SARAF WEBINAR of 1 March 2022
on Commission Implementing Regulation (EU) Regulation 2021/808
FAQ
Coordinated replies (3 EURLs, EC) to questions raised during the webinar

Regulation

Questions on implementation and repealing

Q1	**Are Guidelines for the implementation of decision 2002/657/EC 2 repealed or not ?**
A1	*Commission Implementing Regulation (EU) 2021/808 entered into force on 10 June 2021. All methods validated after this date must follow the rules set out in this Implementing Regulation, that means, they should follow the new EURL guidances dedicated to Implementing Regulation (EU) 2021/808. Previously validated methods according to Commission Decision 2002/657/EC can still be used until their revalidation/new validation according to Implementing Regulation (EU) 2021/808, at the latest until 10 June 2026. These "old" methods can follow the guidances for Commission Decision 2002/657/EC until that time (revalidation/new validation).*
Q2	**What about validated methods before R 2021/808 accredited under flexible scope? New analytes or matrix can be included in the method with the requirements CD 2002/657?**
A2	*New analytes/matrices validated after 10 June 2021 must follow the rules laid down in Commission Implementing Regulation (EU) 2021/808. This principle shall be applied also to "old" methods which are revalidated for new matrix/analyte etc.*
Q3	**Is it possible to provisionally add a new analyte to a 02/657 validated method keeping 657 quality controls for the whole procedure?**
A3	*No, with an extension of the method for a new analyte (new validation), after 10 June 2021 you must follow the rules laid down in Commission Implementing Regulation (EU) 2021/808.*
Q4	**When will EU regulation 2021/808 be valid?**
A4	*Commission Implementing Regulation (EU) 2021/808 entered into the force on 10 June 2021.*

Questions on RPA and regulatory limits

Q5	**What would be the RC for chloramphenicol in feed? 0,15 µg/kg?**
A5	*There is no RPA for chloramphenicol in feed established. However EFSA states that the RPA for food of animal origin is also appropriate to be applied to feed.*
Q6	**Which guideline for RPA?**
A6	*EFSA has published Methodological principles and scientific methods to be taken into account when establishing Reference Points for Action (RPAs) for non-allowed pharmacologically active substances present in food of animal origin, see https://www.efsa.europa.eu/en/efsajournal/pub/5332*
Q7	**How can we calculate RPA?**
A7	*RPA are established in Commission Regulation (EU) 2019/1871.*
Q8	**Is the RPA for Malachite and Leucomalachite green of 0.5 applied individually or as combined? If so, do we need to consider half of 0.5 individually?**
A8	*RPA 0.5 µg/kg for the sum of malachite green and leucomalachite green will be applicable from 28 November 2022. Until that time, the RPA for these substances is 2 µg/kg. The limit is valid for the sum of malachite green and leucomalachite green (not needed to consider half of the limit individually for each of the substances).*
Q9	**What is the RPA for crystal violet and its leuco form?**
A9	*Currently, there is no RPA established for crystal violet and its leuco form. Only a MMPR is proposed as from the EURL Guidance document.*

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Question on MMPR

Q10	**MMPR was mentioned in presentation, however the same is not mentioned in 808/2021, so will it have a legal stand?**
A10	*MMPR is mentioned in a EURL Guidance document. It has no legal status. MMPR is suggested by the EURLs on the basis of state of the art analysis (and it shall be regularly reviewed to ensure that it corresponds to the lowest levels, which are achievable, taking into account the most recent scientific developments). It is considered as a laboratory benchmark with the purpose to harmonise the level at which compounds can be analysed in the Member States.*

Questions on screening method validation

Q11	**Would it be possible for the ANSES speakers to share the draft of the future 2022 screening guidance?**
A11	*At the moment it is not possible to share the guidance, which is currently under review. It will be available in the coming months.*
Q12	**Are DNSH metabolites of Nifurasol mandatory to be included in Screening method? Presently we have not come across any vendors having DNSH ELISA Kits.**
A12	*Indeed there are no ELISA kits commercialised for the screening of DNSH. However the RPA includes DNSH (Commission Regulation (EU) 2019/1871). Therefore DNSH should be controlled.*
Q13	**As per CRL 2010, two approaches are available for calculating cut off level. Does it still become applicable in the new guidance document for screening?**
A13	*In the coming-soon revised guidance for screening methods, it is proposed to keep only the approach of Annex II to that guidance (calculation of threshold T and cut-off value Fm) because this approach is more statistically valid. Furthermore it is the approach used by the majority of the users.*

Questions on specific compounds considerations

Q14	**Cephalosporins are an important class of antibacterial agents in use today for both humans and animals. What is your view on this?**
A14	*Cephalosporines are considered to be essential antibiotics for humans and they needed to be used in animals as less as possible to prevent AMR.*
Q15	**For pesticides regulated under Regulation 396/2005, e.g. Fipronil, which will be included in the new Group A3, can they still have validation according to the pesticide regulation SANTE/12682/2019 or should they have NEW validation under the proposed requirements of 2021/808 (CCalfa, etc)?**
A15	*Fipronil can be used as phytosanitary insecticide or as a veterinary medicinal product. If the samples are taken as a consequence of the treatment of the animals, they should follow the rules for interpretation of the results according to Commission Implementing Regulation (EU) 2021/808. If the method is validated according to pesticides, the laboratory has to prove that the result complies with the rules for residues of veterinary medicinal products, i.e. with CCα.*
Q16	**There have been a number of high profile food safety disputes in trade over the past decade e.g WTO between US and EU hormone-treatment beef. What are your views?**
A16	*This question does not relate with the scope of Commission Implementing Regulation (EU) 2021/808 (as a topic of this training session). In any case, EU food law shall pursue the general objectives of a high level of protection of human life and health and the EU hormone ban is justified to ensure that high level of consumer health and safety.*

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Q17	**Is the addition of Nifursal (DNSH) in Nitrofuran metabolites necessary to validate the Method?**
A17	*Addition of new analyte (extension of the method) requires new validation, that means that rules laid down in Commission Implementing Regulation (EU) 2021/808 must be followed. If there is a well established method in routine use - and the method is under control and validation data are still correct (proven e.g. with participation in PTs and by control charts) - then the new critieria might be applied to the old validation data and the new analyte might be added according to the method extension guidance (also by applying the new critieria); a new validation report/method description has to be released clearly describing the applicability/fitness for purpose of the method (with the reference to Commission Implementing Regulation (EU) 2021/808).*
Q18	**Addition of Nifursal(DNSH) in Nitrofurans makes it necessary to validate the Method. For validation, do we need to follow EC 808 or EC 657?**
A18	*Addition of new analyte (extension of the method) requires new validation, that means that rules laid down in Commission Implementing Regulation (EU) 2021/808 must be followed. If there is a well established method in routine use - and the method is under control and validation data are still correct (proven e.g. with participation in PTs and by control charts), then the new critieria might be applied to the old validation data and the new analyte might be added according to the method extension guidance (also by applying the new critieria); a new validation report/method description has to be released clearly describing the applicability/fitness for purpose of the method (with the reference to Commission Implementing Regulation (EU) 2021/808).*
Q19	**"Art 107 of Reg. 2019/6 states:** **Antimicrobial products shall not be used in animals for the purpose of promoting growth nor to increase yield** **1. Does that incude anticoccidials?** **2. If so, how checked 3C?** **3. Might anticoccidials wil come under Art 118?"**
A19	*This question does not relate with the scope of Commission Implementing Regulation (EU) 2021/808 (as a topic of this training session). We are not able to provide a reply; this question should be addressed to our colleagues from the Commission who are responsible for veterinary medicinal products.*
Q20	**Regarding medroxyprogesterone acetate does the MRPL still apply?**
A20	*The MRPL no longer applies to medroxyprogesterone acetate, the MMPR (with the same level) is used for harmonisation of the official contols on this substance.*

Question on new control and monitoring plans in 2023

Q21	**For third countries, Plan 1 and 2 must be submitted in March each year?**
A21	*Yes, the plans must be submitted by 31 March of each year.*

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Technical aspects

Questions on calibration points

Q22	**What should be the calibration points for multi residue methods for CAP, DYES, NFM, NITROIMIDAZOLES?**
A22	*In general, for multimethods, calibration curves have to be adapted to the requirements of the different analytes. According to the Commission Regulation (EU) 2019/1871, new RPA have been set for chloramphenicol (0.15 µg/kg), nitrofurans (0.5 µg/kg) and the sum of malachite green and leucomalachite green (0.5 µg/kg) (applicable as from 28 November 2022; until that time, the MRPL values from Commission Decision 2002/657/EC are applicable). Therefore, calibration points shall be set in line with Commission Implementing Regulation (EU) 2021/808 for prohibited or unauthorised substances, i.e. below the regulatory RPA. Validated parameters are at least set at 0.5 ; 1.0 and 1.5 times the RPA according to paragraph 2.2 of Commission Implementing Regulation (EU) 2021/808. Depending on the applied validation approach it might be also possible to have a common calibration curve (starting e.g. at 0.25 µg/kg - or at 0.05 µg/kg if also chloramphenicol should be included) - but this depends also on the performance of the available instruments.*
Q23	**As per EU 2021/808, are beginning and end calibration required or not for routine analyses of samples? Also kindly explain the injection order of samples (batch) for routine analysis. Is EC2 repealed or not ?**
A23	See chapter 3 - for quantitative methods, a calibration curve has to be run - either at the beginning or at the end of the analytical sequence - of course both is also possible. Preferably the batch should be built in a way that contamination possibilites are minimised, see also example in chapter 3. For routine analysis of sample, please see the EURL Guidance on the Quality control during routine analysis (ongoing method performance verification). Also analytical sequences (1.2) and calibration for quantitative determination (1.3) are proposed in this document. The document is available at the EURLs Websites: https://eurl-residues.eu/ and also http://eurl-veterinaryresidues.anses.fr/

Questions on the Level of supplementation for validation

Q24	**In case there are higher MRLs which are saturating LCMSMS, what does the regulation say about the validation levels?**
A24	This is not a regulatory concern due to the MRL value at all. This is only an analytical skillness issue. You have to develop your method in such way that criteria for quantification are fullfilled. If you are in the area of saturated LC-MS/MS response, the method is not suitable for reliable quantification - i.e. reduce the sample amount or increase the dilution.
Q25	**For analytes regulated as "sum of" (e.g. sulphonamides) what should be the levels of validation?**
A25	*Preferably 1/10 of the MRL if feasible (for the single substances). For possible sums such as for sulfonamides, it is urged to validate calibrating each analyte at lower levels than the 1/2 MRL. A range between 1/10th MRL and 1/4 MRL might be suitable in most cases.*
Q26	**Can more clarity (maybe levels with an example) be brought on at and above RPA or LCL at equidistant steps ?**
A26	*If the RPA is 0.15 ng/g, it can be e.g. 0.05 / 0.10 / 0.15 / 0.20 / 0.25 ng/g*
Q27	**Method 1 for CCa says "at and above the RPA" or "at and above the MRL" for the calibration curve. So we cannot use the replicates data of 0.5 RPA and 0.1MRL for CCa calculations, because both these points are lower**
A27	*It can also be considered including the 0.5 RPA when it represents also your chosen LCL below the RPA or even lower if you decide to control at 0.25 RPA for example. Same approach*

	is valid for MRL substance, the 0.5 MRL can be embarked if this is part of your routine calibration. For MRL substances, CCα needs to be calculated starting from the MRL.

Questions on LCL

Q28	**The concept of LCL is similar to QL, that is, is it the first point of the calibration curve?**
A28	Yes, it is the lowest concetration at which the measuring system is calibrated
Q29	**How can we establish LCL?**
A29	*By preliminary experiments during your development and implementation of the method - it could be the concentration at which > 50 % of the spiked samples can be confirmed.*
Q30	**Can we say LCL as LOQ?**
A30	*No, it is a different concept and it could lead to confusion. However your estimated LOQ may become your LCL if you wish and only if it can be fully validated as such under Commission Implementing Regulation (EU) 2021/808.*

Questions on STC

Q31	**How can we go for precision study and its criteria in semi quantitative method of STC?**
A31	*For semi-quantitative methods, the Commission Implementing Regulation (EU) 2021/808 states that "The precision requirements of Chapter 1.2.2.2 do not need to be met for semi-quantitative screening methods. However, the precision shall be determined to prove the suitability of the method for avoiding false compliant analytical results." (see Table 5).* *Therefore the precision can be studied at several concentrations (see 2.2.1.2.), but does not need to fulfill the full criteria of precision of quantitative methods. The STC could be one of these concentrations. If the quantitative value of the screening method is used to make a decision, then go for confirmation: yes or no? Then the STC has to be chosen in a way that the β-error criterion is fulfilled at the MRL.*
Q32	**Trueness criteria for STC**
A32	*Trueness has to be determined only for quantitative screening methods and then criteria of Commission Implementing Regulation (EU) 2021/808 have to be fulfilled. The Commission Implementing Regulation (EU) 2021/808 states that "Quantitative screening methods, used for both screening and confirmatory purpose shall meet the same requirements for accuracy, range, and precision as described in 1.2.2.1 and 1.2.2.2.". Trueness shall be determined at several concentrations (see 2.2.1.2.). The STC could be one of these concentrations. It is important that the β-error criterion is fulfilled at the MRL.*

Question on measurement uncertainty

Q33	**At what level can measurement uncertainty be calculated ?**
A33	It has to be calculated at the level of interest, so then it can be at RPA, MRL or MMPR depending on your substance status. Ideally you have an uncertainty function for a concentration range.

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Question on CCb calculation and application

Q34	**Is it necessary to perform CCβ for quantitative routine vet residue method analysis in a multi-residue method?**
A34	If the quantitative routine method is used as a screening method, it is necessary to evaluate its CCβ for the screening step. The CCβ is the key parameter for the suitability of a screening method, i.e. the data have to be available for all analytes. There might be examples in which the CCβ is set e.g. at general concentration level, e.g 0.5 MRL (but again: this has to be proven with validation data).

Questions on CCa calculation and application

Q35	**Can Method 3 (i.e LCL) be used for estimating CCα for compounds having RPA or applicable only for unauthorized compound having no RPA**
A35	Yes, as long as LCL is below or very close to RPA. CCα needs to be below or equal to RPA.
Q36	**If applying standard additions to suspect non-compliant samples, how can CCα be applied with the new approach? ...as the matrix effect has been included in CCα calculation (using 21 different negatives), whereas the use of standard addition is specifically matrix matching for the sample itself.**
A36	*Only of interest for MRL substances - and in that case, there are probably few cases, in which this might give a different decision; if the standard addition is applied, the concentration range after standard addition should be within the validated concentration range (or close to it), then the CCα of the validation might be used reasonably.*
Q37	**For authorised substances in matrix/species combinations for which no MRL has been set must two CCα be determined (0.1 x Cascade MRL and LCL) and should these levels in turn be used to generate WLr and WLR data?**
A37	*Yes two CCα of confirmation can be determined for this kind of substances: the cascade MRL one and the "non-cascade" MRL set at 0.1 × cascade MRL if analytically feasible (which would be probably also the LCL).*

Question on standard addition

Q38	**Where standard addition is used due to lack of an appropriate internal standard, are there any guidelines on how this should be handled with respect to validation and CCα establishment?**
A38	No, not yet. Currently a working group is formed of NRLs and EURLS to discuss this - there should be validation data for a reasonable concentration range around the level of interest - and a CCα should be calculated. If the standard addition is applied at later occasions, the concentration range after standard addition should be within the validated concentration range (or close to it), then the CCα of the validation might be used reasonably. This technical issue (still under review) will be explained in a future EURL Guidance to be attached to the Commission Implementing Regulation (EU) 2021/808.

Questions on matrix effect determination

Q39	**Relative matrix effect study can do at the initial stage of validation?**
A39	Yes.

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Q40	**Just to clarify - do the relative matrix effects need to be checked regardless of whether internal standard is used? The information/calculations in the regulation is a little confusing.**
A40	*The use of IS is very often in line with efforts in reducing the variations due to matrix effects. Anyhow, the matrix effects always need to be checked and might be already automatically taken into account by application of an appropriate validation scheme.*
Q41	**Will it be acceptable to calculate Relative Matrix Effect using different approaches to that proposed in the Reg 2021/808?**
A41	*Yes, as long as they give comparable information and are trustworthy.*

Questions on stability

Q42	**Are there any studies on the stability of B1 antibiotics (both in solution and in matrices) that comply with 2021/808? If there are, I would be grateful if someone could send it to me.**
A42	Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2011 Dec;28(12):1657-66.. Berendsen et all. Determination of the stability of antibiotics in matrix and reference solutions using a straightforward procedure applying mass spectrometric detection; JAOAC International, 96, 2, 2013, 1-10, GAUGAIN M., CHOTARD M.P., VERDON E. Stability study for 53 antibiotics in solution and in fortified biological matrices by LC-MS/MS. Some information is available at the common 3 EURL websites as well (http://eurl-veterinaryresidues.anses.fr/).
Q43	**Are there any studies on the stability of B1 antibiotics (both in solution and in matrices) that comply with 2021/808? We need it for the re-validation of both our B1 screening and confirmatory methods.**
A43	*Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2011 Dec;28(12):1657-66.. Berendsen et all. Determination of the stability of antibiotics in matrix and reference solutions using a straightforward procedure applying mass spectrometric detection; JAOAC International, 96, 2, 2013, 1-10, GAUGAIN M., CHOTARD M.P., VERDON E. Stability study for 53 antibiotics in solution and in fortified biological matrices by LC-MS/MS. Some information is available at the common 3 EURL websites as well (http://eurl-veterinaryresidues.anses.fr/).*

Question on precision determination

Q44	**As per the validation precision study must be carried out in Semi quantitative**
A44	For semi-quantitative methods, the Commission Implementing Regulation (EU) 2021/808 states that "The precision requirements of Chapter 1.2.2.2 do not need to be met for semi-quantitative screening methods. However, the precision shall be determined to prove the suitability of the method for avoiding false compliant analytical results." (see Table 5). (The use of mass spectrometry is not mandatory.) Therefore the precision can be studied at several concentrations (see 2.2.1.2.), but does not need to fulfil the full criteria of precision of quantitative methods. The STC could be one of these concentrations. If the quantitative value of the screening method is used to make a decision, then you go for confirmation: yes or no? Then the STC has to be chosen in a way that the β-error criterion is fulfilled at the MRL.

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Question on trueness determination

Q45	What is the trueness criteria for screening method
A45	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

Q46	Whether Internal Standard is/must be used for all confirmatory methods irrespective for Group A or Group B substances
A46	The use of 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

Q47	Absolute recovery need not be calculated if Internal Standard is used as well as Matrix based calibration is used. Is it correct or only in those case Internal Standard is not used?
A47	That is correct.

Question on identification criteria

Q48	Is it Ion ratio or relative Ion ratio?
A48	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 %.