Proficiency test – Nitroimidazoles in plasma and milk – NIIM1021

for Official Residue Control Laboratories

PROTOCOL

1. General information

This proficiency test shall assist the participating laboratories in verifying their analytical proficiency. Therefore, the selected methods, the number of parallel analyses performed and the analytical procedures used should closely correspond to those normally applied within routine work. Each laboratory can freely choose which methods to apply (screening and confirmation methods). Laboratories only performing a screening analysis are requested to indicate how a positive result is confirmed (by means of which method and by which laboratory). 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.

One sample set consists of three samples of lyophilised plasma (0.5 g, corresponding to about 10 g reconstituted material) and one sample of lyophilised milk (2.5 g corresponding to about 20 g of reconstituted material). 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/NIIM1021SampleReceipt

First information on laboratory performance will be provided in the form of a preliminary report which will be made available in late November 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:

- 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.

Since the analysis of milk for nitroimidazoles is a new requirement the results for the milk samples will be evaluated in the form of a research study. In the experience of the EURL Berlin, many plasma

methods are suitable for the analysis of milk samples for nitroimidazoles. Participants are therefore asked to apply their routinely used plasma methods to the milk sample, if they do not yet have validated milk method in their portfolio.

For the proficiency assessment, only the results for the plasma samples will be considered whereas scores for the milk sample will be given for information, only.

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

12.08.2021	***Official announcement of proficiency test NIIM1021***
03.09.2021	***Deadline for registration***
~~***05.10.2021***~~ ***04.10.2021***	***Sample shipment***
	Analysis of samples
~~***19.11.2021***~~ ***25.11.2021***	***Deadline for submission of results***
End of November 2021	***Dispatch of preliminary reports to participants***
	Participants are asked to check whether the results they submitted are correctly reproduced in the preliminary report
Middle of December 2021	***Deadline for corrections to preliminary report***
	Preparation of final report on results
March 2022	***Dispatch of report on results (pdf data file) and follow-up questionnaire to the participants***
April 2022	***Deadline for returning follow-up questionnaires***
Summer 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 -20 °C immediately upon receipt. The homogeneity was assessed for a quantity of 0.5 g reconstituted plasma and 1.0 g reconstituted milk and it is therefore recommended to use no smaller sample quantity per single analysis. The sample stability of the lyophilised material was assessed for -20 °C, +4 °C and room temperature and can be guaranteed for at least three months at -20 °C. It is therefore safe to assume that no sample and analyte degradation will occur during the course of the proficiency test if the samples are stored at -20 °C. Stability tests for mid-term and long-term stbility are currently in progress. All stability tests were carried out in the absence of light.

The test items contain an unknown number and concentration of incurred residues of nitroimidazoles. The matrix is indicated by the sample coding (Table 1). An overview of the coding, species, matrix and dry weight is given in Table 1.

Table 1: Overview of samle codes, species, matrix and dry weight.

sample code	*species*	*matrix*	*dry weight /%*
BM	bovine (cow)	milk	12.15
PP	porcine (pig)	plasma	7.79
MP	meleagrine (turkey)	plasma	6.40
CP	galline (chicken)	plasma	4.63

All test items are provided as lyophilised samples. A reconstitution protocol is available from the EURL website: https://eurl-residues.eu/eurl-bvl/bvl-standard-shipment/

4. Remarks on the analysis

The four matrix samples consist of incurred material which was produced in cooperation with the holding for test animals of the Federal Institute for Risk Assessment (BfR). For the production of the test items, incurred materials were mixed with blank material before the batch was subjected to homogenisation and lyophilisation steps.

The test items are to be analysed for nitroimidazoles applying routine analytical methods of the participant's choice. 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. Laboratories performing a screening, as well as a confirmatory analysis should carry out completely independent parallel analyses.

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. Results for other nitroimidazoles and their metabolites can be provided additionally, but will not be part of the proficiency assessment. However, if a sufficient number of results in provided by the participants, the proficiency test parameters will be calculated.

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

minimum required	*recommended*	*optional*
Please refer to the list of substances available from the FIS-VL		

5. Report of the analytical results

The deadline for submitting the test results is **19 November 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 2). Both files (LA2 and LAB) need to be submitted.
- 1 result / method description form (Annex 1) 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.

Please exclusively use the provided result files and/or screening result form for indicating your results.

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 µg/kg or 1.23 µg/kg).
- The indicated results should refer to:
    - the active drug without counter ions
    - the reconstituted material
- The investigated and detected analytes are to be referred to by the names given in the RingDat files. In addition, the CC_α (confirmatory methods) and CC_β (screening methods) of the applied test methods are to be indicated.
- 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 (only on request)

- 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

Enclosures

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)