EQAsia EQA8 trial

Protocol

Identification and antimicrobial susceptibility testing (AST) of *Escherichia coli, Klebsiella pneumoniae, Acinetobacter spp.* and *Staphylococcus aureus* test strains

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1 INTRODUCTION

The EQAsia project aims to strengthen the provision of External Quality Assessment (EQA) services across the One Health sector in South and Southeast Asia. Therefore, a comprehensive and high-quality EQA program for antimicrobial resistance (AMR) is offered to all the National Reference Laboratories/Centres of Excellence in the region since 2021. The EQA trials are organized by the consortium of EQAsia and supported by the Fleming Fund.

The **EQAsia EQA8 trial** includes four EQA panels each composed of seven test strains – *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter spp.*, and *Staphylococcus aureus*, respectively. Each of the four panels includes five strains of the targeted species and two non-target strains. Participating laboratories are asked to perform identification of all seven test strains from the panels they signed up for, as well as antimicrobial susceptibility testing (AST) only on the five target strains in each panel.

Additionally, AST of the relevant reference strains for quality control (QC) is also part of each EQA trial round. The QC reference strains supplied during previous EQA rounds are *Escherichia coli* ATCC 25922/CCM 3954, *E. coli* NCTC 13846/CCM 8874 (for colistin), *Pseudomonas aeruginosa* ATCC 27853/CCM 3955, *S. aureus* ATCC 25923/CCM 3953 (for disk diffusion) and *S. aureus* ATCC 29213/CCM 4223 (for MIC). These reference strains are original CERTIFIED cultures provided free of charge, and should be used for future internal quality control for antimicrobial susceptibility testing in your laboratory. Therefore, please take proper care of these strains.

2 OBJECTIVES

The main objective of this EQA is to support laboratories to assess and, if necessary, improve the identification and antimicrobial susceptibility testing of pathogens, specifically *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter spp.*, and *Staphylococcus aureus*. Therefore, the laboratory work for this EQA should be performed using the methods routinely used in your own laboratory.

3 EQA8 OUTLINE

3.1 Shipping and receipt of strains

Your laboratory is one of the 38 human health and animal health laboratories from South and Southeast Asia participating in EQA8. In April 2024, you are expected to receive a parcel containing one or more of the following panels:

- ***Escherichia coli* panel** - seven test strains of which five are *E. coli* and two are non-target species. The *Escherichia coli* ATCC 25922/CCM 3954 and *E. coli* NCTC 13846/CCM 8874 (for colistin) reference strains have been provided in previous EQA rounds.

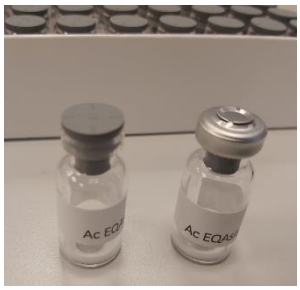
- ***Klebsiella pneumoniae* panel** - seven test strains of which five are *K. pneumoniae* and two are non-target species. The *Escherichia coli* ATCC 25922/CCM 3954 and *E. coli* NCTC 13846/CCM 8874 (for colistin) reference strains have been provided in previous EQA rounds.

- ***Acinetobacter spp.* panel** - seven test strains of which five are *Acinetobacter spp.* and two are non-target species. The reference strain to be used for this panel is *Pseudomonas aeruginosa* ATCC 27853/CCM 3955 and it has been provided in a previous EQA round.

- ***Staphylococcus aureus* panel** - seven test strains of which five are *S. aureus* and two are non-target species. The *S. aureus* ATCC 25923/CCM 3953 (for disk diffusion) and *S. aureus* ATCC 29213/CCM 4223 (for MIC) reference strains have been provided in previous EQA rounds.

Please confirm receipt of the parcel through the confirmation form enclosed in the shipment

N.B.!!! All the isolates are shipped lyophilized. The *E. coli* and *S. aureus* isolates are sent in ampoules. The *K. pneumoniae* and *Acinetobacter spp.* isolates are sent in vials.



3.2 Reviving and storage of strains

The **lyophilized strains** must be stored in a dark, cool place. The strains must be sub-cultured and prepared for storage in your strain collection (e.g., in a -80°C freezer). Aseptic technique must be applied throughout. All testing should be performed in a BSL2 level laboratory or in a biosafety cabinet class II.

- **Needed material**:
    - An ampoule cutter or a file (for the ampoules)
    - Tweezers (for the vials)
    - 70% alcohol
    - Sterile Luria Bertani (LB) broth
    - Agar plates (5 to 6 plates per one strain)
    - Autopipette with tips or Pasteur pipettes
    - Inoculating loop
    - Sterile syringe and needle (optional)

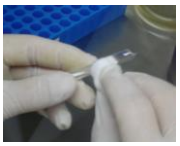
- To open and reconstitute the **ampoules**:

1. Carefully take the ampoule out of the wrap.

   Note: To maintain the vacuum condition, do not break the tip of the ampoule. Otherwise, the air will enter the ampoule and the cotton wool plug will be pushed down and in contact with dried bacterial culture. If it happens, please simply remove the cotton plug with forceps.

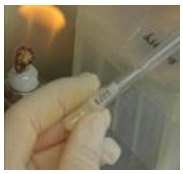
   Note: The ampoule can be cut in the middle or below the cotton wool plug.

2. Wipe the ampoule neck with 70% alcohol-dampened cotton wool.



3. Make a deep score on the around the circumference of the ampoule near the middle of the plug using ampoule cutter or a file. The ampoule should be cut in the middle or below the cotton wool plug.

4. Wrap thick cotton wool around the ampoule and break at the marked area.
5. Remove the pointed end of the ampoule and cotton into a biohazard container.



6. Pipette 0.5 ml of sterile LB broth into the dried cells. Mix gently and carefully to avoid creating aerosols.
7. Transfer one drop of each strain onto one LB agar plate using autopipette or Pasteur pipette. Then, streak the isolate using inoculating loop to get single colonies on plate. The remaining suspension is stored in a screw cap test tube.

8. Incubate the inoculated plates and the suspension tubes at 37°C overnight and observe the bacterial growth.

- To open and reconstitute the **vials:**

1. Flip up the round part of the metal cap using tweezers.
2. The entire metal ring and rubber stopper can be removed.
3. Pipette 0.5 ml of sterile LB broth into the vial with dried cells. Mix gently and carefully to avoid creating aerosols.
4. Incubate the vial for 10-15 minutes at 37°C with the rubber stopper on. Be careful to avoid contamination.
5. Transfer one drop of each strain onto one LB agar plate using autopipette or Pasteur pipette. Then, streak the isolate using inoculating loop to get single colonies on plate. The remaining suspension is stored in a screw cap test tube.
6. Incubate the inoculated plates and the suspension tubes at 37°C overnight and observe the bacterial growth.

OR

2. Alternatively, after step 1 you can keep the metal ring and rubber stopper on the vial. Sterilize the exposed part of the rubber stopper with 70% alcohol.
3. Using a syringe and needle, aseptically take 0.5 ml of sterile LB broth.
4. Insert the needle through the rubber stopper into the vial and inject the content of the syringe.
5. Incubate the vial for 10-15 minutes at 37°C.
6. Take a few drops of the content of the vial using a sterile syringe and needle and inoculate media appropriate for the strain type. Then, streak the isolate using inoculating loop to get single colonies on plate. The remaining suspension is stored in a screw cap test tube.
7. Incubate the inoculated plates and the suspension tubes at 37°C overnight and observe the bacterial growth.

It is furthermore recommended that the strains are stored in your strain collection (e.g., in a -80°C freezer), at least until you have reviewed your results from this EQA trial. The stored test strains should serve as reference if discrepancies are detected during the testing (e.g., they can be used to detect errors such as mislabelling or contamination), and they can also serve as reference material available at a later stage, when needed.

- **Safety precautions**:

All provided strains are considered as UN3373, Biological substance category B. These strains can potentially be harmful to humans and pose a risk due to their possible pan-resistant profile, therefore becoming a challenge in the treatment of a potential human infection. It is the recipient laboratory's responsibility to comply with national legislation, rules and regulations regarding the correct use and handling of the provided test strains, and to possess the proper equipment and protocols to handle these strains. Nevertheless, it is recommended to handle the strains in a BSL2 containment facility using equipment and operational practices for work involving infectious or potentially infectious materials. The containment and operational requirements may vary with the species, subspecies, and/or strains, thus, please take the necessary precautions.

Please consult the Pathogen Safety Data Sheets (PSDSs) produced by the Public Health Agency of Canada. The PSDSs of each pathogen can be found in the bottom of the page. These PSDSs are technical documents that describe the hazardous properties of human pathogens, and provide recommendations for the work involving these agents in a laboratory setting.

3.3 Identification of *Escherichia coli, Klebsiella pneumoniae, Acinetobacter spp.,* and *Staphylococcus aureus* test strains

Each of the four panels in this EQA round contains five target species. i.e. five *E. coli* isolates in the *E. coli* panel. The remaining two isolates in each panel are non-target species – their identification differs from the five target species.

Please follow the routinely used methods in your own laboratory for **identification** of all panel strains.

3.4 Antimicrobial susceptibility testing of *Escherichia coli, Klebsiella pneumoniae, Acinetobacter spp.,* and *Staphylococcus aureus* test strains, and of the reference strains

The strains identified as *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter spp.* and *Staphylococcus aureus* (five isolates from each panel), as well as the appropriate reference strains, should be tested for susceptibility towards as many as possible of the antimicrobials indicated in the test form and in **Tables 1-4**. Note that some of the antimicrobials (highlighted) could be omitted by the Human Health laboratories. Please use the methods routinely used in your own laboratory.

The reference range values used in this EQA for interpreting MIC and disk diffusion results are in accordance with current zone diameter and MIC breakpoint values developed by CLSI (M100, 33rd Ed.). When not available, EUCAST clinical breakpoints (Tables v. 13.0, 2023) or epidemiological cut off values (https://mic.eucast.org/) were used instead. The breakpoint values for *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter spp.* and *Staphylococcus aureus* can be found in **Tables 1-4**, respectively. **Please make sure to use the correct table for the interpretation**.

Table 1. Breakpoints for interpretation of MICs and zone diameters for *E. coli*

The highlighted antimicrobials could be omitted by the Human Health laboratories.

Antimicrobials	Reference values MIC (µg/mL)			Reference values Disk diffusion (mm)		
	S	I	R	S	I	R
Amikacin, AMK	**≤ 4**	**8**	**≥ 16**	**≥ 20**	**17-19**	**≤ 16**
Ampicillin, AMP	≤ 8	16	≥ 32	≥ 17	14-16	≤ 13
Azithromycin, AZI	≤ 16	-	≥ 32	≥ 13	-	≤ 12
Cefepime, FEP	≤ 2	4-8	≥ 16	≥ 25	19-24	≤ 18
Cefotaxime, FOT	≤ 1	2	≥ 4	≥ 26	23-25	≤ 22
Cefotaxime + clavulanic acid, F/C	NA	NA	NA	NA	NA	NA
Cefoxitin, FOX	≤ 8	16	≥ 32	≥ 18	15-17	≤ 14
Ceftazidime, TAZ	≤ 4	8	≥ 16	≥ 21	18-20	≤ 17
Ceftazidime + clavulanic acid, T/C	NA	NA	NA	NA	NA	NA
Chloramphenicol, CHL	≤ 8	16	≥ 32	≥ 18	13-17	≤ 12
Ciprofloxacin, CIP	≤ 0.25	0.5	≥ 1	≥ 26	22-25	≤ 21
Colistin, COL	-	≤ 2	≥ 4	NA	NA	NA
Doripenem, DOR	≤ 1	2	≥ 4	≥ 23	20-22	≤ 19
Ertapenem, ETP	≤ 0.5	1	≥ 2	≥ 22	19-21	≤ 18
Gentamicin, GEN	**≤ 2**	**4**	**≥ 8**	**≥ 18**	**15-17**	**≤ 14**
Imipenem, IMI	≤ 1	2	≥ 4	≥ 23	20-22	≤ 19
Levofloxacin, LEVO	≤ 0.5	1	≥ 2	≥ 21	17-20	≤ 16
Meropenem, MERO	≤ 1	2	≥ 4	≥ 23	20-22	≤ 19
Nalidixic acid, NAL	≤ 16	-	≥ 32	≥ 19	14-18	≤ 13
Piperacillin/tazobactam, PT4	≤ 8/4	16/4	≥ 32/4	≥ 25	21-24	≤ 20
Sulfamethoxazole, SMX	≤ 256	-	≥ 512	≥ 17	13-16	≤ 12
Tetracycline, TET	≤ 4	8	≥ 16	≥ 15	12-14	≤ 11
Tigecycline, TGC*	≤ 0.5	-	≥ 1	≥ 18	-	≤ 17
Tobramycin, TOB	**≤ 2**	**4**	**≥ 8**	**≥ 17**	13-**16**	≤ 12
Trimethoprim, TMP	≤ 8	-	≥ 16	≥ 16	11-15	≤ 10
Trimethoprim/sulfamethoxazole, SXT	≤ 2/38	-	≥ 4/76	≥ 16	11-15	≤ 10

Reference values are based on Enterobacterales breakpoints from CLSI M100, 33rd Ed.

*Reference values are based on Enterobacterales clinical breakpoints from www.eucast.org (Tables v. 13.0, 2023)

Table 2. Breakpoints for interpretation of MICs and zone diameters for *K. pneumoniae*

The highlighted antimicrobials could be omitted by the Human Health laboratories.

Antimicrobials	Reference values MIC (µg/mL)			Reference values Disk diffusion (mm)		
	S	I	R	S	I	R
Amikacin, AMK	**≤ 4**	**8**	**≥ 16**	**≥ 20**	**17-19**	**≤ 16**
Ampicillin, AMP	≤ 8	16	≥ 32	≥ 17	14-16	≤ 13
Azithromycin, AZI	≤ 16	-	≥ 32	≥ 13	-	≤ 12
Cefepime, FEP	≤ 2	4-8	≥ 16	≥ 25	19-24	≤ 18
Cefotaxime, FOT	≤ 1	2	≥ 4	≥ 26	23-25	≤ 22
Cefotaxime/clavulanic acid, F/C	NA	NA	NA	NA	NA	NA
Cefoxitin, FOX	≤ 8	16	≥ 32	≥ 18	15-17	≤ 14
Ceftazidime, TAZ	≤ 4	8	≥ 16	≥ 21	18-20	≤ 17
Ceftazidime/clavulanic acid, T/C	NA	NA	NA	NA	NA	NA
Chloramphenicol, CHL	≤ 8	16	≥ 32	≥ 18	13-17	≤ 12
Ciprofloxacin, CIP	≤ 0.25	0.5	≥ 1	≥ 26	22-25	≤ 21
Colistin, COL	-	≤ 2	≥ 4	NA	NA	NA
Doripenem, DOR	≤ 1	2	≥ 4	≥ 23	20-22	≤ 19
Ertapenem, ETP	≤ 0.5	1	≥ 2	≥ 22	19-21	≤ 18
Gentamicin, GEN	**≤ 2**	**4**	**≥ 8**	**≥ 18**	**15-17**	**≤ 14**
Imipenem, IMI	≤ 1	2	≥ 4	≥ 23	20-22	≤ 19
Levofloxacin, LEVO	≤ 0.5	1	≥ 2	≥ 21	17-20	≤ 16
Meropenem, MERO	≤ 1	2	≥ 4	≥ 23	20-22	≤ 19
Nalidixic acid, NAL	≤ 16	-	≥ 32	≥ 19	14-18	≤ 13
Piperacillin/tazobactam, PT4	≤ 8/4	16/4	≥ 32/4	≥ 25	21-24	≤ 20
Sulfamethoxazole, SMX	≤ 256	-	≥ 512	≥ 17	13-16	≤ 12
Tetracycline, TET	≤ 4	8	≥ 16	≥ 15	12-14	≤ 11
Tigecycline, TGC*	≤ 2	-	≥ 4	NA	NA	NA
Tobramycin, TOB	**≤ 2**	**4**	**≥ 8**	**≥ 17**	13-**16**	≤ 12
Trimethoprim, TMP	≤ 8	-	≥ 16	≥ 16	11-15	≤ 10
Trimethoprim/sulfamethoxazole, SXT	≤ 2/38	-	≥ 4/76	≥ 16	11-15	≤ 10

Reference values are based on Enterobacterales breakpoints from CLSI M100, 33rd Ed.
*Reference values are based on *K. pneumoniae* epidemiological cut off values from https://mic.eucast.org/ on January 2023.

Beta-lactam and carbapenem resistance

The following tests for detection of ESBL-, AmpC-, and carbapenemase-producing phenotypes are recommended for ***E. coli*** and ***K. pneumoniae***:

- Reduced susceptibility to cefotaxime (FOT) and/or ceftazidime (TAZ): it indicates that the bacterial strain may be an ESBL-, AmpC, or carbapenemase-producer. These strains should be tested for ESBL-, AmpC, or carbapenemase-production by confirmatory tests.

- Confirmatory test for ESBL production: it requires the use of both cefotaxime (FOT) and ceftazidime (TAZ) alone, as well as in combination with a β-lactamase inhibitor (clavulanic acid). Synergy can be determined by broth microdilution methods, Gradient Test or Disk Diffusion:

    - It is defined as a ≥ 3 two-fold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs. its MIC when tested alone (Gradient Test 3 dilution steps difference; MIC FOT: FOT/Cl or TAZ: TAZ/Cl ratio ≥ 8).

    - A positive synergy testing for Disk Diffusion is defined as ≥ 5 mm increase of diameter of FOT or TAZ in combination with clavulanic acid (FOT/Cl or TAZ/Cl) compared to testing them alone. The presence of synergy indicates ESBL production.

- Detection of AmpC-type beta-lactamases: it can be performed by testing the bacterial culture for susceptibility to cefoxitin (FOX). Resistance to FOX indicates the presence of an AmpC-type beta-lactamase.

- Confirmatory test for carbapenemase production: it requires the testing of meropenem (MERO) and combination disk test method incl. meropenem ± various inhibitors, i.e. boronic acid, dipicolinic acid or EDTA, cloxacillin.

It should be noted that some resistance mechanisms do not always confer clinical resistance. Therefore, the classification of the phenotypic results (**Figure 1** below) should be based on the "EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance", Version 2.0, July 2017, and the most recent EFSA recommendations – The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018. EFSA Journal 2020;18 (3) https://doi.org/10.2903/j.efsa.2020.6007

1. ESBL-Phenotype		
	MIC (mg/L)	Zone Diameter (mm)
FOT or TAZ	> 1	< 21 (FOT); < 22 (TAZ)
MERO	≤ 0.12	≥ 25
FOX	≤ 8	≥ 19
FOT/CLV and/or TAZ/CLV	SYNERGY	SYNERGY

2. AmpC-Phenotype		
	MIC (mg/L)	Zone Diameter (mm)
FOT or TAZ	> 1	< 21 (FOT); < 22 (TAZ)
MERO	≤ 0.12	≥ 25
FOX	> 8	< 19
FOT/CLV and/or TAZ/CLV	NO SYNERGY	NO SYNERGY

3. ESBL + AmpC-Phenotype		
	MIC (mg/L)	Zone Diameter (mm)
FOT or TAZ	> 1	< 21 (FOT); < 22 (TAZ)
MERO	≤ 0.12	≥ 25
FOX	> 8	< 19
FOT/CLV and/or TAZ/CLV	SYNERGY	SYNERGY

4. Carbapenemase-Phenotype		
	MIC (mg/L)	Zone Diameter (mm)
MERO	> 0.12	< 25

5. Other Phenotypes		
	MIC (mg/L)	Zone Diameter (mm)
1)		
FOT or TAZ	> 1	< 21 (FOT); < 22 (TAZ)
MERO	≤ 0.12	≥ 25
FOX	≤ 8	≥ 19
FOT/CLV and/or TAZ/CLV	NO SYNERGY	NO SYNERGY
2)		
FOT or TAZ	≤ 1	≥ 21 (FOT); ≥ 22 (TAZ)
MERO	≤ 0.12	≥ 25
FOX	> 8	< 19

Susceptible		
	MIC (mg/L)	Zone Diameter (mm)
FOT or TAZ	≤ 1	≥ 21 (FOT); ≥ 22 (TAZ)
MERO	≤ 0.12	≥ 25
FOX	≤ 8	≥ 19

Figure 1: Adapted from EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2020 – The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018 – and in accordance with the EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance, Version 2.0, July 2017.

Genotypic testing by PCR and/or sequencing may be necessary to correctly categorize a bacterial test strain as either ESBL-, AmpC, and/or carbapenemase-producer, but it is **not** required as part of this EQA.

Table 3. Breakpoints for interpretation of MICs and zone diameters for *Acinetobacter spp.*

The highlighted antimicrobials could be omitted by the Human Health laboratories.

Antimicrobials	Reference value MIC (µg/mL)			Reference value Disk diffusion (mm)		
	S	I	R	S	I	R
Amikacin, AMK	≤ 16	32	≥ 64	≥ 17	15-16	≤ 14
Cefepime, FEP	≤ 8	16	≥ 32	≥ 18	15-17	≤ 14
Cefotaxime, FOT	≤ 8	16-32	≥ 64	≥ 23	15-22	≤ 14
Ceftazidime, TAZ	≤ 8	16	≥ 32	≥ 18	15-17	≤ 14
Ciprofloxacin, CIP	≤ 1	2	≥ 4	≥ 21	16-20	≤ 15
Colistin, COL	-	≤ 2	≥ 4	NA	NA	NA
Doripenem, DOR	≤ 2	4	≥ 8	≥18	15-17	≤ 14
Doxycycline, DOX	≤ 4	8	≥ 16	≥13	10-12	≤ 9
Gentamicin, GEN	≤ 4	8	≥ 16	≥ 15	13-14	≤ 12
Imipenem, IMI	≤ 2	4	≥ 8	≥ 22	19-21	≤ 18
Levofloxacin, LEVO	≤ 2	4	≥ 8	≥ 17	14-16	≤ 13
Meropenem, MERO	≤ 2	4	≥ 8	≥ 18	15-17	≤ 14
Minocycline, MIN	≤ 4	8	≥ 16	≥ 16	13-15	≤ 12
Piperacillin/tazobactam, PT4	≤ 16/4	32/4-64/4	≥ 128/4	≥ 21	18-20	≤ 17
Tigecycline, TGC*	≤ 0.5	-	≥ 1	NA	NA	NA
Tobramycin, TOB	≤ 4	8	≥ 16	≥ 15	13-14	≤ 12
Trimethoprim/sulfamethoxazole, SXT	≤ 2/38	-	≥ 4/76	≥ 16	11-15	≤ 10

Reference values are based on *Acinetobacter spp.* breakpoints from CLSI M100, 33rd Ed.

*Reference values are based on *Acinetobacter spp.* epidemiological cut off values from https://mic.eucast.org/ on January 2023.

Table 4. Breakpoints for interpretation of MICs and zone diameters for *S. aureus*

The highlighted antimicrobials could be omitted by the Human Health laboratories.

Antimicrobials	Reference value MIC (µg/mL)			Reference value Disk diffusion (mm)		
	S	I	R	S	I	R
Cefoxitin, FOX	≤ 4	-	≥ 8	≥ 22	-	≤ 21
Chloramphenicol, CHL	≤ 8	16	≥ 32	≥ 18	13-17	≤ 12
Ciprofloxacin, CIP	≤ 1	2	≥ 4	≥ 21	16-20	≤ 15
Clindamycin, CLI	≤ 0.5	1-2	≥ 4	≥ 21	15-20	≤ 14
Erythromycin, ERY	≤ 0.5	1-4	≥ 8	≥ 23	14-22	≤ 13
Fusidic acid, FUS*	≤ 1	-	≥ 2	≥ 24	-	≤ 23
Gentamicin, GEN	≤ 4	8	≥ 16	≥ 15	13-14	≤ 12
Kanamycin, KAN*	≤ 8	-	≥ 16	≥ 18	-	≤ 17
Linezolid, LZD	≤ 4	-	≥ 8	≥ 21	-	≤ 20
Penicillin, PEN	≤ 0.12	-	≥ 0.25	≥ 29	-	≤ 28
Quinupristin/dalfopristin, SYN	≤ 1	2	≥ 4	≥ 19	16-18	≤ 15
Rifampin, RIF	≤ 1	2	≥ 4	≥ 20	17-19	≤ 16
Sulfamethoxazole, SMX	≤ 256	-	≥ 512	≥ 17	13-16	≤ 12
Tetracycline, TET	≤ 4	8	≥ 16	≥ 19	15-18	≤ 14
Trimethoprim, TMP	≤ 8	-	≥ 16	≥ 16	11-15	≤ 10
Vancomycin, VAN	≤ 2	4-8	≥ 16	NA	NA	NA

Reference values are based on *Staphylococcus aureus* breakpoints from CLSI M100, 33rd Ed.
*Reference values are based on *Staphylococcus aureus* clinical breakpoints from www.eucast.org (Tables v. 13.0, 2023).

4 SUBMISSION OF RESULTS VIA THE INFORMATICS MODULE

We recommend that you write your results in the enclosed test forms as it will help you when transferring results onto the online platform.

The detailed 'Guideline for reporting results in the EQAsia Informatics Module' is available for download directly from the EQAsia website. Please follow the guideline carefully.

Login to the Informatics Module:

Access the Informatics Module (incognito window) via the following link https://eqasia-pt.dtu.dk/

When first given access to login to the Informatics Module, your **personal loginID and password** is sent to you by email.

Note that the primary contact person for a participating institution is registered both as primary and secondary contact. Should you like to add another person as the secondary contact, please contact eqasia@food.dtu.dk

When you submit your results, remember to have by your side the completed test forms (template available for download from the EQAsia website). If the same reference strain is used for different pathogens, please enter the results (even if the same) for all the pathogens.

Results must be submitted no later than June 7th, 2024.

If you have troubles entering your results or if you experience technical problems with the informatics module, please contact the DTU team directly at eqasia@food.dtu.dk, explaining the issues that you encountered.

Before submitting your final input for all the organisms, please ensure that you have filled in all the relevant fields as **you can only 'finally submit' once**! 'Final submit' blocks further data entry.

After submission, the Informatics Module will allow you to view and print a report with your submitted results.

5 EVALUATION OF RESULTS

The scores for the submitted results will be released after the submission deadline has passed. Then, you will be able to access the evaluation of your results. Results in agreement with the expected interpretation are categorised as '4' (correct), while results deviating from the expected interpretation are categorised as '3' (incorrect, minor), '1' (incorrect, major) or '0' (incorrect, very major).

SCORES		Obtained Interpretation		
		Susceptible	Intermediate	Resistant
Expected Interpretation	Susceptible	4	3	1
	Intermediate	3	4	3
	Resistant	0	3	4

0	Incorrect: very major
1	Incorrect: major
3	Incorrect: minor
4	Correct

Once the results have been evaluated, you will be able to access your certificate via the EQAsia Informatics Module. You will be notified by email when the certificate is available. The certificate will contain score for identification and for susceptibility testing for each of the panels for which you submitted results. Performance rate for each panel will also be shown on the certificate.

The EQAsia project team would like to thank you once again for your participation in this EQA round!