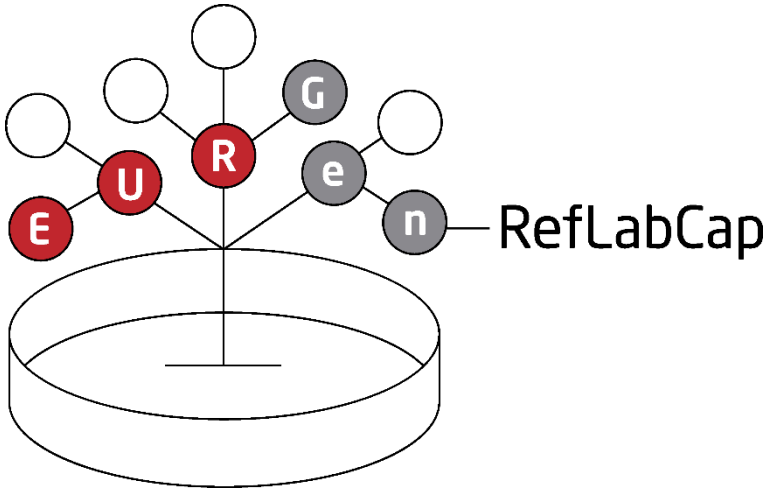
EURGen-RefLabCap Webinar

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

Monday, 7 November 2022

14:00-15:00 CET

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



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Meeting agenda

1. Presentation of the guidance document

2. Upcoming task

3. Discussion

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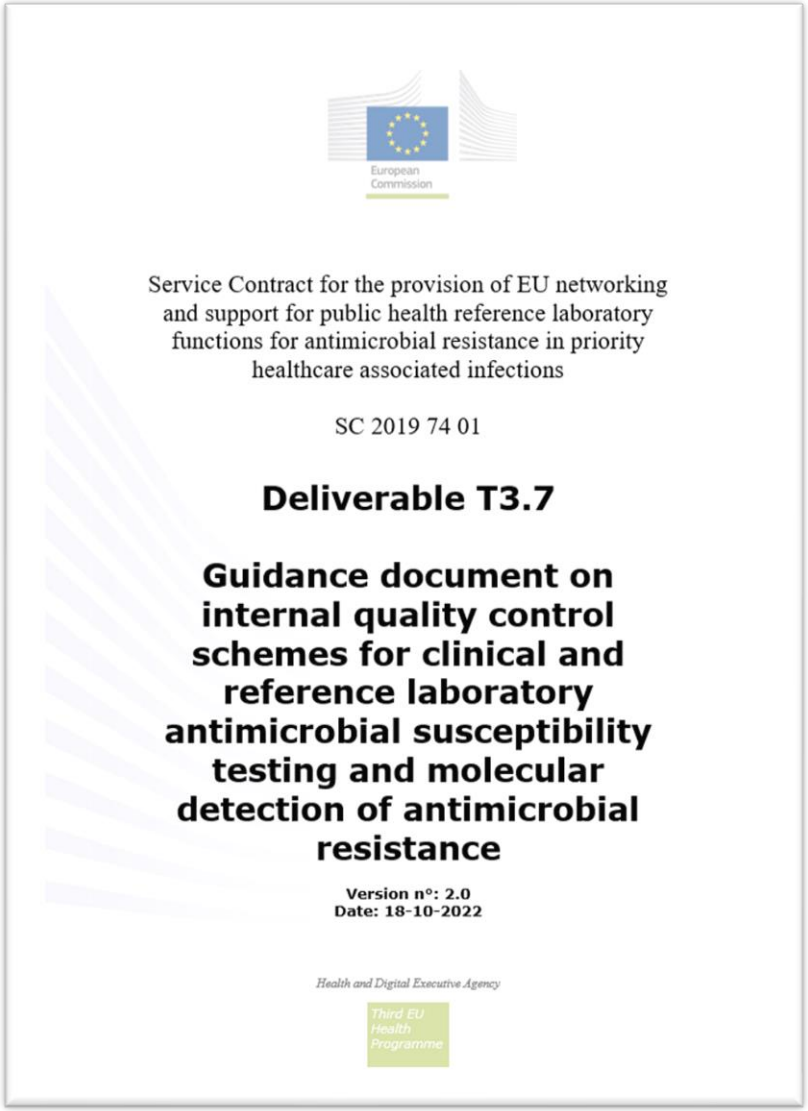
Background and aim



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

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°: 2.0
Date: 18-10-2022

Health and Digital Executive Agency

Third EU Health Programme

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Background and aim

- Assistance to NRLs and other laboratories on the techniques for Internal Quality Control
- Collection of the most recently available information from different regulatory agencies and other sources
- Description of the standardized / recommended methods for antimicrobial susceptibility testing of CRE and CCRE
- Proposed methods for detecting relevant antimicrobial resistance determinants – excluding WGS

Reliable and accurate results for diagnostics and surveillance purposes of CRE and CCRE

Data that are comparable within Europe for surveillance purposes

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BACKGROUND AND AIM

TABLE OF CONTENTS

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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

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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 15189:2012

"Medical laboratories - Requirements for quality and competence"

This International Standard, based upon ISO/IEC 17025 and ISO 9001, specifies requirements for competence and quality that are particular to medical laboratories[1)]. It is acknowledged that a country could have its own specific regulations or requirements applicable to some or all its professional personnel and their activities and responsibilities in this domain.

Medical laboratory services are essential to patient care and therefore have to be available to meet the needs of all patients and the clinical personnel responsible for the care of those patients. Such services include arrangements for examination requests, patient preparation, patient identification, collection of samples, transportation, storage, processing and examination of clinical samples, together with subsequent interpretation, reporting and advice, in addition to the considerations of safety and ethics in medical laboratory work.

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO 15189

1 Scope

This International Standard specifies requirements for quality and competence in medical laboratories.

This International Standard can be used by medical laboratories in developing their quality management systems and assessing their own competence. It can also be used for confirming or recognizing the competence of medical laboratories by laboratory customers, regulating authorities and accreditation bodies.

NOTE International, national or regional regulations or requirements may also apply to specific topics covered in this International Standard.

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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: 4) Management requirements; 4.2) Quality management system

4.2 Quality management system

4.2.1 General requirements

The laboratory shall establish, document, implement and maintain a quality management system and continually improve its effectiveness in accordance with the requirements of this International Standard.

The quality management system shall provide for the integration of all processes required to fulfil its quality policy and objectives and meet the needs and requirements of the users.

The laboratory shall:

a) determine the processes needed for the quality management system and ensure their application throughout the laboratory;

b) determine the sequence and interaction of these processes;

c) determine criteria and methods needed to ensure that both the operation and control of these processes are effective;

d) ensure the availability of resources and information necessary to support the operation and monitoring of these processes;

e) monitor and evaluate these processes;

f) implement actions necessary to achieve planned results and continual improvement of these processes.

4.2.2 Documentation requirements

4.2.2.1 General

The quality management system documentation shall include:

a) statements of a quality policy (see 4.1.2.3) and quality objectives (see 4.1.2.4);

b) a quality manual (see 4.2.2.2);

c) procedures and records required by this International Standard;

d) documents, and records (see 4.13), determined by the laboratory to ensure the effective planning, operation and control of its processes;

e) copies of applicable regulations, standards and other normative documents.

NOTE The documentation can be in any form or type of medium, providing it is readily accessible and protected from unauthorized changes and undue deterioration.

4.2.2.2 Quality manual

The laboratory shall establish and maintain a quality manual that includes:

a) the quality policy (4.1.2.3) or makes reference to it;

b) a description of the scope of the quality management system;

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO 15189

Example: 5) Technical requirements; 5.6) Ensuring quality of examination results

5.6 Ensuring quality of examination results

5.6.1 General

The laboratory shall ensure the quality of examinations by performing them under defined conditions.

Appropriate pre and post-examination processes shall be implemented (see 4.14.7, 5.4, 5.7 and 5.8).

The laboratory shall not fabricate any results.

5.6.2 Quality control

5.6.2.1 General

The laboratory shall design quality control procedures that verify the attainment of the intended quality of results.

NOTE In several countries, quality control, as referred to in this subclause, is also named "internal quality control."

5.6.2.2 Quality control materials

The laboratory shall use quality control materials that react to the examining system in a manner as close as possible to patient samples.

Quality control materials shall be periodically examined with a frequency that is based on the stability of the procedure and the risk of harm to the patient from an erroneous result.

NOTE 1 The laboratory should choose concentrations of control materials, wherever possible, especially at or near clinical decision values, which ensure the validity of decisions made.

NOTE 2 Use of independent third party control materials should be considered, either instead of, or in addition to, any control materials supplied by the reagent or instrument manufacturer.

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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"

This document has been developed with the objective of promoting confidence in the operation of laboratories. This document contains requirements for laboratories to enable them to demonstrate they operate competently, and are able to generate valid results. Laboratories that conform to this document will also operate generally in accordance with the principles of ISO 9001.

This document requires the laboratory to plan and implement actions to address risks and opportunities. Addressing both risks and opportunities establishes a basis for increasing the effectiveness of the management system, achieving improved results and preventing negative effects. The laboratory is responsible for deciding which risks and opportunities need to be addressed.

The use of this document will facilitate cooperation between laboratories and other bodies, and assist in the exchange of information and experience, and in the harmonization of standards and procedures. The acceptance of results between countries is facilitated if laboratories conform to this document.

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INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO/IEC 17025

1 Scope

This document specifies the general requirements for the competence, impartiality and consistent operation of laboratories.

This document is applicable to all organizations performing laboratory activities, regardless of the number of personnel.

Laboratory customers, regulatory authorities, organizations and schemes using peer-assessment, accreditation bodies, and others use this document in confirming or recognizing the competence of laboratories.

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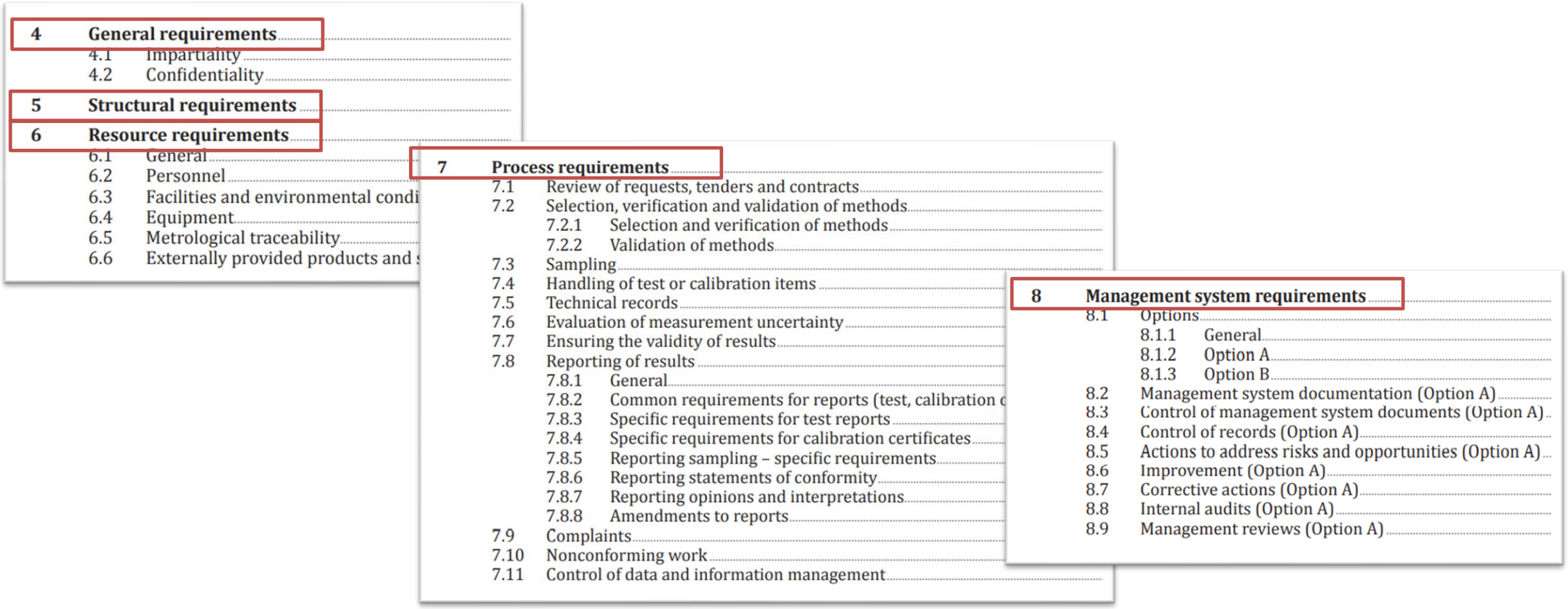
INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO/IEC 17025



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INTERNAL QUALITY CONTROL – IN GENERAL

ISO STANDARDS – ISO/IEC 17025

Example: 4) General requirements; 4.1) Impartiality

4 General requirements

4.1 Impartiality

4.1.1 Laboratory activities shall be undertaken impartially and structured and managed so as to safeguard impartiality.

4.1.2 The laboratory management shall be committed to impartiality.

4.1.3 The laboratory shall be responsible for the impartiality of its laboratory activities and shall not allow commercial, financial or other pressures to compromise impartiality.

4.1.4 The laboratory shall identify risks to its impartiality on an on-going basis. This shall include those risks that arise from its activities, or from its relationships, or from the relationships of its personnel. However, such relationships do not necessarily present a laboratory with a risk to impartiality.

NOTE — A relationship that threatens the impartiality of the laboratory can be based on ownership, governance, management, personnel, shared resources, finances, contracts, marketing (including branding), and payment of a sales commission or other inducement for the referral of new customers, etc.

4.1.5 If a risk to impartiality is identified, the laboratory shall be able to demonstrate how it eliminates or minimizes such risk.

4.2 Confidentiality

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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

While this International Standard is intended for use throughout the currently recognized disciplines of medical laboratory services, those working in other services and disciplines such as clinical physiology, medical imaging and medical physics could also find it useful and appropriate. In addition, bodies engaged in the recognition of the competence of medical laboratories will be able to use this International Standard as the basis for their activities. If a laboratory seeks accreditation, it should select an accrediting body which operates in accordance with ISO/IEC 17011 and which takes into account the particular requirements of medical laboratories.

This International Standard is not intended to be used for the purposes of certification, however a medical laboratory's fulfilment of the requirements of this International Standard means the laboratory meets both the technical competence requirements and the management system requirements that are necessary for it to consistently deliver technically valid results. The management system requirements in Clause 4 are written in a language relevant to a medical laboratory's operations and meet the principles of ISO 9001:2008, *Quality management systems — Requirements*, and are aligned with its pertinent requirements (Joint IAF-ILAC-ISO Communiqué issued in 2009).

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 – 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
- Regularly confirming warnings and new breakpoint tables

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INTERNAL QUALITY CONTROL – FOR AST

Phenotypic antimicrobial susceptibility testing

- Broth microdilution
- Disk diffusion
- Detection of β-lactamases

Molecular detection of antimicrobial resistance

- β-lactam resistance
- Colistin resistance

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – BROTH MICRODILUTION

Phenotypic antimicrobial susceptibility testing

- **Broth microdilution**
- Disk diffusion
- Detection of β-lactamases

Molecular detection of antimicrobial resistance

- β-lactam resistance
- Colistin resistance

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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
  - E.g.: preparation of working solutions of tigecycline no more than 12 hours before testing, adjusting the zinc concentration of the broth medium for testing of carbapenems, depleting iron from the broth medium before testing cefiderocol, and refraining from adding surfactants to the medium when testing colistin

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – BROTH MICRODILUTION

ISO 20776-1:2019

- 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: *Escherichia coli* ATCC 25922

Colistin: *mcr-1*-positive *E. coli* NCTC 13946

β-lactams in combination with β-lactamase inhibitors: *E. coli* ATCC 35218, or *Klebsiella pneumoniae* ATCC 700603, or *K. pneumoniae* ATCC BAA-2814

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – BROTH MICRODILUTION

EUCAST breakpoint tables and warnings

- AST of colistin

  Additional control strain *E. coli* NCTC 13946

  Cation-adjusted Mueller-Hinton broth

  Colistin sulphate salts

  Non-treated polystyrene microdilution trays

  No additives

- AST of carbapenems

  Adjusting the zinc concentration of the broth medium

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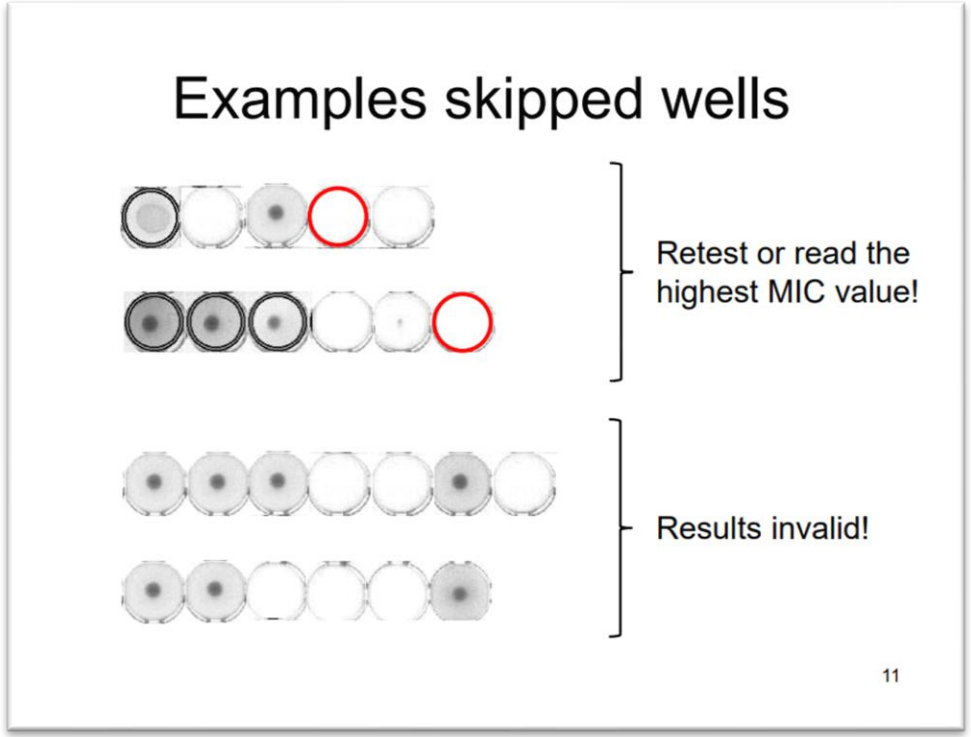
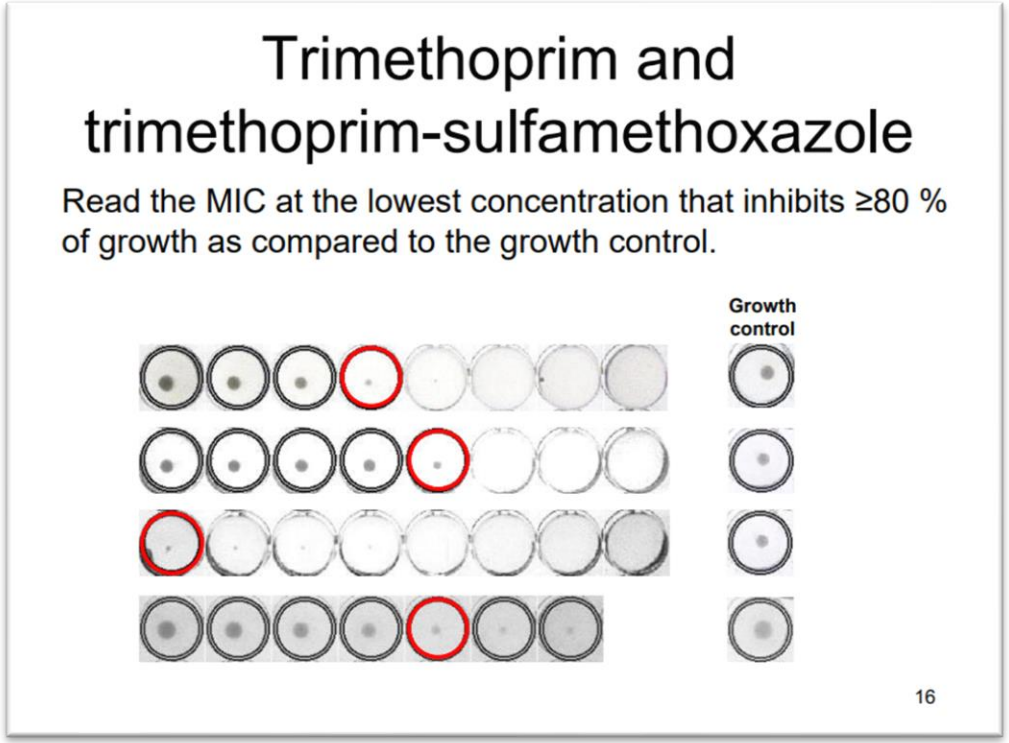
INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – BROTH MICRODILUTION

EUCAST visual guidelines





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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DISK DIFFUSION

Phenotypic antimicrobial susceptibility testing

- Broth microdilution
- **Disk diffusion**
- Detection of β-lactamases

Molecular detection of antimicrobial resistance

- β-lactam resistance
- Colistin resistance

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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 10.0, January 2022"*

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

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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 for Enterobacterales, 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 for certain bacterial species and for certain antimicrobials
  - E.g.: presence of faint growth and/or isolates colonies within the inhibition zone for trimethoprim, ampicillin, ampicillin/sulbactam, amoxicillin/clavulanic acid, temocillin, mecillinam and fosfomycin should be ignored

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DISK DIFFUSION

EUCAST protocol

- Quality control of the agar plates
  - Agar depth is 4 ± 0.5 millimetres and that the surface pH is within the range 7.2-7.4
- Control strains
  - Same as for the BMD protocol

Disk diffusion should not be used for colistin susceptibility testing

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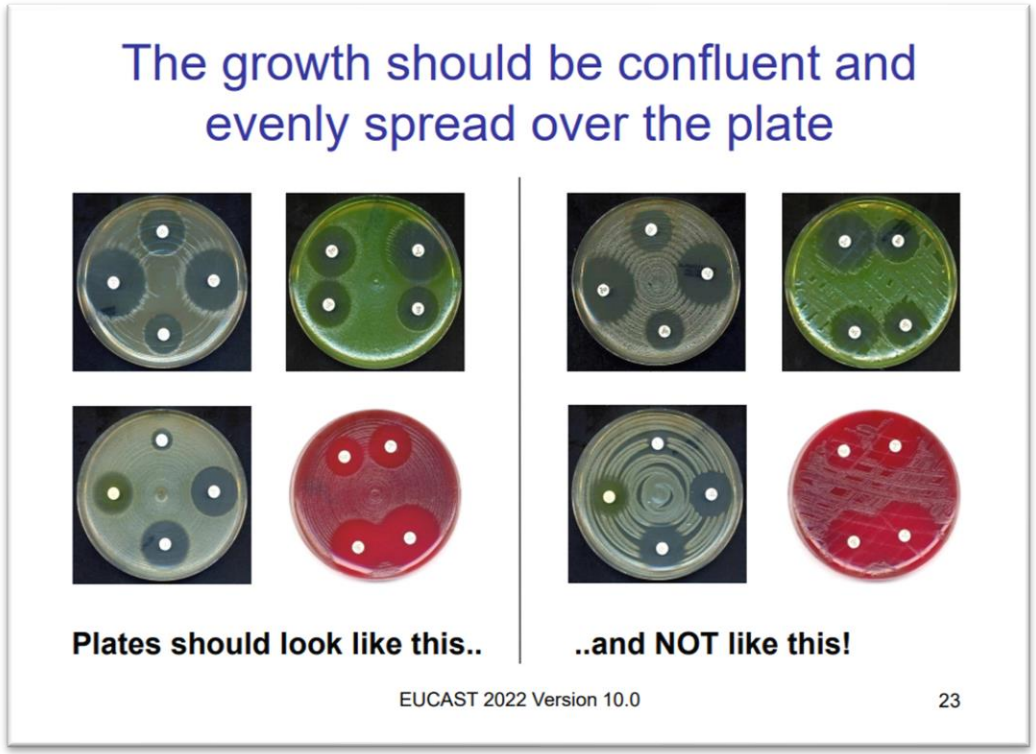
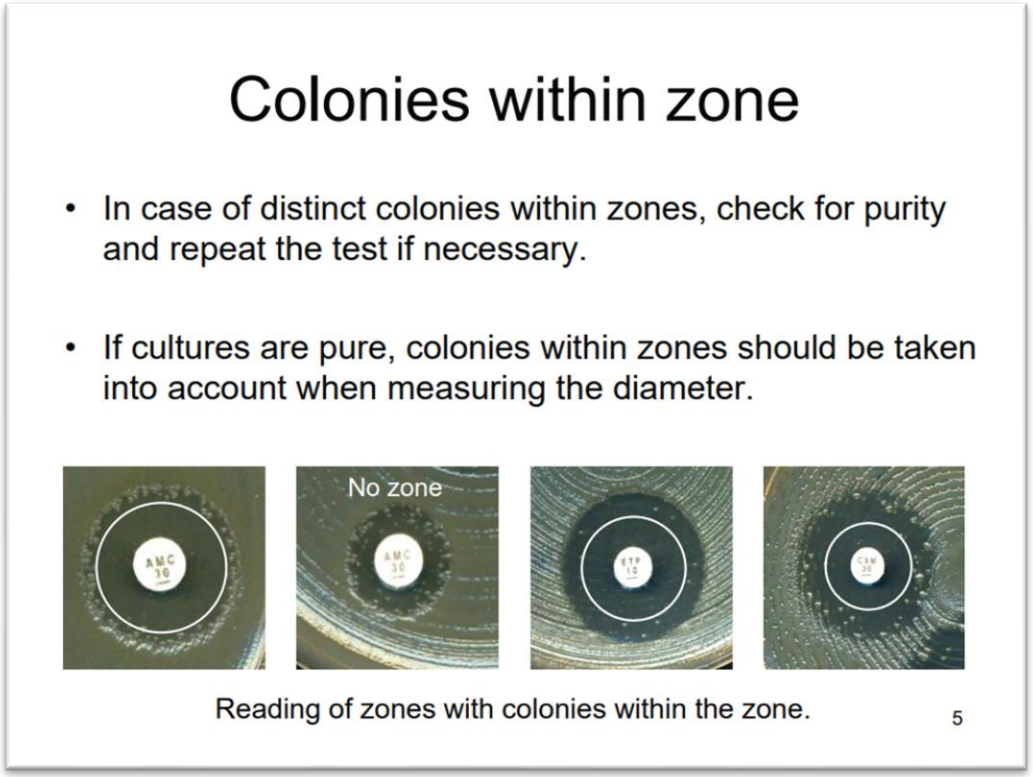
INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DISK DIFFUSION

EUCAST visual guidelines





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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DETECTION OF BETA-LACTAMASES

Phenotypic antimicrobial susceptibility testing

- Broth microdilution
- Disk diffusion
- **Detection of β-lactamases**

Molecular detection of antimicrobial resistance

- β-lactam resistance
- Colistin resistance

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DETECTION OF BETA-LACTAMASES

Proposed methods – EUCAST guidelines

- *"EUCAST guidelines for detection of resistance mechanisms and specific resistances ofclinical and/or epidemiological importance. Version 2.0, July 2017"*

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DETECTION OF BETA-LACTAMASES

EUCAST guidelines

Section

1. Introduction
2. Carbapenemase-producing Enterobacteriaceae
3. Extended-spectrum β-lactamase-producing Enterobacteriaceae
4. Acquired AmpC β-lactamase-producing Enterobacteriaceae
5. Polymyxin resistance in Gram-negative bacilli
6. Carbapenem resistance in *P. aeruginosa* and *Acinetobacter*
7. Methicillin resistant *Staphylococcus aureus*
8. Glycopeptide non-susceptible *Staphylococcus aureus*
9. Vancomycin resistant *Enterococcus faecium* and *Enterococcus faecalis*
10. Penicillin non-wild-type *Streptococcus pneumoniae*

Different methods

We focus on the ones with same protocols and materials as standardized AST

Same information as other documents

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DETECTION OF BETA-LACTAMASES

EUCAST guidelines

- Production of carbapenemases
  - MIC values above the epidemiological cut-off values (ECOFF) for carbapenems
  - Meropenem and ertapenem are recommended as screening agents
  - Through BMD: Carbapenemase-producers have MIC > 0.125 mg/L for either
  - Through DD: Carbapenemase-producers have zone diameters < 28 mm MER or < 25 mm for ERT

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DETECTION OF BETA-LACTAMASES

EUCAST guidelines

- Production of extended-spectrum β-lactamases (ESBL)
  - Resistant to a cephalosporin (e.g. cefotaxime, ceftazidime, cefepime) but susceptible towards the cephalosporin in combination with a β-lactamase inhibitor (clavulanic acid)
  - Through DD: zone diameters at least 5 mm larger for the disk containing the cephalosporin with inhibitor, than the diameter observed for the cephalosporin by itself
  - The ratio between the MIC values of the cephalosporin, and the cephalosporin in combination with the inhibitor will be equal to or higher than 8

Isolate	Cefotaxime MIC (mg/L)	Cefotaxime/clavulanic acid MIC (mg/L)	Ratio between MIC values	ESBL production
Isolate A	32	0.5/4	64	Yes
Isolate B	32	8/4	4	No
Isolate C	32	32/4	1	No

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DETECTION OF BETA-LACTAMASES

EUCAST guidelines

- AmpC-mediated β-lactam resistance
  - Resistant to cefoxitin (MIC > 8 mg/L or zone diameter < 19 mm)
  - Resistant to some cephalosporins (ceftazidime and/or cefotaxime) and the cephalosporins in combination with β-lactamase inhibitors
  - Generally susceptible to cefepime
  - No distinction between organisms that are overexpressing intrinsic AmpC from those that have acquired additional plasmid-mediated ampC genes

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INTERNAL QUALITY CONTROL – FOR AST

PHENOTYPIC AST – DETECTION OF BETA-LACTAMASES

EUCAST guidelines

- Control strains
  - Negative control strain *E. coli* ATCC 25922
  - Positive control strain(s)

Control strain	Mechanism of resistance
Enterobacter cloacae CCUG 59627	AmpC combined with decreased porin expression
K. pneumoniae CCUG 58547 or K. pneumoniae NCTC 13440	Metallo-β-lactamase (VIM)
K. pneumoniae NCTC 13443	Metallo-β-lactamase (NDM-1)
E. coli NCTC 13476	Metallo-β-lactamase (IMP)
K. pneumoniae CCUG 56233 or K. pneumoniae NCTC 13438	Klebsiella pneumoniae carbapenemase (KPC)
K. pneumoniae NCTC 13442	OXA-48 carbapenemase
K. pneumoniae ATCC 25955	Negative control for carbapenemase production
E. coli CCUG 58543	Acquired CMY-2 AmpC
E. coli CCUG 62975	Acquired CMY AmpC and CTX-M-1 group ESBL
K. pneumoniae CCUG 58545	Acquired DHA

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INTERNAL QUALITY CONTROL – FOR AST

MOLECULAR DETECTION – BETA-LACTAM RESISTANCE

Phenotypic antimicrobial susceptibility testing

- Broth microdilution
- Disk diffusion
- Detection of β-lactamases

Molecular detection of antimicrobial resistance

- **β-lactam resistance**
- Colistin resistance

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INTERNAL QUALITY CONTROL – FOR AST

MOLECULAR DETECTION – BETA-LACTAM RESISTANCE

Molecular detection of β-lactam resistance

- Databases: Beta-Lactamase Database (BLDB), other

- Acquired β-lactamases → PCR protocols
  - EuSCAPE multiplex PCR protocol (*bla*$_{KPC}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$ and *bla*$_{NDM}$)
  - Dallene et al. set of 6+1 multiplex/simplex PCR (*bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{OXA}$, *bla*$_{CTX-M}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{KPC}$, *bla*$_{VEB}$, *bla*$_{GES}$, *bla*$_{PER}$ and plasmid-mediated AmpC β-lactamases)

- Chromosomal point mutations → sequencing

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INTERNAL QUALITY CONTROL – FOR AST

MOLECULAR DETECTION – COLISTIN RESISTANCE

Phenotypic antimicrobial susceptibility testing

- Broth microdilution
- Disk diffusion
- Detection of β-lactamases

Molecular detection of antimicrobial resistance

- β-lactam resistance
- **Colistin resistance**

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INTERNAL QUALITY CONTROL – FOR AST

MOLECULAR DETECTION – COLISTIN RESISTANCE

Molecular detection of colistin resistance

- Databases: EURL-AR lists, other

- Acquired *mcr*-genes → PCR protocols
  - EURL-AR multiplex PCR protocol (*mcr-1* to *mcr-5*)
  - Borowiak et al. multiplex PCR protocol (*mcr-6* to *mcr-9*)

- Chromosomal point mutations → sequencing

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INTERNAL QUALITY CONTROL – FOR AST

MOLECULAR DETECTION – BETA-LACTAM AND COLISTIN RESISTANCE

- 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

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Upcoming task

Email consultation for the guidance document

Document was distributed 21 October 2022

2 weeks of email consultation period: 24 October to 7 November → **Extended to 14 November**

Changes will be implemented and the updated document will be shared by email

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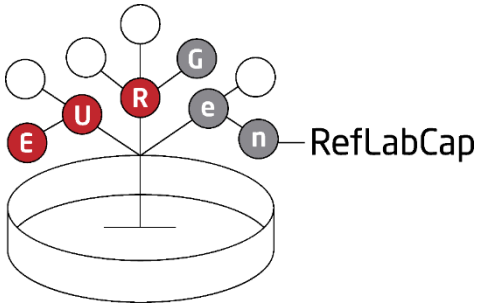
Thank you on behalf of the EURGen-RefLabCap team



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