Protocol for EQAsia EQA5 2022

ID and antimicrobial susceptibility testing of *Campylobacter jejuni* and *C. coli*, *Enterococcus faecium* and *E. faecalis*, and *Streptoccocus pneumoniae* test strains

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 during 2021-22. The EQA is organized by the consortium of EQAsia and supported by the Fleming Fund.

The EQAsia EQA5 2022 includes the antimicrobial susceptibility testing of five *Campylobacter jejuni* / *C. coli*, five *Enterococcus faecium* / *E. faecalis* and five *Streptococcus pneumoniae* strains **identified** among a total of **seven** test strains for each microorganism, which include two non-target species strains.

Additionally, antimicrobial susceptibility testing of the relevant reference strains for quality control (QC) in relation to antimicrobial susceptibility testing is included. The QC reference strains supplied (or that have been supplied in previous EQAS) are: *Campylobacter jejuni* ATCC 33560/ CCM 6214, *Staphylococcus aureus* ATCC 25923/ CCM 3953 (for disk diffusion of the Enterococci), *Enterococcus faecalis* ATCC 29212/ CCM 4224 (for MIC) and *Streptococcus pneumoniae* ATCC 49619/ CCM 4501. 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. Handle and maintain them as suggested in the manual 'Subculture and maintenance of quality control strains' available on the EQAsia website.

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 *C. jejuni* / *C. coli*, *E. faecium* / *E. faecalis* and *S. pneumoniae*. Therefore, the laboratory work for this EQA should be performed using the methods routinely used in your own laboratory.

3 OUTLINE OF THE EQASIA EQA

3.1 Shipping, receipt and storage of strains

In September 2022, it is expected that National Reference Laboratories located in South and Southeast Asia will receive a parcel containing one or more of the following:

- Seven test strains of which five are *C. jejuni* or *C. coli*, in addition to two non-target species strains. The *Campylobacter jejuni* ATCC 33560/ CCM 6214 will be provided as reference strain (if not already received in previous EQAs).

- Seven test strains of which five are *E. faecium* or *E. faecalis*, in addition to two non-target species strains. The *Staphylococcus aureus* ATCC 25923/CCM 3953 (for disk diffusion) and *Enterococcus faecalis* ATCC 29212/ CCM 4224 (for MIC) will be provided as reference strains (if not already received in previous EQAs).

- Seven test strains of which five are *S. pneumoniae*, in addition to two non-target species strains. The *Streptococcus pneumoniae* ATCC 49619/CCM 4501 will be provided as reference strain (if not already received in previous EQAs).

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

All strains are shipped lyophilized. The lyophilized strains must be stored in a dark, dry and cool place. The strains must be sub-cultured and prepared for storage in your strain collection (e.g. in a -80°C freezer). This set of cultures 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 function as reference material available for reference at a later stage, when needed.

For reconstitution of the test strains, please see the document 'Instructions for opening and reviving lyophilised cultures of test strains' on the EQAsia website.

For reconstitution of the QC reference strains, please see the document 'Subculture and maintenance of quality control strains' on the EQAsia website.

All provided strains belong to 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.

3.2 Identification of *C. jejuni / C. coli, E. faecium / E. faecalis* and *S. pneumoniae* test strains

For each test species, two out of the seven test strains related to each bacterial species does not belong to the target species of the EQA trial. To identify the five cultures of the correct target species among the seven test strains, you should use the method routinely used in your own laboratory for **identification** of the organism.

3.3 Antimicrobial susceptibility testing of *C. jejuni / C. coli, E. faecium / E. faecalis* and *S. pneumoniae* test strains, and of the reference strains

The strains identified as *C. jejuni / C. coli*, *E. faecium / E. faecalis* and *S. pneumoniae*, as well as the appropriate reference strains, should be tested for susceptibility towards as many as possible antimicrobials listed in **Tables 1-3**, but always considering their relevance regarding the laboratory's routine work. 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 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, 32nd Ed.). When not available, EUCAST clinical breakpoints (Tables v. 12.0, 2022) or epidemiological cut off values (https://mic.eucast.org/) are used instead. The breakpoint values for *C. jejuni / C. coli*, *E. faecium / E. faecalis* and *S. pneumoniae* can be found in **Tables 1-3**, respectively. **Make sure to use the correct table for the interpretation**.

Interpretation of MIC or disk diffusion results will lead to categorization of the result into one of the categories: **resistant** (R), **intermediate** (I) or **susceptible** (S). In the evaluation report you receive

upon the submission deadline, the obtained interpretations in comparison with the expected interpretation will be evaluated/scored as follows:

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

Table 1. Interpretive criteria for *C. jejuni* / *C. coli* antimicrobial susceptibility testing

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
Chloramphenicol, CHL*	≤ 16	-	≥ 32	NA	NA	NA
Ciprofloxacin, CIP	≤ 1	2	≥ 4	≥ 24	21-23	≤ 20
Ertapenem, ETP**	≤ 0.5	-	≥ 1	NA	NA	NA
Erythromycin, ERY	≤ 8	16	≥ 32	≥ 16	13-15	≤ 12
Gentamicin, GEN*	≤ 2	-	≥ 4	≥ 21	-	≤ 20
Tetracycline, TET	≤ 4	8	≥ 16	≥ 26	23-25	≤ 22

Reference values are based on *Campylobacter jejuni/coli* breakpoints from CLSI M45, 3rd Ed.
*Reference values are based on *C. jejuni* and *C. coli* epidemiological cut off values from https://mic.eucast.org/ on August 2022.
**Reference values are based on EFSA (European Food Safety Authority) recommendation.

Table 2. Interpretive criteria for *E. faecium* / *E. faecalis* antimicrobial susceptibility testing

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
Ampicillin, AMP		≤ 8	-	≥ 16	≥ 17	-	≤ 16
Chloramphenicol, CHL		≤ 8	16	≥ 32	≥ 18	13-17	≤ 12
Ciprofloxacin, CIP		≤ 1	2	≥ 4	≥ 21	16-20	≤ 15
Daptomycin, DAP	*E. faecium*	-	-	≥ 8	NA	NA	NA
	E. faecalis	≤ 2	4	≥ 8	NA	NA	NA
Erythromycin, ERY		≤ 0.5	1-4	≥ 8	≥ 23	14-22	≤ 13
Gentamicin, GEN*		≤ 128	-	≥ 256	≥ 8	-	≤ 7
Linezolid, LZD		≤ 2	4	≥ 8	≥ 23	21-22	≤ 20
Quinupristin/dalfopristin, SYN		≤ 1	2	≥ 4	≥ 19	16-18	≤ 15
Teicoplanin, TEI		≤ 8	16	≥ 32	≥ 14	11-13	≤ 10
Tetracycline, TET		≤ 4	8	≥ 16	≥ 19	15-18	≤ 14
Tigecycline, TGC*	*E. faecium*	≤ 0.25	-	≥ 0.5	≥ 22	-	≤ 21
	E. faecalis	≤ 0.25	-	≥ 0.5	≥ 20	-	≤ 19
Vancomycin, VAN		≤ 4	8-16	≥ 32	≥ 17	15-16	≤ 14

Reference values are based on *Enterococcus* spp. breakpoints from CLSI M100, 32nd Ed.
*Reference values are based on *Enterococcus* spp. clinical breakpoints from "The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, 2022. http://www.eucast.org."

Table 3. Interpretive criteria for *S. pneumoniae* antimicrobial susceptibility testing

Antimicrobials	Reference value MIC (µg/mL)			Reference value Disk diffusion (mm)		
	S	I	R	S	I	R
Amoxicillin/clavulanic acid, AUG2$_{(nonmeningitis)}$	≤ 2/1	4/2	≥ 8/4	NA	NA	NA
Azithromycin, AZI	≤ 0.5	1	≥ 2	≥ 18	14-17	≤ 13
Cefepime, FEP$_{(nonmeningitis)}$	≤ 1	2	≥ 4	NA	NA	NA
Cefotaxime, FOT$_{(nonmeningitis)}$	≤ 1	2	≥ 4	NA	NA	NA
Ceftriaxone, AXO$_{(nonmeningitis)}$	≤ 1	2	≥ 4	NA	NA	NA
Cefuroxime, FUR$_{(parenteral)}$	≤ 0.5	1	≥ 2	NA	NA	NA
Chloramphenicol, CHL	≤ 4	-	≥ 8	≥ 21	-	≤ 20
Clindamycin, CLI	≤ 0.25	0.5	≥ 1	≥ 19	16-18	≤ 15
Ertapenem, ETP	≤ 1	2	≥ 4	NA	NA	NA
Erythromycin, ERY	≤ 0.25	0.5	≥ 1	≥ 21	16-20	≤ 15
Levofloxacin, LEVO	≤ 2	4	≥ 8	≥ 17	14-16	≤ 13
Linezolid, LZD	≤ 2	-	-	≥ 21	-	-
Meropenem, MERO	≤ 0.25	0.5	≥ 1	NA	NA	NA
Penicillin, PEN$_{(nonmeningitis)}$	≤ 2	4	≥ 8	NA	NA	NA
Tetracycline, TET	≤ 1	2	≥ 4	≥ 28	25-27	≤ 24
Trimethoprim/sulfamethoxazole, SXT	≤ 0.5/9.5	1/19-2/38	≥ 4/76	≥ 19	16-18	≤ 15
Vancomycin, VAN	≤ 1	-	-	≥ 17	-	-

Reference values are based on *S. pneumoniae* breakpoint values from CLSI M100, 32nd Ed.

4 REPORTING OF RESULTS AND EVALUATION

We recommend that you write your results in the enclosed test forms and that you read carefully the description in paragraph 5 before entering your results in the informatics module. If the same reference strain is used for different pathogens, please enter the results (even if the same) for all the pathogens. The informatics module will allow you to view and print a report with your reported results. The scores for the results will be released after the result submission deadline; 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).

Results must be submitted no later than November 4th 2022.

If you have trouble in entering your results, please contact the EQA Coordinator directly, explaining the issues that you encountered:

Patrícia T. dos Santos
National Food Institute, Technical University of Denmark
Kemitorvet, Building 204, DK-2800 Lyngby – DENMARK
E-mail: pado@food.dtu.dk

Direct communication with the EQA Coordinator must be in English.

5 HOW TO SUBMIT RESULTS VIA THE INFORMATICS MODULE

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

Access the Informatics Module (**incognito window**) using https://eqasia-pt.dtu.dk. See below how to login to the Informatics Module.

When you submit your results, remember to have by your side the completed test forms (template available for download from the EQAsia website).

Do not hesitate to contact us if you have trouble with the Informatics Module.

Before finally submitting your 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 data entry.

Login to the informatics module:

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 pado@food.dtu.dk.

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