SARAF WEBINAR of 8 December 2022
on Commission Implementing Regulation (EU) Regulation 2021/808
FAQ
Coordinated replies (3 EURLs, EC) to questions raised during the webinar

Regulation

Questions on control/monitoring plans

Q1	**Is there an equivalent of plan 2 for third countries?**
A1	*Commission Delegated Regulation (EU) 2022/2292 states in point C of Part II of Annex I that the third country has to submit the plan in accordance with requirements laid down in Art. 4 of Commission Implementing Regulation (EU) 2022/1646 (risk-based control plan for national production). This means that there is no obligation for the third country to submit a surveillance plan.*
Q2	**Is Commission Implementing Regulation (EU) 2021/808 applicable to methods used for other control purposes, which are not included in the official control scheme of control?**
A2	*This Regulation applies to official controls aimed at verifying compliance with the requirements on the presence of residues of pharmacologically active substances (Art. 1).* *If your methods are used for other purposes than official controls, then the requirements laid down in this Regulation are not mandatory.*
Q3	**What should be the approach of third country regulatory bodies exporting to the EU if values below the decision limit (CCα) are detected in food of animal origin?**
A3	*Article 5(1) of Commission Implementing Regulation (EU) 2021/808 states: The result of an analysis shall be considered non-compliant where it is equal to or above the decision limit for confirmation (CCα).* *This means that findings below CCα are compliant.*
Q4	**Is the third country required to implement both plans 1 & 2 in line with Commission Implementing Regulation (EU) 2022/1646 for import of product of animal origin to EU or only one plan is required as per the current practice?**
A4	*Commission Delegated Regulation (EU) 2022/2292 states in point C of Part II of Annex I that the third country has to submit the plan in accordance with requirements laid down in Art. 4 of Commission Implementing Regulation (EU) 2022/1646 (risk-based control plan for national production).*

Question on RPA

Q5	**Is the new RPA for nitrofuran metabolites, chloramphenicol and malachite green fixed based on risk assessment?**
A5	*Nitrofurans and their metabolites and chloramphenicol are prohibited substances (Table 2 of Annex to Commission Regulation (EU) No 37/2010) and malachite green is an unauthorised substance for food producing animals. No presence of those substances in food of animal origin is allowed. Reference points for action are set at the lowest level which can analytically be achieved by the official control laboratories. The new reference points for action take into account the progress achieved in the last 20 years as regards the sensitivity of the methods of analysis for the analysis of these substances. Detailed information on the scientific background is available here: https://www.efsa.europa.eu/en/efsajournal/pub/5332. Information on risk assessment evaluation of these residues have been delivered by EFSA in scientific opinion reports along the 2014-2016 period. These documents can easily be found on the EFSA website. For chloramphenicol - Scientific Opinion on Chloramphenicol RPA in food and feed - EFSA Journal 2014;12(11):3907. For nitrofurans - Scientific Opinion on nitrofurans and their metabolites in food - EFSA Journal 2015;13(6):4140. For malachite green - Scientific Opinion on Malachite green in food - EFSA Panel on Contaminants in the Food Chain (CONTAM) - EFSA Journal 2016;14(7):4530.*

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Questions on validation process

Q6	**Do the values for trueness, repeatability and recovery have to meet the acceptance criteria at all spiking levels?**
A6	*The values for trueness, repeatability and recovery have to meet the acceptance criteria at all spiking levels relevant to the official control.*
Q7	**CCα is 0.12 ppb and CCβ is 0.20 ppb for chloramphenicol as per Commission Decision 2002/657/EC while now RPA is 0.15 ppb. Can this method be used in these conditions?**
A7	*As long as the validated decision limit for confirmation (CCα) is below the RPA of 0.15 µg/kg, the method is suitable for official controls for EU. The CCβ for screening method needs to be below the RPA.*
Q8	**From this example, does it mean that full series of validation should be done at 0.05 for chloramphenicol for considering it as LCL?**
A8	*Calibrated series of validation for chloramphenicol can be selected in whatever lowest calibration level (LCL) applicable to your instrumentation as long as it is a LCL below the RPA of 0.15 µg/kg and with calculated CCα at the LCL which does not reach or exceed the RPA.*
Q9	**Please explain this French approach in more detail.**
A9	*For further explanation on this French approach for validation according to Commission Implementing Regulation (EU) 2021/808, please forward your questions directly to the two French NRLs: ANSES-Fougeres (for Veterinary Medicinal Products) and ONIRIS-LABERCA (for Growth Promoters).*
Q10	**We need examples of the adaptation of methods to the new regulation. It will not always be necessary to do a complete new validation, will it?**
A10	*There is no need to proceed to a complete revalidation of analytical method validated according to Commission Decision 2002/657/EC as long as you can demonstrate similar performance against the new criteria according to Commission Implementing Regulation (EU) 2021/808.*
Q11	**What does "Official Method" mean? Do all French routine labs have to follow and use these validated and accredited methods?**
A11	*Official Method means a method that has been demonstrated to be validated with acceptable performance in line with the criteria according to Commission Implementing Regulation (EU) 2021/808 and being further accredited under ISO 17025 by the national accreditation body. Such method also has to be approved at the official laboratory by the competent authority of the country.*
Q12	**Why is it necessary to try and validate in a quantitative manner for a forbidden compound as MELA? It is a huge amount of work if you want to do this for all forbidden substances.**
A12	*It is necessary to know from which level on you can reliably identify the compound. You can use the qualitative confirmatory method validation parameters from Table 5 of Annex I to Commission Implementing Regulation (EU) 2021/808.*

Questions on matrix selection

Q13	**Can a single validation include a representative matrix of shrimp and fish or is it necessary to perform separate validation for fish and shrimp matrix?**
A13	*The best approach is to validate one matrix fully and extend the method with the new matrices or species according to the EURL Guidance document on extension of methods. However a sufficient amount of preliminary experiments demonstrating the suitability of the method for all matrices included in the validation could be considered.*
Q14	**Do the samples in the three validation series have to be of the same species? For example, if we need to validate in different species of urine, should we do complete validation in the most representative without being able to include other species?**

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A14	*The best approach is to validate one matrix fully and extend the method with the new matrices or species according to the EURL Guidance document on extension of methods. However a sufficient amount of preliminary experiments demonstrating the suitability of the method for all matrices included in the validation could be considered.*
Q15	**Please clarify the conception of "pooled samples". How did you form the pooled fat sample?**
A15	*The pooled material has been prepared using aliquots of each of the representative fat materials collected for the validation. For a better homogeneity of the pooled material, it has been heated and mixed. Then a first series of analyses was performed in order to evaluate the "matrix effect" parameter. Since all coefficients of variation for the matrix effect were < 20 %, the samples have been pooled randomly for the next series 2, 3 and 4 during the validation.*
Q16	**The EURL Guidance Document on Validation of Confirmation Methods proposes a minimum of 21 different batches for full validation, 7 batches in each series. We need 21 different samples for full validation. Do the samples for series 1 have to be different from the samples of series 2 and 3?**
A16	*Yes, it is far better to have each series of samples collected from different animals/batches/lots/species. The 2 ideas behind this is to have a collection of validated samples: 1 - representative of the scope you consider for your method; 2 - being able to evaluate the variabilities your method will be facing during the routine control.*

Questions on spiking level

Q17	**It is important to qualify the choice of the 3 spiking levels indicated in the document as this seems to be a minimum. Many labs, such as the French guidelines, are using 4 spiking levels. This can be confusing for inexperienced labs.**
A17	*The choice of the 3 spiking levels indicated in the document has to be considered as a minimum requirement. Your suggestion to indicate in the Guidance this relevant statement will be considered.*
Q18	**None of the A3 analytes has a MMPR which level apply for validation, in particular A3b (pesticides)?**
A18	*Substances as for example pesticides falling into A3b substances are not authorised as veterinary medicinal products, but they can be authorised for use in husbandry. These substances have an established MRL or default MRL of 0.01 mg/kg as a pesticide residue.* *If validation is done according to the Guidelines for validation procedures for pesticide residues (SANTE 11312/2021), the level for enforcement is the established MRL or the default MRL (Regulation (EC) No 396/2005), taking into account the measurement uncertainty as described in SANTE 11312/2021.* *If validation is done according to the VMPR, the calculation for CCα shall be done according to point 2.6.2 of Annex I to Commission Implementing Regulation (EU) 2021/808. In this case, the CCα is calculated using the established MRL or default MRL (Regulation (EC) No 396/2005) – as it is done for authorised substances, for example in Table 1 of Commission Regulation (EU) No 37/2010. The measurement uncertainty is with this approach already included in calculation of the CCα, hence the level for enforcement is the CCα (i.e. equal to the established pesticide MRL or default MRL + "measurement uncertainty"). Only in case the level found exceeds the CCα (i.e. above the pesticide MRL + "measurement uncertainty") the concerned foods shall not be placed on the market or shall be withdrawn from the market if already placed on the market.* *In case of a suspicion of unauthorised use of a pesticide as veterinary medicinal product, a competent authority can do the investigation and in case of evidence of an unauthorised use, it can impose sanctions for unauthorised use of the substance. However, as long as the food complies with the pesticide MRL, the food can remain on the market and no withdrawal is performed.*

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Q19	**Commission Delegated Regulation (EU) 2022/1644 includes protein and peptide hormones as well as antiviral substances. Where do we find the list of analytes required for the analysis such as in Commission Regulation (EU) No 37/2010 and in MMPR guide, as well as the levels of interest (MMPR)?**
A19	*The EURL Guidance on MMPRs and the substance list will be extended in the course of 2023. In addition, all substances which can be analysed are in the EFSA catalogue, which will be available at the beginning of 2023 at EFSA websites.*

Questions on matrix effect

Q20	**Are other approaches to calculate the relative matrix effect accepted?**
A20	*Yes, they are accepted if they give comparable information.*
Q21	**How to perform relative matrix effect in case of nitrofuran metabolites when the lab is not having pure NP Standards?**
A21	*Marketed Pure NP Standards can be purchased from several companies.*
Q22	**From what is stated in 808/2021, no criteria is set for the matrix effects. Same for absolute recovery. Could you please provide an information where the 20% criteria for the matrix effects is coming from?**
A22	*The matrix effect is part of the in-house reproducibility - if it is greater than 20 %, critieria for quantitative methods can probably not be fulfilled.*
Q23	**Regarding matrix effect criteria is it also accepted being < RSD Reproducilility?**
A23	Yes, this should be the case.
Q24	**The document gives information on relative matrix effects - it only suggests when internal standards are used. Please confirm whether the document requires matrix effects to be determined even when no internal standards are used.**
A24	*Even though only the determination of the relative matrix effect is explicitely described in Commission Implementing Regulation (EU) 2021/808, it is highly recommended to determine the matrix effect, even if no internal standard is used.*
Q25	**What extra information can you get by calculating the matrix effect in contrast to the analysis of the samples for determining the reproducibility? Certainly when the % CV of the data determined for reproducibility is below 20 %?**
A25	*If the in-house reproducibility is correctly determined, the matrix effect is part of this value. Concerning extra information, it is, e.g. for further optimisation or for restrictions in applicability/further method extensions.*

Questions on CCβ

Q26	**Following the definition (39) of 'screening target concentration'(STC), STC can also be equal to CCβ. However one possible calculation (section 2.7, c) is:** **CCβ = STC + k(one-sided, 95 %) × (combined) standard measurement uncertainty at or above the STC.** **I think that this is contradictory.**
A26	*This is clarified in the screening guidance - there are approaches where STC is equal to CCβ (approaches without calibration curves).*
Q27	**For qualitative screening methods, do we need to validate on 0.5 MRL or 0.1 MRL to determine CCβ?**
A27	*It depends on the quality of the method - 0.5 MRL might be sufficient.*

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Q28	**For screening semi-quantitative analysis, will the requirement for S/N > 3 only apply to the quantifying transition as second transition is for confirmation?**
A28	*Yes (for screening methods, no second transition is needed).*
Q29	**In many places in the presentation, CCβ is shown for confirmatory method, however as per table 5 of Commission Implementing Regulation (EU) 2021/808, CCβ is not applicable but only CCα is applicable?**
A29	*CCβ in the Commission Implementing Regulation (EU) 2021/808 is defined as the capacity of detection with critical concentration for screening an analyte. There is no use anymore to consider and calculate the other CCβ for confirmation that was also proposed in the former Commission Decision 2002/657/EC. CCα is the important parameter for the evaluation of a sample (decision for compliance); CCβ characterises the method - and focuses on false negative results.*
Q30	**Should method 3 be used for quantitative screening method only?**
A30	*Yes, this is correct.*
Q31	**Why do you need to check for false compliant results during precision experiments if there are already no false compliant results during spiking experiments at STC during validation of a semi-quantitative screening method?**
A31	*Question is not clear, we cannot provide a reply.*

Questions on CCα

Q32	**How are the results treated when the reported results are between CCα and RPA?**
A32	*For substances for which RPA is set out, the rules laid down in Commission Regulation (EU) 2019/1871 apply.* *Art. 5 states: Food of animal origin, containing residues of a pharmacologically active substance in a concentration at or above the reference point for action, shall be considered not to comply with Union legislation and shall not enter the food chain. Food of animal origin containing residues of a pharmacologically active substance in a concentration at a level below the reference point for action shall not be prohibited from entering the food chain. Where the results of official controls identify residues of prohibited or non-allowed substances at ANY level (i.e.* ***above, equal to or below the reference points for action****), the competent authority shall carry out the investigations to determine whether there has been illegal treatment with a prohibited or non-allowed pharmacologically active substance.*
Q33	**If we go through Commission Implementing Regulation (EU) 808/2021, there is no specific formula as CCα= CCβ + k(one-sided, 99 %) × (combined) standard measurement uncertainty at CCβ.** **Instead, the following formula is mentioned:** **CCα = LCL + k(one-sided, 99 %) × (combined) standard measurement uncertainty at LCL**
A33	*Yes, this is correct.*
Q34	**In order to calculate the CCα with method 3 you need to use the combined measurement uncertainty. For the validation of a qualitative confirmatory method, you do not need to determine precision and trueness. Can we use instead the standard deviation on our response to calculate CCα?**
A34	*More information is needed to answer the question.*
Q35	**In case we have to lower the CCα limit and add a new species, can it be done with a simplified validation in a single experiment?**
A35	*The EURL Guidance on method extension proposes strategies for integrating new species into a method as well as amending new concentration levels. Whether it is possible to extend a method is always a case by case decision.*

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Q36	**In the example of chloramphenicol, the CCa is calculated taking into account the within-laboratory reproducibility 30 % at the level 0.05 µg/kg. However, the Commission Implementing Regulation (EU) 808/2021 states that combined standard measurement uncertainty has to be used. Could it be that there is an error in the example?**
A36	*No, it is not an error in the proposed example. In fact, your method calculated combined standard measurement uncertainty shall comply with and be lower than this criterion of within-lab reproducibility of 30 % (when concentration is lower than 10 ppb like for chloramphenicol in the example).*
Q37	**Is LOD & CCα equal? If not, do we need to establish them separately?**
A37	*It is not equal; LOD and CCα are entirely different concepts and need to be determined separately. In the frame of Commission Implementing Regulation (EU) 2021/808, LOD does not play a role and hence does not need to be determined.*
Q38	**The decision limits obtained through method 1 shall be verified by analysing blank matrix fortified at the calculated decision limit. Is there any specific criteria for verification as the same is not provided in Commission Implementing Regulation (EU) 808/2021?**
A38	*No, the check shall avoid the calculation of unrealistically low CCα far below the lowest spike level (due to extrapolation).*
Q39	**When you have a CCα slightly higher than MRL and your sample concentration falls between MRL and CCα, would you consider that compliant or not?**
A39	*The decision criterium is CCα.The CCα is calculated according to point 2.6.2.a of Annex I to Commission Implementing Regulation (EU) 2021/808. In this case, the MRL is already included in this calculation and the measurement uncertainty is taken into account. Therefore, the sample result is then compared with CCα directly (not with the MRL).* *Art. 5 of Commission Implementing Regulation (EU) 2021/808 states: The result of an analysis shall be considered non-compliant where it is equal to or above the decision limit for confirmation (CCα).*

Questions on uncertainty calculation

Q40	**Can this formula be used for combined standard measurement uncertainty as part of calculation for CCα?** **Combined std MU @ LCL = k * RSD * conc, where k=2 or do you just use the % RSD from within laboratory % CV?"**
A40	*The combined standard MU is without a coverage factor.*
Q41	**How was U (trueness) uncertainty calculated?**
A41	*It comes usually from spiking experiments (since reference materials are rarely available).*

Questions on stability

Q42	**As is not linked to the method but more to the stability of the sample before analysis, is it possible that EURLs share their data?**
A42	*Stability data are provided to PT (proficiency tests) participants along the various EURL-PT organised each year by the 3 EURLs and for years now. Setting up a database for stability data is part of the 2023 EURLs workplan.*
Q43	**How can stability data from EURLs be accessed? Is that data to be expanded to most analytes/matrices?**
A43	*See the reply above. Please contact the EURL with specific questions.*

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Q44	**For the determination of analyte stability in matrix, do we have to check stability for all compounds? (interaction between analytes, not reflecting the reality with samples containing a limited number of analytes)?**
A44	*If feasible, then yes (and interaction may also take place in the standard solutions).*
Q45	**Is it acceptable to prove the stability for different families of analytes with a screening method and consider that the stability is also correct for all corresponding confirmatory methods or others methods with the same targeted families?**
A45	*This question cannot be answered straightfully. Stability is effectively a parameter strictly depending on the analyte behavior in the chemical solution and/or biological matrix of interest. But there are different cases depending on the chemical families of analytes that are considered and also depending on the suggested screening and confirmatory methods where chemistry for extraction, purification, detection is applied.*
Q46	**The stability seemed to focus on ultra-low temperatures. Is there any flexibility for other conditions?**
A46	*Ultra low is only the reference temperature - the other temperatures are relevant in practice.*

Question on calibration

Q47	**At what level should the calibration graph start ?**
A47	It depends: for B-substances usually at 0.1 * MRL or ML, for A-substances as low as possible (ALARA principle). Include zero and then five points in equidistant steps around MRL, RPA or ML.

Question on software/Excel tool

Q48	**Can the excel tool be shared for the calculation of CCα and CCβ for Group A and Group B compounds?**
A48	Yes, it can; please contact the EURLs.
Q49	**Is there any chance to get software for calculation of CCα and CCβ?**
A49	*Yes, please contact the EURLs.*
Q50	**Why does the European Commission not furnish to all EU official laboratories an "official" and free software package to elaborate validation data obtained following Commission Implementing Regulation (EU) 2021/808?**
A50	*The European Commission does not own such software package. Support is offered by EURLs.*

Question on on-going performances

Q51	**At what level do we have to keep internal QC ?**
A51	*For example, see the "EURL Guidance Document on the Quality control during routine analysis (ongoing method performance verification)" which can be found on the EURL websites.*

Question on method extension

Q52	**Can clarity be brought on proportional and constant deviation used as criteria for extension of method validation to different matrices as per EURL guidance document?**

A52	It is not easy to explain it in a few words - it is an estimation of the size of predefined factorial effects on the analytical result. You vary factors on two levels - you have for each factor level 4 experiments (runs) and you can calculate for each of the 4 runs a mean calibration curve. y=ax+b characterises a calibration curve - and the deviation of proportional (a) and the deviation of constant (b) of the two calibration curves gives an estimate of the size of the factor effect (and a potential concentration dependent factor effect). So if e.g. the matrix is a factor level, it can be shown that the matrix does not have a significant effect on the result (or an acceptable effect on the result). For examples see the "EURL Guidance document on the extension of quantitative confirmation methods" which can be found on the EURL websites.

FROM 2002/657 TO 2021/808

Q53	**If the laboratory has validated the method compliant with Commission Decision 2002/657/EC and needs to expand the range, can it do so in simplified validation or is it necessary to study not only one concentration level but two?** **In case you have to lower the CCα and add a new species, can it be done in a simplified validation in a single experiment?**
A53	It depends. See for examples on the "EURL Guidance document on the extension of quantitative confirmation methods" which can be found on the EURL websites.
Q54	**What is the reference method for the analysis of DNSH along with other metabolites of nitrofurans?**
A54	*There is no reference method. Methods which fulfill the criteria can be used, for example there is a method from the EURL Anses-Fougeres published in Food Chemistry 342 (2021) from Guichard et al. : "Confirmation of five nitrofuran metabolites including nifursol metabolite (DNSH) in meat and aquaculture products by liquid chromatography-tandem mass spectrometry" https://doi.org/10.1016/j.foodchem.2020.128389.*

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Question on trueness determination

Q55	**What is the trueness criteria for a screening method**
A55	Only for quantitative screening, see point 2.2 of Commission Implementing Regulation (EU) 2021/808. Trueness has to be determined only for quantitative screening methods and criteria of 2021/808 has to be fulfilled. The Commission Implementing Regulation (EU) 2021/808 states that "Quantitative screening methods, used for both screening and confirmation shall meet the same requirements for accuracy, range, and precision as described in 1.2.2.1 and 1.2.2.2.". It is important, that the β-error criterion is fulfilled at the level of interest.

Question on internal standard

Q56	**Must an Internal Standard be used for all confirmatory methods irrespective for Group A or Group B substances?**
A56	The use of an internal standard is not mandatory, but highly recommended as a guarantee to help fulfilling the criteria of performance according to Commission Implementing Regulation (EU) 2021/808. Anyway, it is useful to use it for A substances especially to avoid false negatives (and to be sure of positive results), for B substances in order to have a reliable quantification.

Question on recovery

Q57	**Absolute recovery need not be calculated if an Internal Standard is used as well as a Matrix based calibration is used. Is it correct or only in those case Internal Standard is not used?**
A57	That is correct.

Question on identification criteria

Q58	**Is it Ion ratio or relative Ion ratio?**
A58	It is a relative ion ratio (relative to the base ion / base transition) - and also a maximum allowed relative deviation. 1.2.4.1 - Relative intensities: The ion ratio of the analyte to be confirmed shall correspond to those of the matrix-matched standards, matrix-fortified standards or standard solutions at comparable concentrations, measured under the same conditions, within ± 40 % relative deviation. For example, the highest transition is set to 100 %, the second transition is 50 % of this highest transition. The maximum allowed deviation would be +/- 40 % , this means the relative intensity in the second transition would be acceptable between 30 % and 70 %.