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 – version 2 of December 2015

Contract period: January 2016 – December 2017

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LEGAL FUNCTIONS AND DUTIES


1. OBJECTIVES FOR THE PERIOD JANUARY - DECEMBER 2016

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 2016

A. General Tasks
   Article 32, paragraph 1 (e)

   1. Meeting 4 EU-RLs, EU-RLs residues management,
      Upon specific requests from DG-Santé

   2. Technical and scientific support to the Commission,
      Upon specific requests from DG-Santé and/or from FVO along the year 2016
      a – A review of the Decision 2002/657/EC as requested from the Commission and from the expert committee on residues of veterinary medicinal products as of its June 23, 2015 meeting

   3. Compilation of annual reports and cost estimates,
      Documents to be released to DG-Santé by September 2016 (Programme 2017 including performance indicators) and by March 2016 (Technical report of programme 2015)
      Documents to be released to DG-Santé by September 2017 (Programme 2018 including performance indicators) and by March 2017 (Technical report of programme 2016)
4. Co-operation with European / International organisations,
   DG-SANTE/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. FOLLOW-UP OF THE DATABASE FOR THE REFERENCE MATERIALS
   PREPARED DURING EU-RL ORGANIZED PROFICIENCY TESTING STUDIES
   • Expected Output: 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.

2. UPDATED SURVEY ON MICROBIOLOGICAL INHIBITORY METHODS AND
   THEIR PERFORMANCE FOR SCREENING ANTIMICROBIALS IN
   FOODSTUFTS

   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 has been additionally engaged at the EU-RL to assess the performance claimed by
   the EU-MS NRLs for the analytical methods in place for various foodstuffs. This issue had
   been already carried out for meat in 2014-2015 period (see report of July 2015) and also
   follow-ups to the CEN TC-275 WG6 for specific recommendations. Now it will be further
   extended to an evaluation of additional matrices: milk, eggs, aquaculture products, and
   honey and in various Countries starting with EU Member States at least.
   • Expected Output: By the end of 2016 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 will also be further proposed in
     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 methods for antimicrobials (authorized
   or banned) in different matrices (muscle, milk, eggs, fish, honey …) or for
   banned dyes in aquaculture products.

Considering the advances in mass spectrometric high resolution technologies (i.e. time-of-flights and orbital traps instruments) and the current level of acquisition by EU-MS NRLs of such instruments over the past 5 years, it is now our collective concern to evaluate and to demonstrate the possible future of the analytical strategies to screen and to confirm VMP residues in food by means of this new innovative instrumentation. This study will be implemented as a collaborative study with the aim to strictly assess the LC-HRMS instruments’ capabilities to be used as reliable screening tools. Samples spiked with various veterinary medicinal products will be prepared and blindly distributed to the participants to the collaborative study. The analyses will focus on Full Scan MS schemes using if possible the 4 different types of HRMS instruments (ToF, Orbitrap, Hybrid-ToF, and Hybrid-Orbitrap). The participants will be authorized to use their own strategy of separative LC conditions. This comparative study we intend to propose at least to the network of EU-MS NRLs but also possibly to selected official laboratories from Countries outside EU will be undertaken over a 2-year period. It will be intended to produce enough data to assess parameters such as detection capabilities, false-positive rates and false-negative rates for the forthcoming and may-be generalizing strategies in LC-HRMS screening of veterinary medicinal product residues in food from animal origin. The study will also provide details on the analytical performances of analyses operated by such instrumentation (LC-HRMS) and further contribute to a set of EU recommendations on criteria dedicated to the evaluation of analytical performance of methods for control (screening / confirmation) by HRMS systems.

Objectives: 1 - To demonstrate the current state-of-the-art of the NRLs network for the use of LC-HRMS instrumentation. 2 – To deliver the data raised by a collaborative NRLs interlaboratory round for full scan testings in spiked meats. 3 – To recommend the criteria of performance for screening and for confirmation of antimicrobial residues in food operated by means of LC-HRMS instruments.

- Expected outputs: Will be delivered to the network of NRLs the report of the state-of-the-art in LC-HRMS instrumentation in EU by end 2016 (Output 1); the report of the collaborative interlaboratory study by end 2017 (Output 2); a set of EU-RL recommendations by end 2017 to introduce the HRMS strategies into the regulatory framework including Decision (EC) no 657/2002 (Output 3); reports will be posted in due times on our EU-RL website to the attention of the network of NRLs (Output 4). A proposed publication will also be submitted to a peer-reviewed international scientific journal in 2017 (Output 5).

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.

**Objectives:** The present proposed study is aimed at developing further the strategy of investigation for possible C3G-C4G critically important antimicrobials such as ceftiofur (C3G) and cefquinome (C4G). As objective 1, will be considered for evaluation the development of possible analytical methods for ceftiofur/cefquinome metabolites and/or ceftiofur/cefquinome end-products aiming at controlling analytical evidence of not authorized veterinary treatments in poultry. As the objective 2, the non-authorized presence of ceftiofur and cefquinome in poultry will also be investigated by means of an animal experimental study on laying hens, analyses including hen liver, muscle, eggs, feathers, and droppings. As objective 3, 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.

- **Expected outputs for 2016:** As follow-up to advances in the project along the year 2015, will be delivered a final report of this study including data from the 3 main objectives and to be presented to the network of NRLs by means of a communication at the 2016 EU-RL annual workshop (Output 1). During the symposium dedicated to the veterinary drug residue analysis, namely Euroresidue VIII (May 2016), a communication will also be displayed to the scientific community (Output 2). The report will also be posted in due time on our EU-RL website to the attention of the network of NRLs (Output 3). A proposed publication will also be submitted to a peer-reviewed international scientific journal in 2017 (Output 4).


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...).

**Objectives:** 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 possible biomarker(s) of metabolic effects of, at least, the triarylmethane treatments in aquaculture products.

- **Expected outputs:** As follow-up to advances in the project along the year 2015, 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 (Output 1). The SOP will also be further posted on our EU-RL website to the attention of the network of NRLs (Output 2). Also will be delivered to the network of NRLs the strategy and the results of the metabolomic approach applied to triarylmethanes, and for malachite green at least (Output 3). During the symposium dedicated to the veterinary drug residue analysis, namely Euroresidue VIII (May 2016), a communication will also be displayed on this issue to the scientific community (Output 4). A proposed publication will also be submitted to a peer-reviewed international scientific journal in 2017 (Output 5).

### 6.4 - Extending LC-HRMS screening analysis to all Group B1 antimicrobial residues in different species/products and validating according new regulations (2017)

The context of the evolution of screening strategies put the new LC-HRMS analytical systems at the first place of advanced technologies dedicated to VMP residue control in balance with the now well-known LC-MS/MS instruments. Considering the network of NRLs and Field laboratories in the E.U., the EU-RL considers of high interest to develop and propose to the network of NRLs a multi-antimicrobial Group B1 method integrating as many antibiotic VMP residues as possible within a single Full Scan High Resolution Mass Spectrometric instrument. This project could be performed with one of the new generation of LC-HRMS systems the EU-RL is about to purchase by end of 2016.

**Objectives:** 1 - To develop a full-scan high resolution mass spectrometric method. 2 - To validate the performance of the method in line with the new standardized criteria according to the revised Decision (EC) no 657/2002.

- **Expected outputs for 2017:** A Standard Operating Procedure to be presented to the network of NRLs (Output 1); a report of validation according to new revised Decision (EC) no 657/2002 to be drafted (Output 2); a hands-on training to be delivered during the next annual workshop (Output 3); SOP and validation report to be posted in due times on our EU-RL website to the attention of the network of NRLs (Output 4); a communication to the International Scientific Community to be delivered in Symposium and/or through a International Peer-reviewed Scientific Journal(Output 5).
6.5 - Considering development of a unique analytical method for controlling banned antimicrobial substances including in priority CAP, NIFU and DYES (2017-2018)

There is more and more concern in reducing the number of samples to be controlled in regard to the numerous analytical methods implemented into the annual national residue control plans of the 28 EU-MS. In order to try reducing this number of implemented analytical methods, it is of interest to combine them when possible but with keeping high standard level of reliability of the official control. Therefore the EU-RL considers of particular need to evaluate a new challenge to merge as many banned antimicrobial substances as possible within the very same unique routine control method.

Objectives: 1 - To develop a LC-MS/MS method capable of including low Reference Point for Action (RPA) level of control for chloramphenicol, nitrofurans metabolites and possibly in the aquaculture products the triphenylmethane dyes and there leucobase (malachite green, …). 2 - To validate the performance of the method in line with the new standardized criteria according to the 2017-forecast revised Decision (EC) no 657/2002

- Expected outputs: A report of evaluation of the capability to develop combined CAP, NIFU, DYE residue method (Output 1 - 2017); To deliver a Standard Operating Procedure presented to the network of NRLs (Output 2 – 2017-2018); a report of validation according to new revised Decision (EC) no 657/2002 will be drafted (Output 3 – 2017-2018); a hands-on training will be delivered during the next annual workshop (Output 4 - 2018); SOP and validation report to be posted in due times on our EU-RL website to the attention of the network of NRLs (Output 5 - 2018); a communication to the International Scientific Community to be delivered in Symposium and/or through an International Peer-reviewed Scientific Journal (Output 6 - 2018).

6.6 - Validation of new confirmatory LC-MS/MS monitoring of beta-lactam residues in meat and/or milk (2017-2018)

Beta-lactam antibiotics are the most important VMP products used in food-producing livestock animals and in milking cows-sheep-goats. Additionally some of them (3rd & 4th generation cephalosporins) are considered critical antibiotics in regard to the still-relevant antimicrobial resistance issue. Therefore, the EU-RL considers the quality of the confirmatory control of these residues in meat and in milk is still an issue to be improved within the EU-MS NRLs network.

Objectives: 1 - To develop in 2017 a LC-MS/MS method capable of including MRL beta-lactam substances in meat; 2 - To validate the performance of the method in meat in line with the new standardized criteria according to the 2017-forecast revised Decision (EC) no 657/2002; 3 - To develop in 2018 a LC-MS/MS method capable of including MRL beta-lactam substances in milk; 4 - To validate the performance of the method in milk in line with the new standardized criteria according to the 2017-forecast revised Decision (EC) no 657/2002.
• **Expected outputs**: To deliver a Standard Operating Procedure in meat presented to the network of NRLs (Output 1 – 2017); a report of validation according to new revised Decision (EC) no 657/2002 will be drafted (Output 2 – 2017); to deliver a Standard Operating Procedure in milk presented to the network of NRLs (Output 3 – 2018); a report of validation according to new revised Decision (EC) no 657/2002 will be drafted (Output 4 – 2018); a hands-on training will be delivered during the next annual workshop (Output 5 - 2018); SOP and validation report to be posted in due times on our EU-RL website to the attention of the network of NRLs (Output 6 – 2017-2018); a communication to the International Scientific Community to be delivered in Symposium and/or through an International Peer-reviewed Scientific Journal (Output 7 - 2018).

6.7 - **Assessment of the long-term stability of Group-A6 and Group-B1 proficiency testing materials prepared and stored by the EU-RL for the EU-NRLs needs (2017-2018)**

Numerous testing materials have been prepared at the EU-RL level since past 20 years. Some of them have been stored and kept frozen (-20°C) for a while now and there is still a need to complement the EU-RL Proficiency Testing material preparation activity with providing to the network of EU-MS NRLs a certain set of high-quality reference materials as it has been requested by some of them along the past years to check the reliability and the variations of their analytical VMP residue control methods. The stability of these testing materials made of different biological matrices (meat, milk, eggs, honey, fish, shrimp, poultry, urine, and feed) and naturally incurred with Gr-A6 or Gr-B1 antibiotic VMP residues is still an issue for many of them.

**Objectives**: To reward the preparation of as many as possible PT Materials. To implement a systematic and adequate long-term control of the stability of the PT Materials after their first usage during a PT round. To update a Reference Material database posted onto the EU-RL website to the attention of the network of EU-NRLs and CC official labs.

• **Expected outputs**: To deliver a Standard Operating Procedure for PT Reference Materials (Output 1); To update a Reference Material database (Output 2); To post on the EU-RL website the database (Output 3); To post on the EU-RL website the procedure to request access to the PT Reference Materials (Output 4).

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, postage into the EU-RL website and when advised published in relevant scientific journals.

Screening methods are used to detect the presence of a substance or class of substances at the level of interest. These methods should have the capability for a high sample throughput and are used to sift large numbers of samples for potential non-compliant results. 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.

Two different electrochemical biosensors have been identified which are able to perform multiplex screening of antibiotics:

- A commercial system named Vantix™ (from Vantix Diagnostics™) is a potentiometric biosensor, claimed to provide a reproducible platform on which sensitive and robust assays can be developed. The main advantages of this platform are firstly a reduced investment, secondly each user could perform his own development, using antibodies and antibiotic-enzyme conjugates and thirdly the sample preparation is usually very manageable (even no preparation for milk). The combs are constituted of 12 channels, which allow analysing 12 analytes simultaneously or 12 samples or a mix. The system will be evaluated in 2016 to perform the first developments of analytical methods for the specific screening of chloramphenicol residues in different matrices (eg. honey, eggs, and aquaculture products). The objective in 2017 will be to go on with the development of new antibiotic residue methods on this technology (eg. tetracyclines, quinolones, sulfonamides, aminoglycosides, macrolides) for a multi-residue screening in milk, honey, tissues (including aquaculture products) and eggs.

- An amperometric biosensor has been developed by a Spanish research team. Their research interests focus on analytical electrochemistry, nanostructured electrochemical interfaces and electrochemical and piezoelectric sensors and biosensors. Disposable amperometric magnetosensors, involving a mixture of modified-magnetic beads, for the multiplex screening of cephalosporin, sulfonamide and tetracycline antibiotic residues in milk has been recently published in a peer-reviewed journal. The multiplexed detection relies on the use of a mixture of target specific modified magnetic beads and application of direct competitive assays using horseradish peroxidase (HRP)-labeled tracers. At first step, this amperometric
biosensor had already been applied to the detection of individual families (beta-lactams, tetracyclines) in milk. The cost of amperometric biosensor is low. Moreover, the development of methods is manageable, using antibodies and antibiotic-enzyme conjugates. Finally, sample preparation seems to be much reduced. In 2016, the interest of a new partnership for technological transfer with the Spanish team in Madrid will be investigated. The objective in 2017 will be to reinforce this collaboration by a technical evaluation of the system in our laboratory. Then, the development of specific and/or multi-residue methods will be started in 2017.

**Objectives:** Our action in 2016 will be to evaluate these two systems. Moreover will be considered to investigate the interest of new partnership with the Spanish team in Madrid or any other research teams or instrument manufacturers like Vantix Diagnostics™ in order to demonstrate actual capabilities of these systems applicable to the screening of antibiotics. Our actions in 2017 will be: 1 - to follow-up with the evaluation of the Vantix Diagnostics™ system in order to develop new multiplex methods applicable to the screening of antibiotics in different matrices; 2 - to continue the partnership with the Spanish team in Madrid. The objective will be to evaluate their multiplex electrochemical biosensor for the screening of different families of antibiotics in several matrices (milk, tissues, honey, eggs, etc). 3 - Finally the performances of these 2 systems will be compared.

- **Expected outputs:** Two reports of evaluation will be delivered by the end of 2017 to the network of NRLs if possible (Output 1). The evaluation reports will then be posted on our EU-RL website to the attention of the network of NRLs (Output 2). A formal presentation of the advances on the project will be offered during the 2 annual workshops organized in 2016 and in 2017 to the attention of the EU-NRL experts (Output 3). External communication to the worldwide scientific community might be also further considered by means of symposium presentations and/or scientific publications in peer-reviewed journals (Output 4).

7.2 – Evaluation of a new multiplex rapid method to screen antibiotics specifically in Milk products

The Evidence Investigator (Randox, UK) is an innovative multiplex system based on chemiluminescence detection which 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 a new kit (2015) named InfiniPlex® developed by Randox for the screening of at least 77 antibiotics in milk (authorized and banned substances). This kit will be evaluated according to the European guideline for the validation of screening methods (2010). Usually, microbiological commercial tests (tube tests) are used for the multi-antibiotic screening in milk. The main drawbacks of these kits are always a lack of detection for some authorized families of antibiotics (e.g. aminoglycosides, quinolones). The InfiniPlex® kit is of great interest because its spectrum of detection is much larger and the announced detection capabilities are mostly
below MRLs. Moreover even banned substances (eg. dapsone, chloramphenicol) could be screened with this kit at the same time.

**Objective:** Evaluation of the performance of the multiplex rapid method aimed at screening antibiotic substances in milk products (InfiniPlex®).

- **Expected outputs:** One report of validation will be delivered by the end of 2016 to the network of NRLs (Output 1). The validation report will then be posted on our EU-RL website to the attention of the network of NRLs (Output 2). A formal presentation of the advances on the project will be offered during the workshop organized in 2017 to the attention of the NRLs experts (Output 3). A scientific publication in a peer-reviewed journal might be also further considered (Output 4).

7.3 – Evaluation of the 4 rapid method kits to screen the 4 nitrofuran banned substances as their metabolites in Aquaculture Products

The screening of nitrofuran metabolites with immunoassays is an interesting alternative to LC-MS/MS methods because of a lower investment in the analytical equipment. Since 2004, ELISA kits for the screening of nitrofuran metabolites have been evaluated and validated in differences matrices in our laboratory. Two ELISA kits for the screening of residues of 2 nitrofuran metabolites (AOZ, AMOZ) from r-Biopharm (Germany) have been evaluated in shrimps and poultry meat (chicken, turkey). Moreover, 4 test kits for the screening of residues of nitrofuran metabolites (AOZ, AMOZ, AHD and SEM) from Shenzhen Lvshiyuan Biotechnology Co. Ltd (China) have been evaluated in porcine muscle.

The evaluation of the performance of 4 commercially available kits from r-Biopharm (Germany) aimed at screening the nitrofuran substances in aquaculture products is planned for 3 main reasons:

- The performance of these 4 ELISA kits could be compared to the performance of the multiplex approach for the screening of nitrofuran metabolite in aquaculture products,
- The ELISA kits for the screening of AHD and SEM are brand-new commercial kits (2013), and the performance of the previously tested ELISA kit before for SEM was not satisfactory in relation to the MRPL level,
- The ELISA kits for the screening of AOZ and AMOZ have been fully validated in shrimps and it is of interest to extend in other aquaculture products (tuna, cod, salmon, tilapia, pangasius, etc),
- These screening technologies also generally fits better with the first need of the low-equipped official laboratories especially in the EU-trading-linked-third countries we are more and more asked to work with as the Reference EU laboratory for the antibiotic VMP residue control.
- A VMP residue EU-RL guideline for the validation of the screening methods was published in 2010. It has started reinforcing the evaluation of the performance of the screening methods
**Objective**: Evaluation of the performance of 4 commercially available ELISA kits and one chemiluminescence immunobiosensor aimed at screening the nitrofuran substances in aquaculture products.

- **Expected outputs**: One report of evaluation per kit will be delivered by the end of 2017 to the network of NRLs (Output 1). The evaluation reports will then be posted on our EU-RL website to the attention of the network of NRLs (Output 2). A formal presentation of the different advances on the project will be offered during the 2 annual workshops organized in 2016 and 2017 to the attention of the NRLs experts (Output 3). External communication to the worldwide scientific community might be also further considered by means of symposium presentations and/or scientific publications in peer-reviewed journals (Output 4).

7.4 – Evaluation of 2 rapid method kits to screen the pharmacological dye residues in Aquaculture Products

Few screening methods are commercialized and have been evaluated for the screening of dyes in aquaculture products. Two different ELISA kits have been evaluated for the screening of dyes in fish and shrimp in 2009. One of the kit gave satisfactory results (detection capability below the MRPL of 2 µg/kg) while the other kit was not able to detect malachite green below its MRL. The aim of the project is to evaluate the performance of 2 other suppliers of ELISA kits (Randox, UK; Abraxis, US) for the detection of residues of malachite green, leuco malachite green and crystal violet in aquaculture products and to validate the kits according to the decision EC/2002/657.

**Objectives**: Evaluation of the performance of 2 commercially available ELISA kits aimed at screening the dye residues in aquaculture products.

- **Expected outputs**: One report of evaluation per kit will be delivered by the end of 2017 to the network of NRLs (Output 1). The evaluation reports will then be posted on our EU-RL website to the attention of the network of NRLs (Output 2). A formal presentation of the advances on the project will be offered during the workshop organized in 2017 to the attention of the NRLs experts (Output 3). External communication to the worldwide scientific community might be also further considered by means of a symposium presentation and/or a scientific publication in a peer-reviewed journal (Output 4).

C. **Quality Assurance and Quality Control**

*Article 32, paragraph 1 (b, c)*

8. Organisation of proficiency tests PT (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

A - The non-authorized substances of choice for this PT shall be the Dyes as a comeback after the last interlaboratory analysis of Dyes metabolites implemented in 2013. The matrix of choice might be selected from different possible aquaculture products (fish, shrimps, prawns...). However, as an innovation in the process of controlling antimicrobial banned substances, it will also be considered to extend the PT to monitor complementary the residues of other banned substances: Chloramphenicol and/or Nitrofurans in aquaculture products.

**Objective:** Providing to the NRLs network one PT including testing for several dye residues including their metabolites in aquaculture products and possibly for other banned antimicrobials in aquaculture products (Chloramphenicol, Nitrofurans).

- **Expected output:** Will be delivered to the participants and to DG-SANTE desk officer by the end of 2016 or first trimester of 2017 a final report on the results obtained by the participating laboratories (Output 1). The report will also be posted in due time on our EU-RL website to the attention of the DG-SANTE exclusively (Output 2).

B - As a follow-up of new PT organization launched in 2016, the non-authorized substances of choice for this PT shall be again a combination of several banned substances chosen among the groups A6, B2f and B3e, i.e. chloramphenicol, nitrofurans, carbadox/olaquindox and dyes. The matrix of choice might be selected from different possible species/products with options proposed in relation to the appropriate issues in 2017. The EU-NRLs method(s) to be controlled will be all considered collectively.

**Objective:** Providing to the NRLs network one PT including testing for several non-authorized substance residues including their possible metabolites in specific species/products of concern chosen either for their domestic monitoring or for their import control.

- **Expected output:** Will be delivered to the participants and to DG-SANTE desk officer by the end of 2017 or first trimester of 2018 a final report on the results obtained by the participating laboratories (Output 1). The report will also be posted in due time on our EU-RL website to the attention of the DG-SANTE exclusively (Output 2).

8.2 Antimicrobials from Group B1

A - The MRL-based authorized antimicrobials of choice should be representative compounds of at least one family of antimicrobials, i.e. penicillins, cephalosporins, tetracyclines, aminoglycosides, macrolides, sulfonamides, (fluoro)quinolones, amphenicols which are registered in Annex I of Regulation 37/2010/EC. The matrix of choice this year should be honey products. The possible 2-step strategy of analysis (screening + confirmation) will be evaluated during this PT.

**Objective:** Providing to the NRLs network a 2-step PT (screening+confirmation) including testing for several B1 group antimicrobial residues in honey products.

- **Expected output:** Will be delivered to the participants and to DG-SANTE desk officer by the end of 2016 a final report with including all the data obtained by
the participating laboratories (Output 1). The report will also be posted in due time on our EU-RL website to the attention of the DG-SANTE exclusively (Output 2).

B - The group B1 MRL-based authorized antimicrobials of choice should be representative compounds of at least one of the following families of antimicrobials, ie. penicillins, cephalosporins, tetracyclines, aminoglycosides, macrolides, sulfonamides, (fluoro)quinolones, amphenicols which are registered in Annex I of Regulation 37/2010/EC. The matrix of choice this year should be aquaculture products. The possible 2-step strategy of analysis (screening + confirmation) will be evaluated during this PT.

**Objective:** Providing to the NRLs network a 2-step PT (screening+confirmation) including testing for several B1 group antimicrobial residues in aquaculture products (fishes and/or shrimps).

- **Expected output:** Will be delivered to the participants and to DG-SANTE desk officer by the end of 2017 a final report with including all the data obtained by the participating laboratories (Output 1). The report will also be posted in due time on our EU-RL website to the attention of the DG-SANTE exclusively (Output 2).

**8.3 Proficiency test in relation with coordinated monitoring programme**

No coordinated monitoring programme for 2016 nor 2017 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](http://www.cofrac.fr)).

- **Expected outputs:** Production of at least 3 new PT testing materials per each of the forecast PTs over 2016 and 2017.

**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 5.1*).

- **Expected output:** Update of the List of the current EU-RL PT testing materials to be posted in due time on our EU-RL website to the attention of the EU-MS NRLs.

**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-Fougères training courses specific toward scientists from Member States and/or Accessing Countries and/or Candidate Countries, only upon request and agenda to be agreed between the Parties:

- A 2-week training session for one scientist or a 1-week training session for 2 scientists in 2016 in screening and/or confirmation of antimicrobial residues in meat and/or other relevant products (milk, fish, honey, egg, ...).
- A 2-week training session for one scientist or a 1-week training session for 2 scientists in 2017 in screening and/or confirmation of antimicrobial residues in meat and/or other relevant products (milk, fish, honey, egg, ...).

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

11.1 Projection of 4 visits to EU-NRLs from the Member States over 2016-2017.

11.2 International missions in several symposia, seminars and workshops for enhancing dissemination of scientific information in the field of antibiotic residues in food.

11.3 Follow-up and improvement of the 14-year-old EU-RL Website and specific management of its recent end-2015 transfer into the new global Anses-EU-RLs mini-website platform built under the Anses-format and 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 over 2016-2017 but responding according to the commercial availability or non-availability of the substances.

13. Analysis of official samples

As EU-RL, the Anses-Fougères will continue over 2016-2017 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. 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 August 2016 (for 2016 EU-MS NRMPs) and by the end of August 2017 (for 2017 EU-MS NRMPs).

15. Organisation of annual workshops to the attention of EU-MS NRLs

A workshop for the attention of the experts from the network of NRLs in charge of antimicrobial residue control in food will be organized.

In 2016, the programme will be dedicated to the advances in the screening of antibiotics in food products from animal origin and particularly focussed on the delivering of the new converging standard operating procedure for screening by LC-MS/MS of a set of 80 antibiotics in meat which has been developed/updated over the period 2013-2015. News on the advances on the screening LC-HRMS technologies and on new guidance updating/repealing the Decision 657/2002 regarding validation of analytical residue methods will also be delivered during this workshop.

In 2017, the programme will be dedicated to the advances of the EU-RL team activities in the various scientific-technical-regulatory projects developed during the 2015-2017 period. For instance, among others the 2 main issues will be the news on the advances on the screening LC-HRMS technologies in regard to antibiotic VMP residues on the one hand and on new guidance updating/repealing the Decision 657/2002 regarding validation of analytical residue methods on the other hand.

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