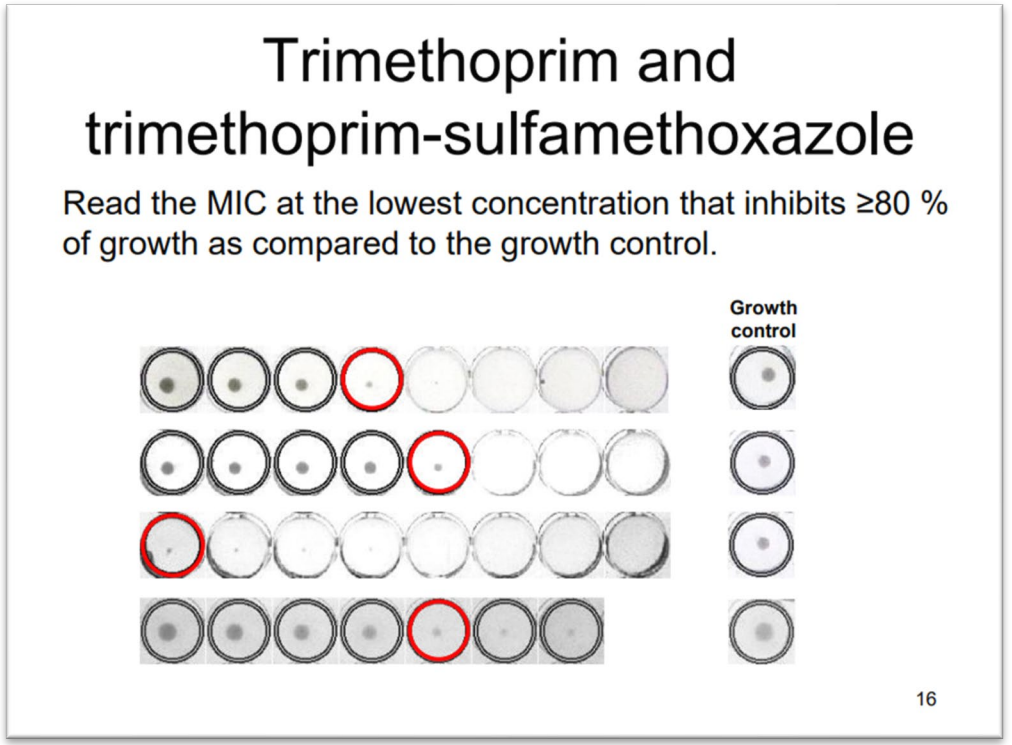
INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – BROTH MICRODILUTION

EUCAST visual guidelines





EUCAST reading guide for broth microdilution, Version 4.0. Växjö, Sweden: 2022.

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DISK DIFFUSION

Phenotypic antimicrobial susceptibility testing

- Broth microdilution ✓
- **Disk diffusion**

Molecular detection of antimicrobial resistance

- PCR protocols
- Whole-genome sequencing

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DISK DIFFUSION

Standard protocol – EUCAST protocol

- *"Antimicrobial susceptibility testing - EUCAST disk diffusion method. Version 11.0, January 2023"*

EUCAST documents

- Clinical breakpoint tables
- Warnings page
- Visual guides (e.g. how to confirm adequate growth and determine zone diameters)

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DISK DIFFUSION

EUCAST protocol

Contents		Page
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3	Preparation of inoculum	8
4	Inoculation of agar plates	10
5	Application of antimicrobial disks	11
6	Incubation of plates	12
7	Examination of plates after incubation	14
8	Measurement of zones and interpretation of susceptibility	15
9	Quality control	17
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Antimicrobial susceptibility testing - EUCAST disk diffusion method, Version 11.0. Växjö, Sweden: 2023.

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DISK DIFFUSION

EUCAST protocol

- How to prepare and store the agar plates
- How to obtain the bacterial inoculum, inoculate the surface of the agar and incubate the plates
  - Incubation at 35 ± 1°C during 18 ± 2 hours, stacking no more than five agar plates
- How to read the zone diameters
- Lists of situations that require special attention, including the adjustment of medium composition, or incubation conditions, or reading details for certain bacterial species and for certain antimicrobials:
  - Disregard faint growth and single colonies within the inhibition zones for trimethoprim and fosfomycin, respectively
  - Variations in the concentration of divalent cations (Ca2+ and Mg2+) cause drifts in the inhibition zones for aminoglycosides

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DISK DIFFUSION

EUCAST protocol

- Quality control of the agar plates and disks

- Control strains
  - Same as for the BMD protocol
  - And additionally for testing of piperacillin and ticarcillin in *P. aeruginosa* → *E. coli* ATCC 25922

Disk diffusion should not be used for colistin susceptibility testing

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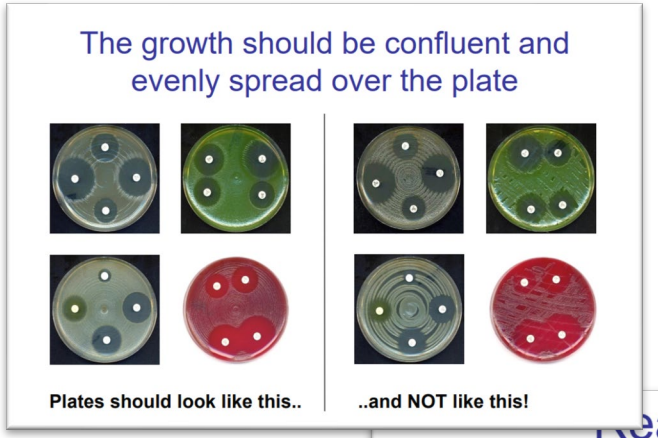
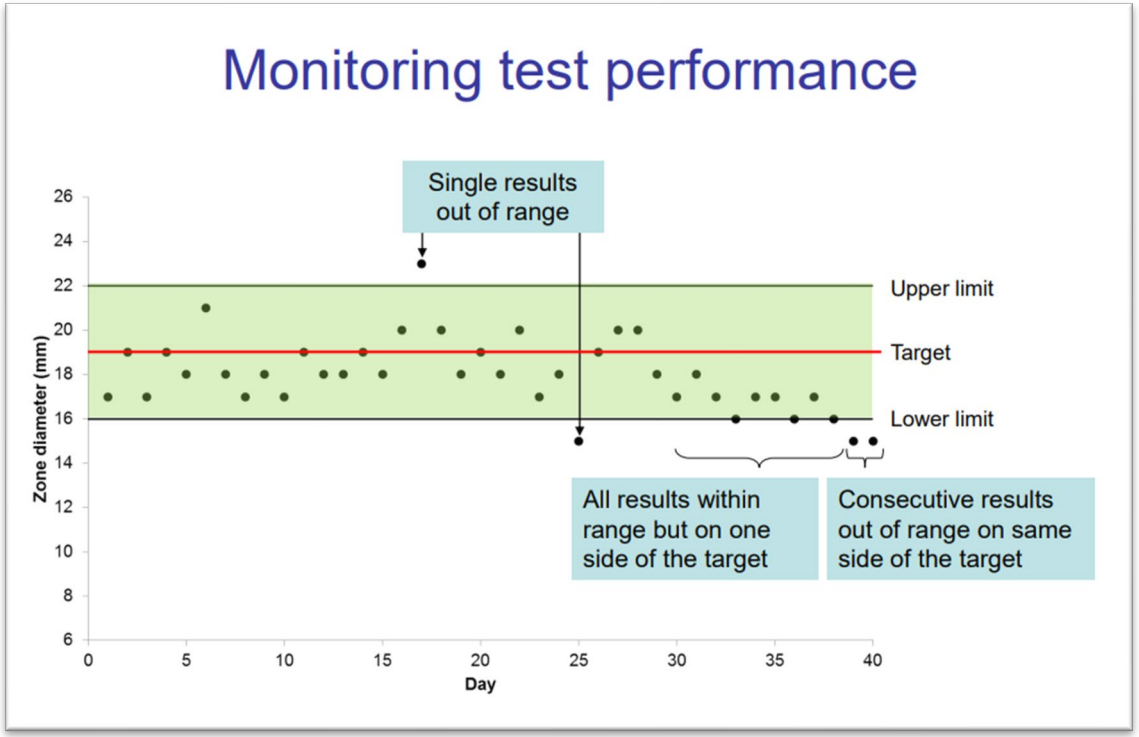
INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DISK DIFFUSION

EUCAST visual guidelines





EUCAST disk diffusion method for antimicrobial susceptibility testing, Version 11.0. Växjö, Sweden: 2023.

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST

Phenotypic antimicrobial susceptibility testing

- Broth microdilution ✓
- Disk diffusion ✓

Concrete examples of documentation for IQC

Molecular detection of antimicrobial resistance

- PCR protocols
- Whole-genome sequencing

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – CONCRETE EXAMPLES OF DOCUMENTATION FOR IQC

In the previous slides

- **Standard / recommended** methods for AST

We achieve the most accurate results possible

- Quality control strain for **each iteration**

We make sure that there was no random error on this day

≠

Quality control of the methods

To detect systematic deviations or other general problems with the methods or local set-up

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – CONCRETE EXAMPLES OF DOCUMENTATION FOR IQC

Quality control of the methods

- **Examples** in the Appendices 1 – 4 of the guidance document

  Laboratories should not adjust their methods to follow the Appendices, especially if the methods are accredited and/or if they consistenty produce results for control strains within the accepted ranges

- Based on **DTU** SOP

  Combination of different protocols, may not follow one specific protocol entirely

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Appendix 1 - Example of method overview documentation for internal quality control

- Ensures all operators follow the same protocol
- Ensures there's no confusion regarding species- or panel- specific details
- Document should be revised as needed (e.g. when purchasing material from different manufacturers)

Method overview for broth microdilution

Bacteria	Agar	Culture [1]	MIC panel	Solvent for McFarland suspension	Bouillon	Transfer from McFarland	Inoculum to reconstitute wells	Inoculator programme [2]	Incubation
E. coli	TSA 5% blood	W	EUVSEC3	dem. water	CAMHB	10 µl	50 µl/ well	1	36-37°C 18-20 h
Pseudomonas	TSA 5% blood	W	EUVSEC3	dem. water	CAMHB	10 µl	50 µl/ well	1	36-37°C 18-20 h
Acinetobacter	TSA 5% blood	W	EUVSEC3	dem. water	CAMHB	10 µl	50 µl/ well	1	36-37°C 18-20 h
ESBL suspect	TSA 5% blood	W	EUVSEC2	dem. water	CAMHB	10 µl	50 µl/ well	1	36-37°C 18-20 h
[Other relevant species]									

1) F: Fresh overnight culture must be used. W: The culture may be refrigerated up to 3 days before use.
2) Sensititre autoinoculator equipment number 1234 only.

Document approved by:
Approval date:

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Appendix 2 - Example of batch of reagents, materials and equipment documentation for internal quality control

- Allows for identification of material-specific deviations
- Ensures traceability
- Can aid in stock management

Batch and equipment documentation for internal quality control of broth microdilution

Batch and equipment control is carried out for every test iteration.

Table 1. Batch of reagents, materials and equipment

Date/initials						
Dem. water						
CAMHB						
TSA 5% blood						
EUVSEC3						
EUVSEC2						
Inoculator 1234						
Inoculator 5678						
McFarland std.						
Dosing heads						
Incubator AB12						
Incubator CD34						
[other]						
[other]						

Remarks:

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Appendix 3 - Example of method control documentation for internal quality control

- Should be performed regularly to ensure general method conformity
- Should also be performed when new batches of relevant material are received (e.g. new media) – ensures no batch-specific deviations
- Should be revised as needed (e.g. when purchasing material from different manufacturers, and every year when new guidelines are published)

Method control for broth microdilution

Method control is carried out every week of a test period.
Method control is performed for every new batch of panels or media.
Test forms for quality control must be attached.
Results sheets for all test isolates must be attached.

Table 1. Reference strains to be used for weekly method control and for control of new batches of panels or media

Reference strain		***E. coli* ATCC 25922**	***E. coli* NCTC 13846**	***P. aeruginosa* ATCC 27853**
Media		CAMHB	CAMHB	CAMHB
MIC panel (Sensititre™)	**EUVSEC3**	X	X	X
	EUVSEC2	X		

Table 2. Acceptance intervals (mg/L) for approval of method, panels or media

Antimicrobials	**Reference strain**		
	***E. coli* ATCC 25922**	***E. coli* NCTC 13846**	***P. aeruginosa* ATCC 27853**
Amikacin	0.5-4	--	1-4
Ampicillin	2-8	--	--
[...]	*[...]*	*[...]*	*[...]*
Ciprofloxacin	0.004-0.016	--	0.125-1
Clindamycin	--	--	0.5-4
Colistin	0.25-2	2-8	--
[...]	*[...]*	*[...]*	*[...]*

Purpose: [ ] weekly control [ ] panel batch control [ ] media batch control [ ] _____

Panel code: _____
Panel batch: _____
Panel expiration date: _____

Broth code: _____
Broth batch: _____
Broth expiration date: _____

Performed by: _____
Date: _____

Read by: _____
Date: _____

Remarks:

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Appendix 4 - Example of documentation for quality control of each AST **iteration** ("test form")

- There should be a "test form" for each combination of control strain + panel
- Allows for quick evaluation of conformity with accepted ranges
- Allows for long-term evaluation of trends in deviations
- Avoids errors because accepted range is coloured
- Should be revised for new combinations of QC strains+panels, and every year when new guidelines are published

"Test form" for quality control for broth microdilution

Quality control is carried out at least once a day when testing is performed.

Control strain: *Escherichia coli* ATCC 25922
Panel: EUVSEC3
Broth medium: CAMHB
Volume per well: 50 µl
Accepted ranges: Green (EUCAST QC tables v13.0, valid from 01/01/2023)

	1	2	3	4	5	6	7	8	9	10	11	12
A	AMP 32	AZI 64	AMI 128	GEN 16	TGC 8	TAZ 8	FOT 4	COL 16	NAL 64	TET 32	TMP 16	SMX 512
B	AMP 16	AZI 32	AMI 64	GEN 8	TGC 4	TAZ 4	FOT 2	COL 8	NAL 32	TET 16	TMP 8	SMX 256
C	AMP 8	AZI 16	AMI 32	GEN 4	TGC 2	TAZ 2	FOT 1	COL 4	NAL 16	TET 8	TMP 4	SMX 128
D	AMP 4	AZI 8	AMI 16	GEN 2	TGC 1	TAZ 1	FOT 0.5	COL 2	NAL 8	TET 4	TMP 2	SMX 64
E	AMP 2	AZI 4	AMI 8	GEN 1	TGC 0.5	TAZ 0.5	FOT 0.25	COL 1	NAL 4	TET 2	TMP 1	SMX 32
F	AMP 1	AZI 2	AMI 4	GEN 0.5	TGC 0.25	TAZ 0.25	CHL 8	CHL 16	CHL 32	CHL 64	TMP 0.5	SMX 16
G	MERO 0.03	MERO 0.06	MERO 0.12	MERO 0.25	MERO 0.5	MERO 1	MERO 2	MERO 4	MERO 8	MERO 16	TMP 0.25	SMX 8
H	CIP 0.015	CIP 0.03	CIP 0.06	CIP 0.12	CIP 0.25	CIP 0.5	CIP 1	CIP 2	CIP 4	CIP 8	POS CON	POS CON

Code	Antimicrobial agent (15)	Test range (mg/L)
AMI	AMIKACIN	4-128
AMP	AMPICILLIN	1-32
AZI	AZITHROMYCIN	2-64
FOT	CEFOTAXIME	0.25-4
TAZ	CEFTAZIDIME	0.25-8
CHL	CHLORAMPHENICOL	8-64
CIP	CIPROFLOXACIN	0.015-8
COL	COLISTIN	1-16
GEN	GENTAMICIN	0.5-16
MERO	MEROPENEM	0.03-16
NAL	NALIDIXIC ACID	4-64
SMX	SULFAMETHOXAZOLE	8-512
TET	TETRACYCLINE	2-32
TGC	TIGECYCLINE	0.25-8
TMP	TRIMETHOPRIM	0.25-16
POS	POSITIVE CONTROL	2x

Performed by: _____
Date: _____

Read by: _____
Date: _____

Remarks:

A similar layout can be used as "result sheet" to record the actual test results for each isolate:

- without colour

- without having the headers pre-filled

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – PCR PROTOCOLS

Phenotypic antimicrobial susceptibility testing

- Broth microdilution ✓
- Disk diffusion ✓

→ **Concrete examples of IQC** ✓

Molecular detection of antimicrobial resistance

- **PCR protocols**
- Whole-genome sequencing

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INTERNAL QUALITY CONTROL – FOR AST

MOLECULAR DETECTION – PCR PROTOCOLS

Molecular detection of resistance through PCR

- Databases: Beta-Lactamase Database (BLDB), EURL-AR list of *mcr*-genes, ResFinder, AMRFinderPlus

- Acquired AMR genes → PCR protocols
  - Protocols for detection of β-lactamases
  - Protocols for detection of *mcr*-genes

- Chromosomal point mutations → sequencing

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INTERNAL QUALITY CONTROL – FOR AST

MOLECULAR DETECTION – PCR PROTOCOLS

- Quality control
  - Use all positive control strains described in the chosen PCR protocol
  - Always include a negative control
  - Do not combine different PCR protocols into a larger multiplex
  - Do not use terms “susceptible” or “resistant” → report results as presence or absence of the genes included in the protocols
  - Create method overview documentation and record the batch of reagents, materials and equipment

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – WHOLE-GENOME SEQUENCING

Phenotypic antimicrobial susceptibility testing

- Broth microdilution ✓
- Disk diffusion ✓

→ **Concrete examples of IQC** ✓

Molecular detection of antimicrobial resistance

- PCR protocols ✓
- **Whole-genome sequencing**

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INTERNAL QUALITY CONTROL – FOR AST

MOLECULAR DETECTION – WHOLE-GENOME SEQUENCING

Molecular detection of resistance through WGS

- Databases: Same as before
  - Other bioinformatics tools? → confirm databases before using

- Techniques described in EURGen-RefLabCap WGS protocol
  - Harmonisation of QC is difficult due to diversity of options
  - Main QC parameters and respective thresholds proposed in the protocol

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INTERNAL QUALITY CONTROL – FOR AST

MOLECULAR DETECTION – WHOLE-GENOME SEQUENCING

- Quality control
  - Create method overview documentation and record the batch of reagents, materials and equipment
  - Record the version and/or date of the bioinformatics tools and databases that are used for analysis of raw sequence data
  - Store the raw sequence data permanently
  - Apply well-defined QC thresholds for raw data and for assemblies

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – WHOLE-GENOME SEQUENCING

Phenotypic antimicrobial susceptibility testing

- Broth microdilution ✓
- Disk diffusion ✓

→ **Concrete examples of IQC** ✓

Molecular detection of antimicrobial resistance

- PCR protocols ✓
- Whole-genome sequencing ✓

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Questions and discussion

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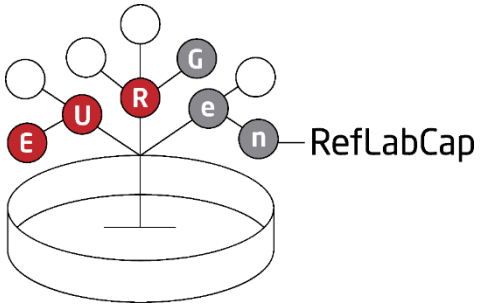
Thank you on behalf of the EURGen-RefLabCap team



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