EURL-PH-AMR
EU Reference Laboratory for public health on Antimicrobial Resistance
Grant agreement 101194806

Protocol for the 2026 phenotypic external quality assessment (EQA) exercise of performance of laboratories participating in the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net)

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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
Handling and safety instructions	Strains are UN3373, biological substance category B. Follow biosafety level 2 (BSL2) practices. Subculture and process the strains within 48 hours from receipt. Freeze at -80° C for future analysis.	Page 2
Sharing of strains	Strains cannot be re-distributed by the recipient laboratories.	Page 3
Antimicrobials included in this EQA	Adapted from the EURL-PH-AMR Laboratory testing strategy.	Page 3 Annex 1
Performing AST and reporting AST results	Follow the most current EUCAST guidelines. Use the routine AST methods applied in your laboratory. Broth microdilution is the recommended method for most antimicrobials. Disk diffusion is the recommended method for cefiderocol. Use S/I/R to report results for all antimicrobials, including screening agents and antimicrobials with bracketed breakpoints. 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 4 Annex 1
EURL-PH-AMR website with supporting documents	https://www.eurl-ph-amr.dk/eqa-pt	Page 5
EURGen-Net EQA webtool for submission of results	https://EURL-PH-AMR.eqa.dtu.dk	Page 4
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 4
Deadline for submission of results	9 November 2026	Page 4
Contact	eurl-ph-amr@ssi.dk	Page 7
Accreditation	DTU Food is accredited by DANAK (no. 516) under ISO/IEC 17043:2023 to provide proficiency testing schemes including the scheme for EURGen-Net EQA.	Page 1

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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 agreement 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.

The 2026 EURGEN-Net EQA is organised by the National Food Institute, Technical University of Denmark (DTU Food), in their capacity as a consortium member of the EURL-PH-AMR.

DTU Food is accredited by DANAK (no. 516) under ISO/IEC 17043:2023 to provide proficiency testing schemes including the scheme for EURGen-Net EQA.

DANAK
PT Reg.no. 516
Member EA MLA

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

The process for the 2026 EURGen-Net EQA exercise is the same as for the 2025 EQA exercise.

3.1 Overview of the EURGen-Net EQA process

In 2026, 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 2026**.

After the submission deadline, submitted data will be validated and each AST result will be scored according to the system described in Section 5. Participating laboratories will be informed via email when their evaluation report is available for download in the password-protected webtool.

The EURL-PH-AMR will publish an annual report (https://www.eurl-ph-amr.dk/eqa-pt), summarising the results from participating laboratories, with each laboratory anonymised.

3.2 Shipping, receipt, and initial processing of strains

All participating laboratories will receive a parcel containing four swabs from DTU Food. Each swab will contain a pure culture of a bacterial isolate.

The cover letter includes safety and handling instructions.

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

The laboratories are advised to 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.

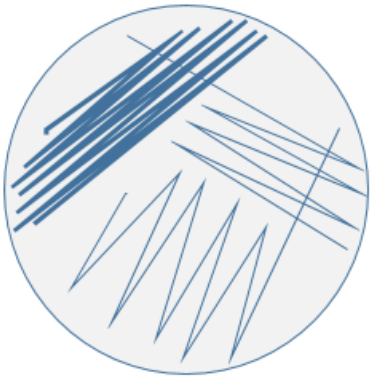


Figure 1. Plating of the test strains

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

1. Strains received for the 2026 EURGen-Net EQA cannot be re-distributed further by the recipient laboratories.
2. DTU Food cannot distribute strains to laboratories after the EQA exercise, e.g. for confirmatory, training, or reference purposes.

3.3 Determination of expected results

To enable scoring of AST results reported by participating laboratories, the EURL-PH-AMR defined the expected AST results for each strain. These expected results were established as a consensus of AST results generated by two laboratories: DTU Food and the EUCAST Development Laboratory, Växjö, Sweden. 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).

Specifically, the expected minimum inhibitory concentration (MIC) values for each strain-antimicrobial combination, and their respective interpretation, were determined by broth microdilution following EUCAST recommendations (i.e. ISO 20776-1:2019 and using MH-F broth for fastidious organisms. For antimicrobials for which disk diffusion is the recommended method, disk diffusion was performed according to the EUCAST disk diffusion manual[1]. All results were interpreted according to the EUCAST clinical breakpoints tables v16.0[2].

The consensus AST results were subsequently shared with ECDC.

During preparation of the test swabs for distribution, DTU Food performed confirmatory AST of the strains by broth microdilution and/or disk diffusion to ensure there were no alterations of the strains.

3.4 Included antimicrobial agents

The organism-antimicrobial combinations included in the EURGen-Net EQA are listed in Annex 1, which are adapted from the EURL-PH AMR Laboratory testing strategy[3].

> ***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 2026 EURGen-Net EQA and can report partial data.*

[1] https://www.eucast.org/bacteria/methodology-and-instructions/disk-diffusion-and-quality-control/
[2] https://www.eucast.org/bacteria/clinical-breakpoints-and-interpretation/clinical-breakpoint-tables/
[3] https://www.eurl-ph-amr.dk/guidance

4. PERFORMING AND REPORTING AST RESULTS

4.1 Performing AST

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.

> ***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".*

All isolates included in the 2026 EURGen-Net EQA should be considered as being obtained from patients with a bloodstream infection.

4.2 Interpretation of AST results

For the purpose of this EQA exercise, all test results must be reported using the categories resistant (R), susceptible, increased exposure (I), and susceptible, standard dosing regimen (S). Follow the instructions below when reporting AST results, including specific adjustments to EUCAST reporting guidelines:

- 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'.

4.3 Login to the webtool

All contacts for a participating laboratory will receive an email containing a link to the webtool, a username, and instructions on how to create or reset a password, and how to get access to the webtool. A laboratory can have more than one registered email address.

4.4 Submitting results in the webtool

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

To report AST results, participating laboratories are recommended to follow the sequential steps below:

1. Carefully read the instructions for the webtool '2026 EURL-PH-AMR EQA Webtool guide' available from: https://www.eurl-ph-amr.dk/eqa-pt
2. First record your results on the 'test forms' for this EQA available from: https://www.eurl-ph-amr.dk/eqa-pt
3. Transfer the results from the test forms into the webtool and submit your results

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.**

The webtool allows laboratories to view and print a report containing the submitted results.

> **Note***: Final submission must be performed for each strain individually.*
> *Results must be submitted in the webtool no later than **10 November 2026**.*

5. EVALUATION OF SUBMITTED RESULTS

5.1 Scoring system

The webtool for the 2026 EURGen-Net EQA will apply the same scoring system that was used in 2025 EURGen-Net EQA.

During the first step in the EQA, if an isolate is identified with the correct species, the interpretation of AST results will be evaluated using the scoring system. Conversely, if the species identification is incorrect, 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 implemented scoring system for the evaluation of interpreted results takes the 'level of difficulty' and the 'severity of error' into account for each organism-antimicrobial combination. The level of difficulty reflects the likelihood of misclassification and is categorized as 'difficult' or 'easy'.

'Difficult' situations include cases where:

- An AST result with a one-fold dilution difference from the expected MIC value, or with a zone diameter difference of ± 3 mm from the expected diameter, would result in a different S/I/R interpretation; and/or
- The expected MIC value or diameter falls within the area of technical uncertainty (ATU); and/or
- The relevant EUCAST clinical breakpoint had been recently changed or newly introduced in the latest EUCAST breakpoint table.

'Easy' situations include cases where:

- An AST result with a one-fold dilution difference from the expected MIC value or within ± 3 mm of the expected diameter would lead to the same S/I/R interpretation; and/or
- The EUCAST clinical breakpoint had not been recently changed or added.

Errors are classified into three categories: very major error (VME), major error (ME) and no error. Both VME and ME are penalised in the scoring system. A VME is defined as reporting false susceptibility, i.e. the expected result is R, but the reported result is S or I; and a ME is defined as reporting false resistance, i.e. the expected results is S or I, but the reported result is R.

Table 1 shows the 2026 EURGen-Net EQA scoring system. 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.

The scoring system does not rank or group organism-antimicrobial combinations according to 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 countries. Such analyses can instead be performed at (sub-)national level using the database of national results that is sent to NECs.

Table 1. Scoring system of the 2026 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.

5.2 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 contains scores for each organism-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.

> ***Note**: All participating laboratories are encouraged to perform a self-evaluation of the accuracy, adequacy, and reliability of the AST methods and procedures used, and to assess whether corrective actions are needed.*

5.3 Data sharing

ECDC will receive the anonymised EQA results. In 2027, EURL-PH-AMR will publish an annual report summarising the 2026 EURGen-Net EQA results on the EURL-PH-AMR website. The laboratories will be anonymised.

6. 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 write in English. If your laboratory is encountering an issue entering results, or accessing the webtool, please provide a sufficiently detailed description of the issue including screen dumps which include the URL to ensure efficient follow-up.

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ANNEX 1 - ANTIMICROBIALS INCLUDED IN THE 2026 EURGEN-NET EQA.

Adapted from the EURL-PH-AMR Laboratory testing strategy[4]

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)
Enterobacterales	Amikacin (AMK) Aztreonam (ATM) Aztreonam-avibactam (Fixed 4 mg/L) (AZA) Cefiderocol (FDC) Cefotaxime (CTX) Ceftazidime (CAZ) Ceftazidime-avibactam (Fixed 4 mg/L) (CZA) Ceftolozane-tazobactam (Fixed 4 mg/L) (CZT) Ciprofloxacin (CIP) Colistin (COL) Ertapenem (ETP) Fosfomycin (FOS) (Only *E. coli*) Gentamicin (GEN) Imipenem (IPM) Imipenem-relebactam (Fixed 4 mg/L) (IMR) Levofloxacin (LVX) Meropenem (MEM) Meropenem-vaborbactam (Fixed 8 mg/L) (MEV) Piperacillin-tazobactam (Fixed 4 mg/L) (TZP) Tigecycline (TGC) (Only *E. coli*) Tobramycin (TOB) Trimethoprim-sulfamethoxazole (ratio 1:19) (SXT)
Pseudomonas aeruginosa (PSEAER)	Amikacin (AMK) Aztreonam (ATM) Cefiderocol (FDC) Ceftazidime (CAZ)

[4] https://www.eurl-ph-amr.dk/guidance

Microorganism	Antimicrobial agent
	Ceftazidime-avibactam (Fixed 4 mg/L) (CZA) Ceftolozane-tazobactam (Fixed 4 mg/L) (CZT) Ciprofloxacin (CIP) Colistin (COL) Imipenem (IPM) Imipenem-relebactam (Fixed 4 mg/L) (IMR) Levofloxacin (LVX) Meropenem (MEM) Meropenem-vaborbactam (Fixed 8 mg/L) (MEV) Piperacillin-tazobactam (Fixed 4 mg/L) (TZP) Tobramycin (TOB)