Proficiency test – Precision and accuracy in the analysis of standard solutions – STRD0521

for Official Residue Control Laboratories

PROTOCOL

1. General information

This proficiency test shall assist the participating laboratories in verifying the concentrations of the standard solutions they apply in routine analysis. Any consultation with other participants is against professional scientific conduct and impairs the intended purpose of this study. Thus, the participants themselves are responsible for gaining a genuine idea of their proficiency in routine analysis.

Each participant receives two vials (1 mL) of mix solutions per substance group. We kindly ask you to confirm the receipt of all samples immediately upon receipt using the online form, the password for which you have received via e-mail:

https://ec.europa.eu/eusurvey/runner/STRD0521SampleReceipt

First information on laboratory performance will be provided in the form of a preliminary report which will be made available in August 2021. Participants are asked to confirm that all results transferred to the proficiency testing provider are complete and correct or point out any errors, which may have occurred. The final report containing detailed information on the proficiency test and the participants' performance will be prepared from these (corrected) data. The participants' performance will be evaluated based on the principles described in the *Common Protocol for Proficiency Testing* available from the FIS-VL. The following modifications will be made:

- For the assigned standard deviation of MRL/ML-compounds, the reproducibility standard deviation will be used, if it is smaller than the HORWITZ standard deviation.
- One point will be deducted from a participant's total score if they submit one or two false positive results. If more than two false positive results are submitted by a participant, two points will be deducted from their overall point score.
- To pass the proficiency test, a minimum of two thirds of the maximum possible point score needs to be achieved. The proficiency assessment will only take the substance groups, the participants registered for, into account.

In the final report participants will only be referred to by their lab codes. The participants consent to the publication of their results in an anonymous form by the organiser of the proficiency test. In addition, the results of the NRLs participating in this proficiency test may be provided in a non-anonymous form to the European Commission (DG SANTE) for internal use, as well as to the competent authorities of the Member States (according to Article 94, 2.(c), Regulation (EU) 2017/625). Analogously, the results of

the German official laboratories may be provided in a non-anonymous form to the German competent authorities for internal use (according to Article 101, 1.(c), Regulation (EU) 2017/625).

To give feedback on the proficiency test, please contact eurlvetdrug@bvl.bund.de for the feedback form. For further information on the conduction of EURL proficiency tests, including details on the point score system used for performance evaluation, please refer to the *Common Protocol for Proficiency Testing* available from the EURL-website: https://eurl-residues.eu/eurl-bvl/bvl-proficiency-tests/.

2. Time Schedule

01.04.2021	***Official announcement of proficiency test STRD0521***
30.04.2021	***Deadline for registration***
07.06.2021	***Sample shipment***
	Analysis of samples
~~***30.07.2021***~~ 06.09.2021	***Deadline for submission of results*** postponed due to issues with the sample shipment
Middle of ~~August~~ 2021 September	***Dispatch of preliminary reports to participants by e-mail***
	Participants checking their results and either correcting or confirming them
Middle of ~~September~~ 2021 October	***Deadline for corrections to preliminary report***
	Preparation of final report on results
January/February 2022 ~~***December 2021***~~	***Dispatch of report on results (pdf data file) and follow-up questionnaire to the participants***
February/March ~~***January***~~ ***2022***	***Deadline for returning follow-up questionnaires***
2022	***Evaluation of PT in the framework of the EURL/NRL-Workshop***

3. Samples

The samples are shipped on cooling packs and should be stored at -25 °C immediately upon receipt. The solutions should be kept in the dark at all times. Every mix solution contains an unknown number and concentration of analytes of a single substance group. The substance group of interest is indicated by the sample coding (Table 1).

Two mix solutions of 1 mL each are provided per substance group. One of these mix solutions labelled "xxxx_A – test mix" should be used to conduct all necessary preliminary experiments, e. g. for the selection of an appropriate calibration interval ("xxxx" codes for the substance group). The second mix solution labelled as "xxxx_B – PT test item" should be used for the quantification exercise. Results shall only be submitted for the analyses conducted on the solutions labelled "xxxx_B – PT test item".

The mix solutions were prepared gravimetrically from stock solutions containing the individual standards and ethanol (anthelmintics, coccidiostats) or a volumetric 9 to 1 mixture of acetonitrile and methanol (NSAIDs).The individual stock solutions were partly prepared in other solvent mixtures and therefore the mix solutions may contain up to 0.2 % DMSO. The analyte concentrations in the undiluted mix solutions are in the range 50-2000 ng/mL and the participants shall decide themselves how they would like to dilute the mixes. We recommend to dilute the solutions at least 1:9 in order to obtain good peak shapes in the chromatogram. Dilutions should be prepared in accordance with the laboratory's routine protocol. **All measurement results shall be reported for the undiluted PT test items in ng/mL.**

An overview of the coding, sample type, main solvent, measurands and concentration range of the solutions is given in Table 1.

Table 1: Overview of samples, solvents, measurands and their concentration ranges in the undiluted mix solutions.

sample code	*sample type*	*solvent*	*measurands*	*concentration range in undiluted solution /(ng/mL)*
ANTH_A	test mix	EtOH	anthelmintics	100-2000
ANTH_B	PT test item	EtOH	anthelmintics	100-2000
COCC_A	test mix	EtOH	coccidiostats	100-1000
COCC_B	PT test item	EtOH	coccidiostats	100-1000
NSAI_A	test mix	ACN/MeOH 9/1	NSAIDs	50-1500
NSAI_B	PT test item	ACN/MeOH 9/1	NSAIDs	50-1500

Homogeneity and stability

For the homogeneity and stability assessment, aliquots of 100 µL of the PT test item solutions were diluted with 900 µL of aqueous eluent. We therefore recommend to use no volume smaller than 100 µL for your dilutions and the subsequent analyses.

A stability study for the PT test item solutions is being conducted for the storage temperatures -25°C, +4°C and room temperature. All storage experiments are carried out in the absence of light.

The influence of any analyte instabilities on the participants' results will be respected via consideration of the arising uncertainties for the calculation of the performance scores. Note that the homogeneity and stability assessment was only conducted for the PT test item solutions.

4. Remarks on the analysis

Each laboratory can freely choose which analytical methods to apply. The selected analytical methods and the laboratory procedures, e. g. for the preparation of dilutions should closely resemble the approaches used in routine work. Detected analytes are to be quantified and confirmed. For this purpose the criteria of Commission Decision 2002/657/EC and Commission Implementing Regulation (EU) 2021/808, respectively, are to be taken into account. If screening as well as confirmatory analysis are carried out, the analyses should be performed independently from one another on separate aliquots.

All participants are asked to analyse the *minimum required*, as well as the *recommended* substances as specified in Table 2. For the performance assessment the marker residues laid down in Commission Regulation (EU) No 37/2010, if available, are relevant. For the proficiency assessment the substance groups the participants registered for will be considered.

Table 2: Classification of anthelmintics, coccidiostats and NSAIDs as minimum required and recommended. If not explicitly given, the relevant residues are the marker residues laid down in Commission Regulation (EU) No 37/2010.

substance group	*minimum required*	*recommended*
B2a - anthelmintics	abamectin closantel doramectin fenbendazole and metabolites ivermectin levamisole albendazole and metabolites moxidectin nitroxinil rafoxanide thiabendazole and metabolites triclabendazole and metabolites	clorsulon emamectin eprinomectin flubedazole and metabolites mebendazole and metabolites oxibendazole and metabolites oxyclozanide
B2b - coccidiostats	diclazuril lasalocid maduramicin monensin narasin nicarbazin as 4,4'-dinitrocarbanilide robenidine salinomycin	amprolium clopidol decoquinate halofuginone ipronidazole nequinate semduramycin toltrazuril
B2e - NSAIDs	phenylbutazone flunixin, flunixin-hydroxide diclofenac 4-methylamino antipyrine tolfenamic acid carprofen ibuprofen naproxen meloxicam	oxyphenbutazone ketoprofen vedaprofen mefenamic acid niflumic acid flufenamic acid 4-formylamino antipyrine

5. Report of the analytical results

The deadline for submitting the test results is **30th July 2021**. Please exclusively use the provided result files and/or result form to indicate your results. The information has to be filled in completely and unambiguously. There are

- 2 result files (LA2 and LAB) for the submission of the results of the confirmatory analysis (see Annex 3). Both files (LA2 and LAB) need to be submitted.
- 1 result / method description form (Annex 2) for the submission of screening results
- Optional: 1 result form for the reporting of additional analytes, available upon request.

For the final report, the results will be used exactly as given by the participants in the result files and screening result form. The kind of correction applied is to be stated. **Results shall only be reported for analyses conducted on the samples labelled "B – PT test items" as the concentrations of the undiluted mix solutions in ng/mL.** Necessary corrections arising from the injection of dilutions of the test items have to be carried out by the participants themselves.

5.1 How to indicate your analytical results

5.1.1 Indicating the results

- It is essential for the evaluation of the proficiency test to state the results of parallel determinations separately and not as mean values. For the confirmatory analysis, a minimum of two and and a maximum of six separate results should be provided.
- If a recovery correction is required, it is to be carried out by the participants themselves. For the evaluation the results will be used as they appear on the result forms, without any further corrections. The kind of recovery correction applied is to be stated on the result forms.
- The quantitative results should be expressed by an analytically justifiable number of significant digits (at least three significant places) – even if the within-laboratory reproducibility makes this look unreasonable (e.g. 123 ng/mL or 1.23 ng/mL).
- The indicated results should refer to:
    - the active drug without counter ions
    - the undiluted PT test item solution
- The investigated and detected analytes are to be referred to by the names given in Annex 3. In addition, the CC_{α} (confirmatory methods) and CC_{β} (screening methods) of the applied test methods are to be indicated.
- For this proficiency test it is also necessary to indicate the manufacturer of the standards you used for quantification of the PT test item solution. If you obtained the standard from the EURL Berlin, we kindly ask you to indicate the number of the standard you received. For example, you use a halofuginone hydrobromide standard you obtained from the EURL Berlin for the determination of the PT test item. Then please indicate the standard number we conveyed in your standard order, e.g. 458/49.

- Confirmed analytical results should also be reported if they are below CC_{α}.
- The requested validation parameters are to be indicated for all analytes detectable with the applied method.

5.1.2 Supplementary information (<u>only on request</u>)

- examples of raw data, e. g. chromatograms and calibration curves
- in-depth description of the applied sample preparation and test methods (including references as far as available)
- test method description of the applied methods

<u>Enclosures</u>

1. Result form for screening results, ANNEX 1
2. Instructions for use of result files, ANNEX 2 and ANNEX 3 (by separate email)
3. RingDat files for confirmatory results (by separate email)