EUROPEAN UNION
REFERENCE LABORATORY

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

Contract period: January 2012 – December 2012

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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 2012

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 2012

A. General Tasks

Article 32, paragraph 1 (e)

1. Meeting 4 EU-RLs, EU-RLs residues management,
2. Technical and scientific support to the Commission,
3. Compilation of annual report and cost estimate,
4. Co-operation with international organisations,
5. Documentation services, inter change of information via the website.

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 Full Scan High-Resolution LC-MS for Screening Antibiotic Residues in Food Products from Animal Origin (2012-2013)

Research and development on non-targeted monitoring of contaminants and residues in food by physico-chemical methods is now getting possible thanks to enhancement of new high resolution mass spectrometry technologies such as time-of-flight instruments (ToF, Q-ToF) and FT-Orbitrap MS instruments. The EU-RL of ANSES- Fougeres acquired such a technology, the LC-LTQ-FT-Orbitrap-MS, and started to investigate in this area of research. During the 2009-2011 period, the main focus of the research was set on evaluating the capacities of the LC-LTQ-FT-Orbitrap-MS. First was engaged the development of an analytical method suitable for the post-targeted identification and characterization of 56 antimicrobials using the exact mass measurement process in full-scan HR-MS. At a mass resolution of 60,000 FWMH, it takes into account the screening of 56 antimicrobials in meat from the following families, sulfonamides, macrolides, penicillins, cephalosporins, tetracyclines, quinolones, aminosides and lincosamides for which a molecular library was started building through a ToxID® software. This work was accepted for publication in 2011 in an international peer-reviewed scientific journal (*Food Additives and Contaminants*). A second route for investigating this new methodology for residue control was also started to be addressed by use of a non-targeted approach to detect unknown inhibitory substances (antibiotic metabolites or degradation compounds) in meat samples that had led to positive responses through screening by a microbiological inhibitory plate test. Several naturally contaminated bovine muscle tissues have been successfully investigated at EU-RL Anses-Fougeres by means of this non-targeted HR-MS analysis in full scan mode using statistical analysis (SIEVE® software).

It is our intention to extend this innovative and powerful research in the food residue field. **Two further studies will be engaged in the next programmes (2012-2013).**

The first study will consider the enlargement of the work done for the screening of 56 antibiotics in meat to other matrices, such as milk or honey. The opening to other families of antibiotics and also to other classes of veterinary medicines chosen among antiparasitics, tranquillizers, non-steroidal anti-inflammatories, anticoccidials will be also considered to extend our molecular database and the HR library associated.

The second study will consider the follow-up of the work dedicated to the non-targeted approach to detect unknown inhibitory substances. Official samples that had led to positive responses through screening by a microbiological inhibitory plate test and for which no identification have been successfully achieved will be further investigated by means of this non-targeted Full scan-HR-MS methodology using statistical analysis (SIEVE® software). This work will be implemented in order to get deeper knowledge on the so-called non-targeted approach and to try detecting new unknown inhibitory substances.

6.2 Multi-antimicrobial family method by LC-MS/MS - from Meat and Milk to Honey Products (2012-2013)

A screening method to detect about 60 antimicrobials in meat and in milk was developed at EU-RL Anses-Fougeres in the 2007-2008 period, disseminated to NRLs through a hands-on training session in end-2007 and also accepted for publication in an international peer-reviewed scientific journal (*Food Additives and Contaminants*) in 2009. The project was started again in 2010 considering a

new LC-MS/MS equipment acquired at the end of 2009. An extension of the method to some other antimicrobials was then developed leading in 2011 to a 80 antimicrobial residue screening method in meat. A transfer of the multi-antimicrobial LC-MS/MS method is on-going and will be further addressed in 2012 to meet the performance for monitoring in the honey matrix.
Discussions have been engaged with the UK-NRL to collaborate over the 2012-2014 period on this topic in connection with the regulatory European/International issue considering "Setting Regulatory Limits for Antibiotics in Honey".

6.3 Confirmatory method for dyes in fish and other aquaculture products.

Following any suspicions in the imported aquaculture products and new developments regarding pharmacologically active dyes possibly found in this kind of products, a particular attention would be paid to evaluate possible extension of the dye residue control. Recently, the methylene blue and the brilliant blue have been considered these possible dyes suspected to be in use especially from imported aquaculture products. This explanatory study could be operated in 2012 in partnership with some interested NRLs from the network and depending on intelligence circulating on that issue. The objective would be to detect and to identify the biomarkers of use to control in incurred aquaculture tissues.

6.4 Confirmatory method for chloramphenicol in urine.

Following new developments in using molecularly imprinted polymers for extraction and purification of chloramphenicol in tricky matrices such as urine and honey, a new LC-MS/MS method will be developed and validated.

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.

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.a Antimicrobials

According to recent findings in regard to accuracy of the LC-MS/MS analytical method dedicated to tetracycline residues in meat, the EU-RL will proceed in 2012 to the organisation of a Proficiency Testing Study dedicated to the

evaluation of the overall strategies in the E.U. for monitoring the tetracycline antimicrobial substances in meat products.

8.b Banned substances

According to discussions with the NRLs network during the June 2011 workshop and regarding the state-of-the-art in the analytical methods dedicated to carbadox and olaquindox residues, the next banned substances of choice should be the carbadox (CBX) and the olaquindox (OLQX) as a comeback after the last 2006 PT for CBX and OLQX and also considering the extension of the network of NRLs since 2004 / 2007.

8.c Proficiency test in relation with coordinated monitoring programme

No coordinated monitoring programme for 2012 is defined by the Commission.

9. Production of incurred sample material

9.a 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.b The list of the EU-RL testing materials will be updated and made available to the NRL-network

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.a Participation to SARAF training courses (June 2011, October 2011).

10.b Organisation of EU-RL-Fougeres training courses for scientists from Member States, Acceding Countries and/or Candidate Countries and from Third Countries, on request.

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

11.a Projection of 3 visits to NRLs from the New Member States

11.b International missions for scientific information dissemination

11.c Follow-up and improvement of the 9-year-old EU-RL Website

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

12.a 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.b Collection of Stability Data on Antimicrobials in Standard Solution and Spiked Food Products according to their specific Analytical Conditions.

As a follow-up of the stability study of antimicrobials in stock standard solutions and in spiked meat samples implemented by EU-RL Anses-Fougeres in 2011, this study being a complement to the validation of the LC-MS/MS antimicrobial screening method for meat and milk, it was decided during its presentation to the NRLs network at the June 2011 EU-RL workshop that the dissemination of these data on antibiotics was of great interest for the overall network of laboratories throughout the European Union and even farther. It was also agreed to evaluate the best way to start collecting and compiling informative data on stabilities already evaluated in several NRLs. The EU-RL Ansès-Fougères will invite all the network of NRLs to answer a questionnaire in order to draft a project to structure the stability data obtained by the EU-NRLs for antibiotic residues in various solutions (stock standard and spiking solutions) and matrices (meat, milk, …) in a report document and/or database made available to the whole network, and possibly to extend this information to a published scientific article or handbook of data for the worldwide scientific community of the drug residues.

13. Analysis of official samples

As a EU-RL, the ANSES-Fougeres will go on 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 on carbadox and olaquindox residue analysis or a training session on the tetracycline analysis in muscle tissues following new findings in the accuracy issue for quantification of tetracyclines by LC-MS/MS.

15. Analysis of the National Residue Monitoring Plans of the 27 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 June 2012.

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