DTU Food
National Food Institute

WHO Collaborating Centre
for Antimicrobial Resistance in
Foodborne Pathogens
www.antimicrobialresistance.dk

PROTOCOL for

serotyping and antimicrobial susceptibility testing of *Salmonella* test strains

1 INTRODUCTION

In 2000, the Global Foodborne Infections Network launched an External Quality Assurance System (EQAS). The EQAS is organized by the National Food Institute, Technical University of Denmark (DTU Food), in collaboration with partners and Regional Sites in WHO GFN.

Various aspects of the proficiency test scheme may from time to time be subcontracted. When subcontracting occurs, it is placed with a competent subcontractor and the National Food Institute is responsible for the subcontractor's work.

The WHO EQAS 2019 includes serotyping and antimicrobial susceptibility testing of eight *Salmonella* strains and antimicrobial susceptibility testing of the *Escherichia coli* ATCC 25922 (CCM 3954) reference strain for quality control (QC).

The above-mentioned QC reference strain is included in the parcel only for new participants of the EQAS who did not receive it previously. The QC reference strain supplied is an original CERTIFIED culture provided free of charge, and should be used for future internal quality control for antimicrobial susceptibility testing in your laboratory. The QC reference strain will not be

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included in the years to come. Therefore, please take proper care of these strains. Handle and maintain them as suggested in the manual 'Subculture and Maintenance of QC Strains' available on the WHO Collaborating Centre website (see www.antimicrobialresistance.dk).

2 OBJECTIVES

The main objective of this EQAS is to support laboratories to assess and if necessary improve the quality of serotyping and antimicrobial susceptibility testing of enteric human pathogens, especially *Salmonella*. A further objective is to assess and improve the comparability of surveillance data on *Salmonella* serotypes and antimicrobial susceptibility reported by different laboratories. Therefore, the laboratory work for this EQAS should be performed using the methods routinely used in your laboratory.

3 OUTLINE OF THE EQAS 2019

3.1 Shipping, receipt and storage of strains

In January 2020 around 200 laboratories located worldwide will receive a parcel containing eight *Salmonella* strains. An *E. coli* ATCC 25922 reference strain will be included for participants who signed up to perform antimicrobial susceptibility testing (AST) and did not receive it previously. All provided strains belong to UN3373, Biological substance category B. Extended Spectrum Beta Lactamase (ESBL)-, AmpC- or carbapenemase-producing strains could be included in the selected material.

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

The *Salmonella* strains are shipped as agar stab cultures whereas the reference strain is shipped lyophilised. On arrival, the agar stab culture must be stored in a dark place at 2°C to 8°C. If receiving a lyophilized reference culture, store in a dark, cool place. The agar stab cultures 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 mis-labelling or contamination).

3.2 Serotyping of *Salmonella*

The eight *Salmonella* strains should be serotyped by using the method routinely used in the laboratory. Also serogroup results will be evaluated, therefore, if you do not have all the necessary antisera for a serotyping, please go as far as you can in the identification and report the serogroup. Serogroups should be reported using terms according to Kauffmann-White-Le Minor (Grimont and Weill, 2007. 9th ed. Antigenic formulae of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*).

Please fill in information concerning the brand of antisera used for typing in the fields available in the database for entering results. In addition, we kindly ask you to report which antisera you think are required to complete the serotyping, if relevant.

3.3 Antimicrobial susceptibility testing of *Salmonella* strains and *Escherichia coli* ATCC 25922

The *Salmonella* strains as well as the *E. coli* ATCC 25922 reference strain should be tested for susceptibility towards as many as possible of the antimicrobials mentioned in the test form. Please use the methods routinely used in your laboratory. Expected results are based on broth microdilution tests.

For reconstitution of the *E. coli* reference strain, please see the document 'Instructions for opening and reviving lyophilised cultures' on the WHO Collaborating Centre website (see www.antimicrobialresistance.dk).

The breakpoints used in this EQAS for interpreting MIC results are in accordance with international recommendations (Table 1). Interpretation of MIC results will lead to categorization of strains into three categories: resistant (R), intermediate (I) and susceptible (S). In the evaluation report you receive upon result submission, you can find that obtained interpretations in accordance with the expected interpretation will be defined as 'correct', whereas deviations from the expected interpretation will be defined as 'minor' (I ↔ S or I ↔ R), 'major' (S interpreted as R) or 'very major' (R interpreted as S).

Testing of gentamicin susceptibility may be valuable for monitoring purposes. Therefore we kindly ask you to disregard, for the purpose of this proficiency trial, that the Clinical and Laboratory Standards Institute (CLSI) guidelines state that *Salmonella* should not be reported as susceptible to aminoglycosides.

Table 1. Interpretive breakpoint for *Salmonella* antimicrobial susceptibility testing

Antimicrobials	Reference value, MIC (µg/mL)			Reference value, Disk diffusion (mm)		
	Susceptible	Intermediate	Resistant	Resistant	Intermediate	Susceptible
Ampicillin, AMP	≤8	16	≥32	≤13	14-16	≥17
Cefotaxime, FOT*	≤1	-	>1	≤27	-	>27
Cefoxitin, FOX	≤8	16	≥32	≤14	15-17	≥18
Ceftazidime, TAZ*	≤1	-	>1	≤22	-	>22
Ceftriaxone, CRO*	≤1	-	>1	≤25	-	>25
Chloramphenicol, CHL	≤8	16	≥32	≤12	13-17	≥18
Ciprofloxacin, CIP	≤0.06	0.12-0.5	≥1	≤20mm (5µg) or <23mm (1µg)**	21-30mm (5µg) or (1µg)**	≥31mm (5µg) or ≥23mm (1µg)**
Colistin, COL***	≤2	-	≥4	Not applicable	Not applicable	Not applicable
Gentamicin, GEN	≤4	8	≥16	≤12	13-14	≥15
Meropenem, MERO*	≤0.12	-	>0.12	<27	-	≥27
Nalidixic acid, NAL*	≤16	-	≥32	≤13	14-18	≥19
Sulfonamides, SMX	≤256	-	≥512	≤12	13-16	≥17
Tetracycline, TET	≤4	8	≥16	≤11	12-14	≥15
Trimethoprim, TMP	≤8	-	≥16	≤10	11-15	≥16
Trimethoprim + sulfamethoxazole, TMP+SMX, SXT	≤2/38	-	≥4/76	≤10	11-15	≥16

Reference values used in this EQAS are according to CLSI (M100, 28th edition), with the following exceptions:
* For the cephalosporins and meropenem, the application of the interpretative criteria is intended to indicate if the microorganism is a presumptive ESBL- or carbapenemase-producer. Reference values for the cephalosporins and nalidixic acid are according to CLSI M100 Table 3A. These interpretative criteria are also applied for *Salmonella* test strains for interpretation of AST results in this EQAS. Reference values for meropenem are based on epidemiological cut off values from www.eucast.org.
** The publication by Cavaco LM and Aarestrup FM (J. Clin. Microbiol. 2009. Sep;47(9):2751-8) provides the background for these interpretative criteria in the WHO GFN EQAS.
*** Reference values for colistin are based on epidemiological cut off values form www.eucast.org for *Escherichia coli*. In the current EQAS these values should be applied for the interpretation of *Salmonella* AST results into the category as susceptible or resistant.

Concerning ciprofloxacin susceptibility tests, the applied breakpoints take into consideration mechanisms of resistance due to plasmid-mediated quinolone resistance genes (e.g. *qnr*-genes) and one-point-mutation in the gyrase gene.

Important notes*: beta-lactam resistance*
The following tests for detection of ESBL-, AmpC-, and carbapenamase-producing phenotypes are optional in relation to the current WHO GFN EQAS.

If choosing to participate in this component of the EQAS, all strains displaying reduced susceptibility to cefotaxime (FOT), ceftazidime (TAZ), and/or ceftriaxone (CRO) should be tested for ESBL-, AmpC, or carbapenemase-production by confirmatory tests. Reduced susceptibility to any of the above-mentioned antimicrobials indicates that the bacterial strain is an ESBL-, AmpC, or carbapenemase-producing phenotype.

Confirmatory test for ESBL production requires the use of both cefotaxime (FOT) and ceftazidime (TAZ) alone, and in combination with a β-lactamase inhibitor (clavulanic acid). Synergy is defined either as i) by microbroth dilution methods or E-test; a ≥ 3 twofold concentration decrease in an MIC for either antimicrobial agent tested in combination with clavulanic acid vs. its MIC when tested alone (E-test 3 dilution steps difference; MIC FOT : FOT/Cl or TAZ : TAZ/Cl ratio ≥ 8) or ii) by disk diffusion; a ≥ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid vs. its zone when tested alone (CLSI M100 Table 2A; Enterobacteriaceae). The presence of synergy indicates ESBL production.

Detection of AmpC-type beta-lactamases 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 requires the testing of meropenem (MER). Reduced susceptibility to MER indicates that the bacterial strain is a carbapenemase-producer.

The classification of the phenotypic results should be based on 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. EFSA Journal 2019;17 (2):5598, 278 pp. https://doi.org/10.2903/j.efsa.2019.5598 (page 41)).

The following summary of these recommendations indicate how the phenotypes should be categorized:

ESBL-phenotype:

- FOT or TAZ > 1 mg/L **AND**
- MERO ≤ 0.12 mg/L **AND**
- FOX ≤ 8 mg/L **AND**
- Synergy for FOT : FOT/Cl and/or TAZ : TAZ/Cl

ESBL+AmpC-phenotype:

- FOT or TAZ > 1 mg/L **AND**
- MERO ≤ 0.12 mg/L **AND**
- FOX > 8 mg/L **AND**
- Synergy for FOT : FOT/Cl and/or TAZ : TAZ/Cl

AmpC-phenotype:

- FOT or TAZ > 1 mg/L **AND**
- MERO ≤ 0.12 mg/L **AND**
- FOX > 8 mg/L **AND**
- No synergy for FOT : FOT/Cl nor TAZ : TAZ/Cl

(note, presence of ESBLs is not excluded)

Carbapenemase-phenotype:

- MERO > 0.12 mg/L

(note, presence of ESBLs or AmpCs is not excluded)

Other-phenotype:

- Not covered by any of the above categories **AND**
- FOT, TAZ, FOX, or MERO > interpretative criteria as susceptible in Table 1 (i.e. exhibits reduced susceptibility)

No ESBL-, AmpC-, or carbapenemase:

- FOT, TAZ, FOX, and MERO ≤ interpretative criteria as susceptible in Table 1 (i.e. exhibits susceptibility)

The genotype obtained by PCR and/or sequencing may be necessary to correctly categorize a bacterial test strain as either of the categories, ESBL-, AmpC, and/or carbapenemase-producer, but is not requested as part of this WHO GFN EQAS.

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 web database. For entering your results via the web, you will be guided through all steps on the screen and you will immediately be able to view and print a report evaluating your results. Results in agreement with the expected interpretation are categorised as 'correct', while results deviating from the expected interpretation are categorised as 'incorrect'.

Results must be submitted no later than 30th April 2020.

If you experience difficulties in entering your results, please contact the EQAS Coordinator directly, explaining the issues that occur.

All results will be summarized in a report which will be publicly available. Individual results will be anonymous and will only be forwarded to the official GFN Regional Centre in your region.

We are looking forward to receiving your results.

If you have any questions or concerns, please do not hesitate to contact the EQAS Coordinator:

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Direct communication with the EQAS organisers must be in English.

5 HOW TO SUBMIT RESULTS VIA THE WEBTOOL

The 'guideline for submission of results via webtool' is available for download directly from the WHO Collaborating Centre website (http://antimicrobialresistance.dk/eqas.aspx). Please follow the guideline carefully.

Access the webtool using this address: https://amr-eqas.dtu.dk. About login to the webtool, see below.

When you submit your results, remember to have by your side the completed test forms (available for download from http://antimicrobialresistance.dk/eqas.aspx).

Do not hesitate to contact us if you experience difficulties with the webtool.

Before finally submitting your input for *Salmonella*, respectively, 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 webtool:

When first given access to login to the webtool, your **personal loginID and password** is sent to you by email. This is relevant for two email addresses connected to each participating laboratory.

Note that:

a) If the EQAS organizers have only one contact person for a participating institution, this person is registered both as primary and secondary contact. Should you like to add another person as the secondary contact, please contact suska@food.dtu.dk
b) If your laboratory signed up with two or more contact points, a primary and a secondary contact has been registered. Should you like to make changes to the primary and secondary contact or should you like more than the two persons to be able to access the webtool, please contact suska@food.dtu.dk.

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