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 2014 – December 2014

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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 2014

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 2013

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 2014

3. Compilation of annual report and cost estimate,

   Documents to be released to DG-SANCO by September 2014 (Programme 2014 including performance indicators) and by March 2014 (Technical report of programme 2013)

4. Co-operation with European / International organisations,

   DG-SANCO/FVO, EMA, EFSA, 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 2014 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 the 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 2012-2013 and regarding the microbiological control of antibiotics in food, a thorough, possibly worldwide, survey will be engaged at the EU-RL to assess the performance claimed by the analytical methods in place for various foodstuffs (meat-flesh / milk and possibly eggs and honey) and in various Countries starting with EU Member States at least.
   Deliverable: **By the end of 2014 to the network of NRLs a comprehensive report surveying this issue – A first milestone will be the communication to the network of NRLs during our 2014 workshop and/or during the next Residue Analysis congress scheduled springtime in Belgium. This topic could also be proposed in 2015 for publication as a review 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 **Confirmatory method for aminoglycosides** continued to open to other matrices than kidney and muscle tissues: i.e. milk. (2013-2014)

The objective of this study was started during year 2013 with development and validation of the analytical LC-MS/MS method to control aminoglycosides in kidney and in muscle tissues. Now the 2014 effort will be to continue developing for the milk matrix. First, chromatographic conditions will be adapted to those veterinary aminoglycoside compounds that have an MRL set for milk in the EU legislation (i.e. gentamicin, neomycin, kanamycin, dihydrostreptomycin, streptomycin, spectinomycin, and apramycin *(no milk MRL)*, paromomycin *(no milk MRL)*). The extraction/purification steps and the reversed-phase chromatography adjusted with an ion-pairing mobile phase for kidney/muscle samples will be evaluated and adjusted for the milk matrix. At a second step of the project, the performances of the developed procedure of the method will be evaluated through a phase of validation to which will be possibly added an evaluation of the performances on naturally incurred fluids. **To be delivered by end 2014 a standard operating procedure of the confirmatory LC-MS/MS method for aminoglycosides in milk and a report of validation of the performance of the method. The SOP and validation report will also be posted in due time on our EU-RL website to the attention of the network of NRLs.**

6.2 **Full Scan High-Resolution LC-MS for Screening Antibiotic Residues** in Food Products from Animal Origin (2013-2014)

This study has been started in 2013 based on the concern for the antibioresistance-involved group of cephalosporins, i.e. the 3rd and 4th generation (C3G-C4G), and specifically the ceftiofur and its complicated depletion in tissues through protein-bound metabolisations. This work aims at getting deeper knowledge for the fate of ceftiofur in animal tissues. Poultry farming, where ceftiofur treatments are forbidden, is the particularly chosen issue for this study. This 18 month study will be continued and achieved in 2014 by means of collecting data from a set of farmed-animal experiments and using different analytical instrumentations: HPLC-UV, LC-MS/MS and LC-HR-Orbitrap-MS. It will be investigated possible ceftiofur metabolites and/or end-products biomarkers in chicks and broilers to be considered as evidence of illegal veterinary treatments. The so-called post-targeted and non-targeted approaches in the full scan HR-MS mode will be applied. **Will be delivered a final report of this study to be presented to the network of NRLs by means of a communication at the 2014 EU-RL workshop and/or during one of the specific symposia dedicated to the veterinary drug residue analysis (Ghent 2014, …). The report will also be posted in due time on our EU-RL website to the attention of the network of NRLs.**

6.3 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 will be undertaken. The LC-Tandem-MS multi-antimicrobial residue method that was developed during the past years at the laboratory of Fougeres for meat, for milk and transferred to the NRLs during the past workshops (2007 and 2011) will be 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 at least our LC-LTQ-Orbitrap instrument and possibly with a new type of instrument to be acquired and selected from the new generations of

Q-Exactive / Q-TOF / Q-Trap / Q-Tandem instruments. 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 confirm 80-120 antimicrobial analytes and possibly including other classes of veterinary drugs. 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 honey and egg products). **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 for the end of 2015. Two milestones will be incorporated to the project and presented as 2 reports of the advances made on the topic and 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 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.**

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 all 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 will replace microbiological methods for screening antimicrobial veterinary drug residues only when they can reach a wide spectrum of detection 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. Efforts should concentrate on developing multiplex assays and producing portable devices for use in the field.
Different technologies have been identified as potentially interesting tools for the screening of antibiotic residues in different food matrices (milk, muscle, honey …). The project here will consist of evaluating at the EU-RL level at least 2 different innovative biosensing technologies emerging from research teams and/or rapid kit building companies worldwide.
The 2 systems hereafter displayed demonstrate examples of interesting biosensing strategies that will be evaluated in our project but not excluding any other emerging system that could be also integrated into our project.
. One such new project is the "MCR3" from r-Biopharm (Darmstad; Germany) which is dedicated to the screening of antibiotic residues in milk (13 antibiotics) and in honey (4 antibiotics). This microarray chip reader (MCR3) is designed as a stand-alone platform, with the goal to quantify multiple analytes in complex matrices of food and fluidic samples for field analysis or for routine analytical

laboratories. The technological scheme is based on an indirect competitive immunoassay format using monoclonal antibodies bound to the surface of the microarray. The antigen/antibody interaction on the surface of the chip can be detected by chemiluminescence (CL) read-out system via CCD camera.
. One other system of interest is the ‘’Residue Plex’’ developed by RNAssays (Utrecht; The Netherlands). The ‘’Residue Plex’’ system is a flow cytometric affinity assay (FCAA). Based on monoclonal and polyclonal antibodies, they are combined with natural capture agents grafted to distinct sized microspheres for binding chemicals such as antibiotics. Discrimination of the individual microspheres is arranged by different internal fluorescent intensities, which lead to multiple analytic results per sample. Residue Plex covers five groups of antibiotic residues, including (fluoro)quinolones, tetracyclines, and quinolones. One advantage is the flexibility of the system, which allows the user to develop their own multiplex assay tailored to their specific needs. The ability to combine simultaneous tests on a single sample, minimal 'hands-on' time, and a faster analysis contribute significantly to lowering the cost per analysis.
To be delivered by the end of 2014 to the network of NRLs a review of the potentially interesting multiplex biosensor systems by means of a report to be inserted into our EU-RL website. A scientific publication in a peer-reviewed journal might be also further considered. A first communication on the advances of the project will be proposed during the 2014 workshop organized to the attention of the NRLs and/or during one of the specific symposia dedicated to the veterinary drug residue analysis (Ghent 2014, …). 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 microbiological method to screen antibiotics in muscle and eggs.

This project aims at delivering to the EU-NRLs network detailed scientific information 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. **To be delivered by end of 2014 a report of the validation conducted to determine relevant screening data and assess performances of this method for the benefit of NRLs. A first presentation of the advances on the project will possibly be presented during the 2014 workshop organized to the attention of the NRLs or during one of the specific symposia dedicated to the veterinary drug residue analysis (Ghent 2014, …). The report will then be posted on our EU-RL website to the attention of the network of NRLs.**

7.3 – Evaluation of a newly developed kit named Evidence AM IV (Randox, UK) for the screening of 37 antibiotic residues (aminoglycosides, macrolides, lincosamides) in honey.

This project will be the continuation for the evaluation of the performance of the Evidence Investigator system dedicated to the control of antibiotic residues in honey. After determining the performance data from AMI (sulfonamides), AMII (antibiotics) and AMIII (banned antibiotics) this additional biochip kit AMIV will permit the assessment of 3 more families of antibiotics screened in honey products. This project is also connected to the studies in parag 7.1 dedicated to the survey of the development of new biosensors with wide spectrum antimicrobial detection. The Evidence Investigator system is a multiplex-based technology to

allow multi-analyte detection in a one step control. **To be delivered to the EU-NRLs network by end of 2014 a report on the evaluation/validation of the method to determine the relevant screening data for a reliable control. A first presentation of the advances on the project will be presented during the 2014 workshop organized to the attention of the NRLs and/or during one of the specific symposia in line with veterinary drug residue controls (Ghent 2014, …). The report 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 **Chloramphenicol** as a comeback after the last interlaboratory analysis of chloramphenicol in 2010 and followed by the nitrofuran metabolites in 2011, the carbadox and the dyes in 2013. The matrix of choice might be porcine meat and urine.)
Will be delivered to the participants by the end of 2014 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 at least one representative compound for each family of antimicrobials, ie. penicillins, cephalosporins, tetracyclines, aminoglycosides, macrolides, sulfonamides, quinolones, amphenicols which are registered in Annex I of Regulation 37/2010/EC. The matrix of choice will be porcine muscle meat or cow milk. The strategy of analysis (screening + confirmation) will be evaluated in this PT.
Will be delivered to the participants by the end of first quarter of 2015 a final report on the results obtained by the participating laboratories.

8.3 Proficiency test in relation with coordinated monitoring programme

No coordinated monitoring programme for 2014 is defined by the Commission.

9. Production of incurred sample material

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 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 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 on 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 Parties.

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

11.1 Projection of 3 visits to NRLs from the Member States and the new member 'Croatia' will be incorporated within these 3 visits.

11.2 International missions for scientific information dissemination

11.3 Follow-up and improvement of the 12-year-old EU-RL Website

It is under preparation for 2014 to move our Anses-Fougeres EU-RL website under the umbrella of the general DG-Anses Website.

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

12.1 Request for Standard substances

All the NRL requests considering standard substances will be investigated but 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 rising from certain NRLs to analyze in their place a part or all of the confirmatory sets of samples coming from their National 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 task requested by 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 would possibly include a training session either on aminoglycoside residues analysis in Kidney/Muscle by LC-MS/MS or on state-of-the-art in microbiological control of antibiotic inhibitory residues in various foods.

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 2014.

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