EUROPEAN UNION
REFERENCE LABORATORY
VMP Residues
A6, B1, B2f, B3e

WORK PROGRAMME OF THE EUROPEAN UNION REFERENCE LABORATORY AT THE FRENCH AGENCY FOR FOOD, ENVIRONMENTAL AND OCCUPATIONAL HEALTH SAFETY

Antimicrobials and dyes

Group of substances: B1, A6, B2f, B3e

Laboratoire de Fougères

Proposal

Contract period: January 2015 – December 2015

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LEGAL FUNCTIONS AND DUTIES

The functions and duties of the Reference Laboratory are described in Article 32 of Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 (Official Journal of the European Union L 165, 30.04.2004, pp. 1-141, corrected and republished in Official Journal of the European Union No L 191, 28.05.2004, pp. 1-52).

1. OBJECTIVES FOR THE PERIOD JANUARY - DECEMBER 2015

A. General tasks

Article 32, paragraph 1 (e)

B. Development and validation of analytical methods

Article 32, paragraph 1 (a, c)

C. Quality assurance and quality control including the organisation and implementation of proficiency tests

Article 32, paragraph 1 (b, c)

D. Technical and scientific support to NRLs and third countries

Article 32, paragraph 1(a, d, e, f)

2. WORKING PLAN FOR THE PERIOD JANUARY - DECEMBER 2015

A. General Tasks

Article 32, paragraph 1 (e)

1. Meeting 4 EU-RLs, EU-RLs residues management,

   Upon specific requests from DG-Sanco

2. Technical and scientific support to the Commission,

   Upon specific requests from DG-Sanco and/or from FVO along the year 2015

3. Compilation of annual report and cost estimate,

   Documents to be released to DG-Sanco by September 2015 (Programme 2016 including performance indicators) and by March 2015 (Technical report of programme 2014)

4. Co-operation with European / International organisations,

   DG-SANCO/FVO, EMA, EFSA, EURACHEM

   AOACI, FAO-AIEA, ISO, CEN, FIL-IDF, IUPAC, …

5. Documentation services, inter change of information via the website.

As part of the points no 4 and no 5, several documents will be elaborated by the EU-RL, or collectively with the EU-RL in collaboration with NRLs and/or expert groups from international organization:

1. **COLLABORATIVE DOCUMENT TO BE CREATED WITH THE NRLs' NETWORK ON THE STABILITIES OF ANTIMICROBIALS IN FOOD CONTROL CONDITIONS**
   Deliverable: **A report on the advances in creation and documentation of a Database** to be made available to the NRL network through the EU-RL website and presented during the 2015 workshop.

2. **FOLLOW-UP OF THE BUILDING OF THE GUIDELINE DOCUMENT FROM CCRVDF ON VALIDATION OF MRM with PARTICIPATION TO THE AD-HOC WORKING GROUP**
   Deliverable: In cooperation with the 2 other EU-RLs for « Residues » (BVL & Rikilt) an **updating report on the advances in the CCRVDF MRM Validation Guideline** and on possible evolution of **European guidelines** for recommending to the network of European laboratories on validation of analytical methods for control of VMP residues in food from animal origin (CD 652/2002/CE and Sanco/2726/2004 rev8 and Guideline for validation of screening methods)

3. **FOLLOW-UP OF THE BUILDING OF THE GUIDELINE DOCUMENT FROM CCRVDF ON TOLERANCE OF RESIDUES IN HONEY PRODUCTS with PARTICIPATION TO THE AD-HOC WORKING GROUP**
   Deliverable: **A yearly updated report on advances in the CCRVDF support document** for evaluation of limits or tolerances of VMP residues in apiaries products to be made available to the network of NRLs through the EU-RL website.

4. **FOLLOW-UP OF THE DATABASE FOR THE REFERENCE MATERIALS PREPARED DURING EU-RL ORGANIZED PROFICIENCY TESTING STUDIES**
   Deliverable: **A yearly updated report on the database of reference materials** created starting from our PT materials and to be made available to the network of NRLs through the EU-RL website.

5. **UPDATED SURVEY ON MICROBIOLOGICAL INHIBITORY METHODS AND THEIR PERFORMANCE FOR SCREENING ANTIMICROBIALS IN FOODSTUFFS**

   As a follow-up of last European meetings held during the period 2012-2014 and regarding the microbiological control of antibiotics in food, a thorough, possibly also extra-EU, survey will be additionally engaged at the EU-RL to assess the performance claimed by the analytical methods in place for various foodstuffs. This issue has been already implemented for meat in 2014 (see report 2014), and now it will be further emphasized for additional matrices: milk, eggs, poultry, fish and honey and in various Countries starting with EU Member States at least.
   Deliverable: **By the end of 2015 to the network of NRLs a comprehensive report surveying this issue – A milestone will be the communication delivered to the network of NRLs during our 2015 workshop and dedicated to the biological screening of antimicrobial residues. This topic could also be further proposed in 2015-2016 for communication in specific congress/symposium and as a possible review publication in an International Scientific Journal.**

B. Development and Validation of Analytical Methods

Article 32, paragraph 1 (a, c)

6. Development and Confirmatory method for antimicrobials in different matrices (muscle, milk, eggs, honey …).

6.1 A multi-antimicrobial family method – **Converging toward a Multi-Matrix / Multi-Antimicrobial Method** using suitable LR/HR-MS instruments (2014-2015)

A comparative evaluation of performance for different one method/one instrument strategies aimed at controlling multi-family antimicrobial residues has been undertaken since 2014. The LC-Tandem-MS multi-antimicrobial residue method that was developed during the past years at the laboratory of Fougeres for meat and for milk and transferred to the NRLs during the 2 past workshops (2007 and 2011) is being reconsidered in this project by including a comparison with the new strategy developed at the EU-RL to control antimicrobials by means of a similar LC-HR-MS multi-family/multi-class residue method. This comparative work will be undertaken with our LC-LTQ-Orbitrap instrument and possibly with a new type of instrument to be acquired in 2014: a LC-QTrap instrument. The achievement of this 2-year project will be to design a MRL-built, time- and cost-effective converging method which will be dedicated at least to reliably screen and identify a set of 80-120 antimicrobial analytes in muscle and in milk. The goal includes analyzing within as few as possible runs per sample and to be reliable enough to monitor in several matrix/species of foodstuffs (meat, milk, and may-be aquaculture products). A comparison of targeted screening methods by LC-LRMS (LC-MS/MS) and by LC-HRMS will be undertaken on real-life routine samples. **The deliverable standard operating procedure with a validation report of the method's performance at least in the muscle matrix will be scheduled after the 2-year work by the end of 2015. Two milestones have been incorporated into the project and will be presented as 2 reports of the advances made on the topic to be discussed with the network of NRLs during our workshops of 2014 and of 2015. Final transfer of the methodology should occur during a training session to be organized at our 2016 annual EU-RL/NRLs workshop. The milestones' reports and the final SOP and validation report will be posted in due times on our EU-RL website to the attention of the network of NRLs.**

6.2 **Full Scan High-Resolution LC-MS for Screening Certain Critically Important Antibiotic Residues in Poultry, Chicks and Eggs** – Metabolomic approaches for the search of biomarkers of illegal treatments (2015-2016)

This study has been started in 2015 based on the concern for the antibioresistance-involved group of cephalosporins, i.e. the 3rd and 4th generations (C3G-C4G) considered as critically important antibiotics (CIA), and specifically the ceftiofur and its complicated depletion in tissues through protein-bound metabolisations. A 2013-2014 work was engaged already aimed at getting deeper knowledge on the fate of ceftiofur in animal tissues. Poultry farming, where ceftiofur treatments are not authorized, was the particularly chosen field of experiment for that study. This 18 month study was achieved in 2014 by means of collecting data from a set of poultry farmed-animal experiments and using different analytical instrumentations: HPLC-UV, LC-MS/MS and LC-HR-Orbitrap-MS (see 2014 workprogramme report). After delivering to the network of NRLs the advances of this study during the EU-RL/NRLs workshops of 2013 and of 2014, results have been posted on the EU-RL website and communicated at international symposium (VDRA-Ghent 2014). They are in the process of submission for publication in international peer-reviewed journals.

The present proposed study is aiming at developing further the strategy of investigation for possible C3G-C4G critically important antimicrobials such as ceftiofur (C3G) and cefquinome (C4G). On the one hand, ceftiofur metabolites and/or ceftiofur end-products biomarkers in poultry chicks including feathers will be considered as evidence of not authorized veterinary treatments in poultry. On the other hand, non-authorized presence of ceftiofur and cefquinome in poultry and in eggs will also be investigated by means of animal experimental studies in laying hens with a comparison of metabolic profiles from

treated and non-treated flocks and search by LC-HRMS for biomarkers of these contaminations in animal and in eggs.
Will be delivered a final report of this study to be presented to the network of NRLs by means of a communication at the 2016 EU-RL workshop and/or during one of the specific symposia dedicated to the veterinary drug residue analysis (Euroresidue 2016, …). The report will also be posted in due time on our EU-RL website to the attention of the network of NRLs.

6.3 **Enhancing the control for multi-dye residues in aquaculture products by targeted method with LC-MSMS and untargeted approach using LC-HRMS** (2015-2016).

After its ban in 2004 in the EU, the occurrence of malachite green and its leucobase in aquaculture products is reducing and monitored for several years now and is also well documented in the literature. Recently, the interest in dye residue control was extended to several other dyes closely related to malachite green, i.e. crystal violet and brilliant green. Still possibly used as biocides in aquaculture products imported from other regions of the world, it is also of interest to develop a strategy of control able to counteract any attempt of misuse with other substances from the same triarylmethane family (group substitution) or from other dye families (phenothiazines, xanthens, phenylazoic dyes...). This study will contribute developing/transferring a multi-dye LC-MSMS method able to extend to the actual 5 compound LC-MSMS method and to cover a 10-15+ compounds. Additionally, a metabolomic approach by LC-HRMS will be evaluated to track a biomarker of metabolic effects of, at least, the triarylmethane treatments in aquaculture products.
To be delivered by end 2016 a standard operating procedure of the confirmatory LC-MS/MS method for multi-dyes in aquaculture products including prawns and a report of validation of the performance of the method. The SOP and validation report will also be posted on our EU-RL website to the attention of the network of NRLs. Also delivered the strategy and the results of the metabolomic approach applied to triarylmethanes (for malachite green at least).

7. Study of screening tests (biomethods and kits).

A continuous evaluation of the performance of different screening kits for antimicrobial or dye residue testing (either microbiological or immunological) proposed by manufacturers to be applied on different matrices will be investigated. The results of these investigations will be released to the network of EU-NRLs by means of workshops, enclosure to the EU-RL website and when advised published in relevant scientific journals.

7.1 - Evaluation and Validation of new technologies for rapid screening of veterinary antimicrobial residues in Foodstuffs (Immunoreceptor biosensors)

One important improvement on biosensor technology in the field of high throughput screening of food contaminants is to develop wide spectrum biosensors. Microbiological methods are generally not sensitive enough to cover a wide spectrum of antimicrobial compounds at their regulatory level of interest and they are time consuming by requiring 24 to 48 hours of analysis for one set of samples. Biosensors may replace microbiological methods for screening antimicrobial veterinary drug residues only when they can reach a wide spectrum of compounds with detection levels below regulatory limits and with employing a technology at a reasonably low cost. One possibility to achieve the objective of wide selectivity is to develop a multi-array or multiplexing technology. Our efforts should concentrate on developers of multiplex assays and producers of portable devices for use in the field. For this purpose, a review of the potentially interesting multiplex

biosensor systems will be published in a report by the end of 2014. Therefore, our action in 2015 will be considered further in selecting items from these reported projects especially from the side of multi-array or multiplexing technologies. Also new commercialized systems with existing kits dedicated to the screening of antibiotics could be evaluated and validated if necessary. Moreover will be considered the interest in new partnerships with research teams or equivalent instrument manufacturers in order to demonstrate capabilities of these systems applicable to the screening of antibiotics.
A new reporting to be delivered by the end of 2015 to the network of NRLs. A scientific publication in a peer-reviewed journal might be also further considered. A first communication on the advances of this 2014-2015 project will be proposed during the 2015 workshop organized to the attention of the NRLs and/or during one of the specific symposia dedicated to the veterinary drug residue analysis (AOAC Europe 2015, …). The review report will be posted on our EU-RL website to the attention of the network of NRLs.

7.2 – Evaluation and validation of a new rapid method to screen antibiotics specifically in Egg products

This project started in 2014 on the brand-new Explorer test (Zeu Inmunotech, Spain) based on a recently developed incubator-reader (all in one device) for the screening of antibiotic residues in muscle and in eggs. This new system allows an automatic reading (incubation stops when the negative control is ready) supported by an optical device delivering objective interpretation. The results of the validation of the test for the screening in muscle will be delivered by end of 2014 together with a report of the validation conducted to determine relevant screening data and assess performances of this method. For 2015, the evaluation and validation of this new test in eggs is scheduled possibly starting by the end of 2014.
Furthermore, in 2015, other technologies for the screening of antibiotics specifically in eggs will be studied and possibly validated. The first approach should be the evaluation and the validation of the Evidence Investigator (Randox, UK). This innovative multiplex system based on chemiluminescence detection allows the detection of multiple analytes simultaneously. The encouraging results obtained during the few past years on the validation of 2 kits (AM I and AM II) in honey for sulfonamides and for multi-antibiotic detection, respectively, allow us now to consider that the screening of antibiotics in eggs could be very sensitive with such a broad spectrum antimicrobial detection. Moreover other immunological/affinity methods (ELISA kits, receptor tests) could be evaluated and validated after a global evaluation of the commercial methods available on the market for eggs.
One report of validation per kit will be delivered by the end of 2015 to the network of NRLs. A scientific publication in a peer-reviewed journal might be also further considered. A formal presentation of the advances on the project will be offered during the workshop organized in 2015 to the attention of the NRLs experts. The validation reports will then be posted on our EU-RL website to the attention of the network of NRLs.

C. Quality Assurance and Quality Control

Article 32, paragraph 1 (b, c)

8. Organisation of proficiency tests (characterisation of the material, packaging, evaluation, report)

According to our agreement with the network of NRLs, the EU-RL will proceed to the organisation of a Proficiency Testing Study dedicated to the evaluation of the strategies for monitoring authorized antimicrobial substances in food products.

8.1 Banned substances from **Group A6**

The banned substance of choice for this PT shall be the **Nitrofurans** as a comeback after the last interlaboratory analysis of nitrofuran metabolites implemented in 2011. The matrix of choice might be selected from either red meat or poultry meat or aquaculture product or honey. **Will be delivered to the participants by the end of 2015 a final report on the results obtained by the participating laboratories.**

8.2 Antimicrobials from **Group B1**

The **MRL-based authorized antimicrobials** of choice should be representative compounds of at least one family of antimicrobials, ie. penicillins, cephalosporins, tetracyclines, aminoglycosides, macrolides, sulfonamides, (fluoro)quinolones, amphenicols which are registered in Annex I of Regulation 37/2010/EC. The aminoglycosides may be specially favoured as a follow-up of the transfer of methodology for their screening and confirmation by LC-MS/MS proposed to the network of NRLs during our last workshop of October 2014. The matrix of choice should be porcine meat and with naturally incurred meat testing material produced. The 2 step strategy of analysis (screening + confirmation) will be evaluated in this PT. **Will be delivered to the participants by the end of the first trimester of 2016 a final report with including all the data obtained by the participating laboratories.**

8.3 Proficiency test in relation with coordinated monitoring programme

No coordinated monitoring programme for 2015 is specifically defined by the Commission.

9. Production of incurred sample materials

9.1 According to the previous point 8, the different reference sampling materials will be produced by the EU-RL in accordance with the standards of PT testing material preparation (homogeneity and stability studies) and under our recognized quality assurance scheme (accreditation N° 1 – 2294 - www.cofrac.fr).

9.2 The list of the EU-RL PT testing materials will be updated and made available to the NRL-network through the EU-RL website. (*See also point 5.4*).

D. Technical and Scientific Support to NRLs in the Member States, the Commission and Third Countries

Article 32, paragraph 1 (a, d, e, f)

10. Analytical support and training

10.1 Participation to SARAF training courses upon request.

10.2 Organisation of EU-RL-Fougeres training courses specific toward scientists from Member States, Acceding Countries and/or Candidate Countries and/or from Third Countries, only upon request and agenda to be agreed between the Parties.

11. Missions to NRLs and Third Countries - diffusion of scientific information

11.1 Projection of 2 visits to NRLs from the Member States.

11.2 International missions in symposia, seminaries and workshops for scientific information dissemination

11.3 Follow-up and improvement of the 13-year-old EU-RL Website and its transfer into a new global Anses-EU-RLs mini-websites built with under an Anses-format and to be fully connected to the Anses-DG public internet system: www.anses.fr.

12. Provisions of standard substances including storage, administration, documentation, shipment, etc

12.1 Request for Standard substances

All the NRL requests considering search for standard substances will be investigated but responding according to the commercial availability or non-availability of the substances.

12.2 Collection from the network of NRLs of assessed Stability Data

A collection of data on stability of Antimicrobials in Standard Solutions and in Spiked Food Products according to their specific Analytical Conditions will be requested from the NRLs who already agreed to participate to this collaborative action (*see also point 5.1*).

13. Analysis of official samples

As EU-RL, the Anses-Fougères will continue with analysing at a reference status some of the official samples coming from the NRLs and at their demand.

The specific requests arising from certain NRLs to analyze in their place a part or all of the confirmatory sets of samples coming from their National Residue Monitoring Plan especially for confirmation of Group B1 compounds will not be accepted as this kind of workload is neither a priority in EU-RL activities nor a specific EU-RL task requested by the Annex V of the Directive 96/23/EC.

14. Organisation of a workshop

A workshop for the attention of the experts from the network of NRLs in charge of antimicrobial residue control in food will be organized. The programme will be dedicated to the state-of-the-art in microbiological control of antibiotic inhibitory residues in various foods and to all the relevant issues behind this type of control including usage of rapid testing methods.

15. Analysis of the National Residue Monitoring Plans of the 28 Member States

According to the request of the Commission, the EU-RL will consult on line the RESIDUE database dealing with proposed National Residue Monitoring Plans for Year N and their Year N-1 results. Existing tables will be loaded at the EU-RL location. Information will be extracted and analysed by a EU-RL scientist to check for the adequateness of methods/matrices/combinations proposed by each of the Member States and at the European level. The EU-RL will publish a report for the Commission before the end of July 2015.

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