EURL-PH-AMR

EU Reference Laboratory for public health on Antimicrobial Resistance

101194806

Protocol for the 2025 phenotypic external quality assessment (EQA) exercise of performance of laboratories participating in the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net)

Funded by the European Union

OVERVIEW OF THE MOST IMPORTANT INFORMATION IN THIS PROTOCOL This table does not replace reading the full protocol.		
Strains included in this EQA	Four strains from bloodstream infections.	Page 4, 8
Handling instructions	Subculture and process the strains within 48 hours from receipt.	Page 5
Storage of strains	Freeze at -80° C for future analysis.	Page 5
Sharing of strains	Strains cannot be re-distributed by the recipient laboratories.	Page 6
Safety instructions	Strains are UN3373, Biological substance, category B. Handle in BSL2 facility.	Page 6
Antimicrobials included in this EQA	Adapted from the EURL-PH-AMR Laboratory testing strategy.	Page 7 Annex 1
Recommendations for performing AST	Use the routine AST methods applied in your laboratory. Follow the most current EUCAST guidelines. Broth microdilution is the recommended method for most antimicrobials. Disk diffusion is the recommended method for cefiderocol.	Page 8 Annex 1
Rules for reporting AST results	Follow the most current EUCAST guidelines. Report S/I/R results for all antimicrobials including screening agents. Do not use results from one antimicrobial to report results for other antimicrobial(s). For reporting results of cefiderocol in *Acinetobacter* spp. use: WT=S; NWT=I; likely R=R.	Page 8
Link to the EURL-PH-AMR website with the protocol, the test forms and the instructions for the webtool	https://en.ssi.dk/surveillance-and-preparedness/international-coorporation/eurl-ph-amr/eqa-pt	Page 9
Link to the EURGen-Net EQA webtool for submission of results	https://EURL-PH-AMR.eqa.dtu.dk	Page 9
Username and password for the webtool	All email addresses registered to each laboratory will receive an email with a link to the webtool, a username, and a description of how to create a webtool password.	Page 9
Deadline for submission of results	**10 November 2025**	Page 4, 9
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1. INTRODUCTION

From 2025 to 2031, the European Union Reference Laboratory for Public Health on Antimicrobial Resistance in bacteria (EURL-PH-AMR) has been commissioned the task to provide phenotypic External Quality Assessment (EQA) exercises for the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net) laboratories by the European Commission (Grant 101194806) under the coordination of European Centre for Disease Prevention and Control (ECDC).

Participation in EURGen-Net EQA exercises promotes production of reliable laboratory results and compliance with ISO 15189:2022 (Medical laboratories — Requirements for quality and competence) or ISO 17025:2017 (General requirements for the competence of testing and calibration laboratories) and provides important information on performance and comparability of the reported test results between participating laboratories and countries.

2. SCOPE AND OBJECTIVES

The scope of the EURGen-Net EQA exercise is to provide external quality assessment of antimicrobial susceptibility testing (AST) to the national reference laboratories in countries participating in EURGen-Net for the microorganisms included in the EURGen-Net surveillance.

The overall objectives are to assess the accuracy of AST results reported by participating individual laboratories, and to evaluate the overall comparability of test results between laboratories.

The EQA exercise will be conducted annually by the EURL-PH-AMR. These annual EQAs provide important information on the accuracy of AST, and the comparability of the AST test results reported by the EURGen-Net participating laboratories. Therefore, the laboratory practices for this EURGen-Net EQA should be the same as the AST method(s) routinely used in the participating laboratory, i.e. automated systems, broth microdilution, disk/tablet diffusion, gradient diffusion, or other method(s).

3. OUTLINE OF THE EQA 2025

In 2025, the EURGen-Net EQA exercise will take place in October-November. Laboratories are requested to identify the species of the four provided strains and report AST results for the bacterial strains covered by the EURGen-Net surveillance. Laboratories should use their routine method(s) and submit results via a password-protected webtool. Results must be submitted no later than **10 November 2025**.

After the submission deadline, DTU Food, in their capacity as a consortium member of the EURL-PH-AMR, validates all received data. Each AST result will be scored according to the system

described in this protocol (see section 8). Participating laboratories will be informed by email when their evaluation report is available for download in the password-protected webtool.

The EURL-PH-AMR will publish an annual report, summarising the results from every participating laboratory, with each laboratory anonymised.

3.2 Shipping, receipt, and initial processing of strains

For the 2025 EURGen-Net EQA, all participating laboratories will receive a parcel containing four swabs from DTU Food. Each swab will contain a pure culture of a bacterial isolate.

Please inspect packages to verify the content and for evidence of breakage and leakage. If this is evident, discard by autoclaving, and contact DTU Food to request a replacement package. The contents of the package consist of four swabs, each with different identification (2025 EURGen-Net 1, 2025 EURGen-Net 2, 2025 EURGen-Net 3, 2025 EURGen-Net 4).

Store the swabs in a dark place at 5°C to 25°C until microbiological analysis.

We suggest that you subculture and process the strains within 48 hours from receipt of the parcel. Subculture the test strains onto non-selective media, e.g. a nutrient agar plate or blood agar plate, as illustrated in Figure 1:

1) Inoculate it on one side of the agar plate using the swab to apply material gently and densely;
2) Turn the plate and use a sterile loop to streak once through the area first inoculated, and allow further streaks to separate the culture, aiming to obtain single colonies;
3) Turn the plate and use a sterile loop to streak once through the second area inoculated, and allow further streaks to separate the culture, aiming to obtain single colonies.

It is recommended to store the strains in your strain collection (e.g. in a -80°C freezer), at least until you have reviewed your results from this EQA exercise. This will allow for repetition of species identification and AST, if needed, in light of your individual performance.

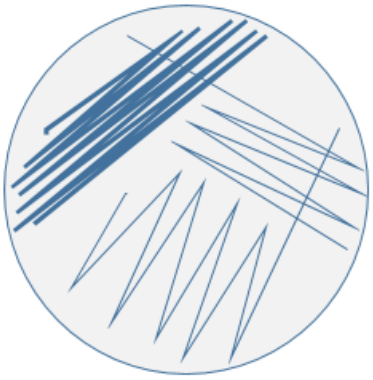

Figure 1: Plating of the test strains

Kindly note, due to the Material Transfer Agreements (MTAs) between DTU and the original providers of the isolates:

1. Strains received for the 2025 EURGen-Net EQA cannot be re-distributed further by the recipient laboratories.
2. It is not possible for DTU Food to distribute strains to laboratories after the EQA exercise, e.g. for confirmatory, training, or reference purposes.

3.3 Safety instructions

All strains used in this iteration of the EURGen-Net EQA are categorized as UN3373, Biological substance, category B. The EQA strains could potentially pose a risk to humans due to their resistance profile and pose a challenge in the treatment of a potential human infection.

Note that it is the responsibility of the recipient laboratory to comply with national regulations and guidelines regarding the correct handling of the provided bacterial cultures and to make use of the proper facilities, equipment, and protocols to handle these strains.

It is recommended to work with the strains in a BSL2 containment facility, using equipment and operational practices for work involving infectious or potentially infectious materials, and take the necessary precautions. It is recommended to wear protective clothing such as a laboratory coat as well as gloves when direct skin contact with infected material is unavoidable. Eye protection must be used where there is a known or potential risk of exposure to splashes. Moreover, all procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet. The use of needles, syringes, and other sharp objects should be strictly limited.

3.4 Antimicrobial susceptibility testing

3.4.1 Derivation of consensus results

To allow for the scoring of AST results reported by participating laboratories (see Table 1), the EURL-PH-AMR, together with ECDC, defined the expected AST results for each strain. These expected results represent an AST consensus derived from two reference laboratories: DTU Food and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Development Laboratory. The reference laboratories used the same AST methodology. Specifically, the expected minimum inhibitory concentration (MIC) values for each strain-antimicrobial combination, and their respective interpretation, were determined by broth microdilution and interpreted using EUCAST clinical breakpoints tables v15.0 (https://www.eucast.org/clinical_breakpoints). Disk diffusion following the most recent

EUCAST recommendations was applied for the relevant strain-antimicrobial combinations. The consensus AST results were reviewed by ECDC.

During preparation of the test swabs for distribution, DTU Food performed confirmatory phenotypic testing of the test strains by broth microdilution and/or disk diffusion.

3.4.2 Instructions for participating laboratories

Participating laboratories should perform AST according to the laboratory's routine procedures, i.e. automated systems, broth microdilution, agar dilution, disk/tablet diffusion, gradient diffusion, or other methods.

Apply the most recent EUCAST clinical breakpoints for the interpretation of the obtained AST results (https://www.eucast.org/clinical_breakpoints/). This allows for categorisation of the test results into the categories resistant (R), susceptible, increased exposure (I), and susceptible, standard dosing regimen (S).

For reporting results of cefiderocol in *Acinetobacter* spp. use: wild-type (WT) = S; non-wild type (NWT) = I; likely resistant (R) = R.

All isolates included in the 2025 EURGen-Net EQA should be considered as being obtained from patients with a bloodstream infection.

> ***Note:*** *if using gradient tests, the obtained MIC values might not refer directly to a two-fold dilution concentration. In this scenario, please be advised to round up the values to the nearest upper two-fold dilution value, to ensure the correct evaluation of the obtained results. For example, an MIC of "0.75 mg/L" according to a gradient test should be reported as "1 mg/L".*

4. INCLUDED ANTIMICROBIAL AGENTS

The organism-antimicrobial combinations included in the EURGen-Net EQA are adapted from the EURL-PH AMR Laboratory testing strategy[1] (Annex 1).

> ***Note:*** *the list in Annex 1 is more inclusive than the panel of antimicrobials likely to be tested by a national reference laboratory during its typical practices. Laboratories that do not test the full panel of antimicrobials are still eligible to participate in the 2025 EURGen-Net EQA and can report partial data.*

In this EQA, the organism-antimicrobial combinations are not ranked by their level of importance to clinical practice, because there are no definitive ranking criteria that are appropriate or applicable for all countries that are eligible to participate in this EQA.

[1] https://en.ssi.dk/surveillance-and-preparedness/international-coorporation/eurl-ph-amr/guidance

5. REPORTING AST RESULTS FOR THIS EQA EXERCISE

AST results can be reported for all organism-antimicrobial combinations included in this EURGen-Net EQA exercise (Annex 1).

To report AST results, we recommend that you follow these sequential steps:

1. Carefully read the instructions for the webtool in Section 6 below;
2. First write your results on the 'test forms' for this EQA (available from: https://en.ssi.dk/surveillance-and-preparedness/international-coorporation/eurl-ph-amr/eqa-pt).
3. Transfer your results from the test forms into the webtool and submit your results (see Section 7). **Results must be submitted in the webtool no later than 10 November 2025.** The webtool allows you to view and print a report that contains your reported AST results.

When analysing the AST results please consider the following:

- For colistin and aminoglycosides (amikacin, gentamicin and tobramycin), assume administration in combination with other agents.
- Breakpoints currently based on epidemiological cut-off (ECOFF) values can be used for interpretation of results, when applicable, if no other relevant EUCAST clinical breakpoints exist.
- Currently, EUCAST recommends using disk diffusion for testing of cefiderocol, but only after consulting the EUCAST Warnings page (Warning 12) about certain media and disks (https://www.eucast.org/ast-of-bacteria/warnings).
- For fosfomycin in *E. coli* it should be assumed that intravenous administration will take place.
- For cefiderocol in *Acinetobacter* spp., report wild-type isolates as 'S'. Report non-wild type isolates which may be associated with impaired clinical response as 'I'. Report likely resistant isolates as 'R'.

6. HOW TO SUBMIT RESULTS VIA THE WEBTOOL

6.1 Login to the webtool

All contacts for a participating laboratory will receive an email containing a link to the webtool, a webtool username, and a description of how to create a webtool password. Users wishing to reset the webtool password may also consult the document 'User guide to reset the EURL-PH-AMR EQA webtool password' available on the EURL-PH-AMR website (https://en.ssi.dk/surveillance-and-preparedness/international-coorporation/eurl-ph-amr/eqa-pt).

6.2 Submitting results in the webtool

The '2025 EURL-PH-AMR EQA Webtool guide' is available for download directly from the EURL-PH-AMR website (https://en.ssi.dk/surveillance-and-preparedness/international-coorporation/eurl-ph-amr/eqa-pt). Please follow the guide carefully.

Before submitting your input for each of the strains, please ensure that you have filled in all the relevant fields as **you can only submit once per strain. Clicking on the button 'Final submit' blocks further data entry.**

Note: Final submission must be performed for each strain individually.

Submission deadline is 10 November 2025

7. EVALUATION OF SUBMITTED EQA RESULTS

7.1 Scoring system

During the first step of the EQA, if the isolate species is identified correctly, the interpretation of AST results will be evaluated using the scoring system. If the species is not identified correctly, the AST results for that isolate will not be evaluated further.

During the second step, the scoring system assesses the reported interpretations of AST results.

The scoring system considers the 'level of difficulty' and 'severity of error' of every permitted organism-antimicrobial combination. The level of difficulty, classified as 'Difficult' or 'Easy', reflects the challenge for participating laboratories to report the expected AST interpretation. 'Difficult' are situations where an AST result with a one-fold difference in dilution from the expected MIC value would have a different interpretation of S/I/R; AND/OR the expected MIC value is inside the area of technical uncertainty (ATU); AND/OR the EUCAST clinical breakpoint was recently changed in, or added to, the latest EUCAST clinical breakpoint table. 'Easy' are situations where an AST result with a one-fold difference in dilution from the expected MIC value will have the same interpretation of S/I/R; AND the EUCAST clinical breakpoint was not recently changed in, nor added to, the latest EUCAST clinical breakpoint table. The scoring of a result reflects the level of difficulty, with errors in 'difficult' results being considered as mild and errors in 'easy' results being considered as severe.

The severity of error is divided into three levels: very major error (VME), major error (ME) and no error. VME is reporting false susceptibility – expecting an R but obtaining an S or I. ME is reporting false resistance – expecting an S or I but obtaining an R. The scoring system penalises VMEs more severely for 'easy' results than for 'difficult' results and does not penalise MEs if the test is considered 'difficult'. The classification of 'no error' includes situations where one susceptibility category (S or I) is expected, but the other susceptibility category is reported. However, this results in a lower score than if the expected susceptibility category is reported.

Table 1 shows the 2025 EURGen-Net EQA scoring system.

The scoring system does not rank or group organism-antimicrobial combinations by their level of importance to clinical practice. This is because there are no definitive criteria for ranking or grouping that are appropriate and applicable to all participating laboratories.

Table 1. Scoring system of the 2025 EURGen-Net EQA exercise

		Difficulty of result and expected interpretation					
		Easy			Difficult		
		R	I	S	R	I	S
Obtained interpretation	R	1	-3 (ME)	-3 (ME)	4	0 (ME)	0 (ME)
	I	-4 (VME)	1	-1	-1 (VME)	4	2
	S	-4 (VME)	-1	1	-1 (VME)	2	4
	Not reported	-	-	-	-	-	-

Legend: R: resistant; I: susceptible, increased exposure; S: susceptible, standard dosing regimen; ME: major error; VME: very major error; - : no data.

7.2 Laboratory feedback reports

By January 2026, the laboratory contact person(s) will receive an email informing them that their evaluation report, including the score, is available for download in the password-protected webtool. The report includes scores for each strain-antimicrobial combination that can be reported.

Upon receipt of the evaluation report, laboratories are recommended to review the score for each strain-antimicrobial combination individually.

7.3 Data sharing

Participating laboratories will receive their own data in the laboratory evaluation reports (see section 7.2). ECDC will receive the anonymised EQA results. In 2026, EURL-PH-AMR will publish an annual report summarising the 2025 EURGen-Net EQA results on the EURL-PH-AMR website.

8. CONTACT

If you would like any support, or have any questions or suggestions, please do not hesitate to contact the EURGen-Net EQA management team by e-mail eurl-ph-amr@ssi.dk. In your communication with the EURGen-Net EQA management team, please use English language. If your laboratory is encountering an issue entering results, or accessing the webtool, please provide a sufficiently detailed description of the issue to ensure efficient follow-up.

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ANNEX 1 - Antimicrobials included in the 2025 EURGen-Net EQA. Adapted from the EURL-PH-AMR Laboratory testing strategy[2]

Microorganism	Antimicrobial agent
***Acinetobacter* species (ACISPP)**	Amikacin (AMK) Cefiderocol (FDC) Ciprofloxacin (CIP) Colistin (COL) Gentamicin (GEN) Imipenem (IPM) Levofloxacin (LVX) Meropenem (MEM) Tobramycin (TOB) Trimethoprim-sulfamethoxazole (ratio 1:19) (SXT)
***Escherichia coli* (ESCCOL)**	Amikacin (AMK) Aztreonam (ATM) Aztreonam-avibactam (fixed 4) (AZA) Cefiderocol (FDC) Cefotaxime (CTX) Ceftazidime (CAZ) Ceftazidime-avibactam (fixed 4) (CZA) Ceftolozane-tazobactam (fixed 4) (CZT) Ciprofloxacin (CIP) Colistin (COL) Ertapenem (ETP) Fosfomycin (FOS) Gentamicin (GEN) Imipenem (IPM)

[2] https://en.ssi.dk/surveillance-and-preparedness/international-coorporation/eurl-ph-amr/guidance

	Imipenem-relebactam (fixed 4) (IMR) Levofloxacin (LVX) Meropenem (MEM) Meropenem-vaborbactam (fixed 8) (MEV) Piperacillin-tazobactam (fixed 4) (TZP) Tigecycline (TGC) Tobramycin (TOB) Trimethoprim-sulfamethoxazole (ratio 1:19) (SXT)
Klebsiella pneumoniae **(KLEPNE)**	Amikacin (AMK) Aztreonam (ATM) Aztreonam-avibactam (fixed 4) (AZA) Cefiderocol (FDC) Cefotaxime (CTX) Ceftazidime (CAZ) Ceftazidime-avibactam (fixed 4) (CZA) Ceftolozane-tazobactam (fixed 4) (CZT) Ciprofloxacin (CIP) Colistin (COL) Ertapenem (ETP) Gentamicin (GEN) Imipenem (IPM) Imipenem-relebactam (fixed 4) (IMR) Levofloxacin (LVX) Meropenem (MEM) Meropenem-vaborbactam (fixed 8) (MEV) Piperacillin-tazobactam (fixed 4) (TZP) Tobramycin (TOB) Trimethoprim-sulfamethoxazole (ratio 1:19) (SXT)
Pseudomonas aeruginosa **(PSEAER)**	Amikacin (AMK) Aztreonam (ATM) Cefiderocol (FDC) Ceftazidime (CAZ) Ceftazidime-avibactam (fixed 4) (CZA) Ceftolozane-tazobactam (fixed 4) (CZT) Ciprofloxacin (CIP) Colistin (COL) Imipenem (IPM) Imipenem-relebactam (fixed 4) (IMR) Levofloxacin (LVX) Meropenem (MEM) Meropenem-vaborbactam (fixed 8) (MEV) Piperacillin-tazobactam (fixed 4) (TZP) Tobramycin (TOB)