Coordinated Research Project (CRP) D5.20.36

“Development of Radiometric and Allied Analytical Methods to Strengthen National Residue Control Programs for Antibiotic and Anthelmintic Veterinary Drug Residues”

Report of the Fourth Research Coordination Meeting (RCM) on the Development of radiometric and allied analytical methods to strengthen national residue control programs for antibiotic and anthelmintic veterinary drug residues

Natal, Brazil, 14-18 April 2014

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1. Introduction

The 4th Research Coordination Meeting (RCM) of the Coordinated Research Project (CRP) on the development of radiometric and allied analytical methods to strengthen national residue control programs for antibiotic and anthelmintic veterinary drug residues was held at the Hotel PraiamarNatal in Natal, Brazil, under the overall organization by the Laboratorio Microbioticos, Campinas from 14 to 18 April 2014. The event was very well organized and coordinated by the local counterpart, Mr. Rodrigo Granja and his efforts were appreciated by all participants.

The meeting was chaired by Thomas W. Kuhn (Agreement holder, Austria) with Mr. Rodrigo Granja (Contract Holder, Brazil) as the Rapporteur and Mr. James Sasanya (IAEA) as Scientific Secretary. Research contract holders Ms. Guihua Liu (China) and Ms. Grace Murilla (Kenya) were unable to attend the meeting in person. Mr. Raj Patel (United Kingdom), Ms. Sarita P. Gobbo Ferrari (Brazil), Ms. Rosario Montes Nino (Spain) and Mr. Alfredo Montes Nino (Brazil) participated as observers.

The RCM was formally opened by Dr. Leandro Feijo, Brasilia’s Director of Food Inspection and the overseer of Brazil’s national residue monitoring program. He delivered a key note speech on the country’s national residue program (as an official risk management tool) and its contribution to the Member State’s food/agricultural exports.

2. Objectives of the 4th RCM.

The meeting commenced with a review of the CRP by the Scientific Secretary including project progress, the scope, objectives and expectations of the 4th RCM. Participants were reminded of the meeting aims including to:

a) Review/evaluate work done since the 3rd RCM and the final outputs;

b) Summarize overall work done (individual/group);

c) Review/discuss recent advances in analytical technologies and residue monitoring/control;

d) Prepare report, technical document (TECDOC) including publications and, a manual composed of standard operating procedures (SOPs);

e) Further promote interaction/networking on current/remaining and future research opportunities;

f) Identify strategies to share/transfer technology developed.

The main objective of the CRP was to assist National Reference Laboratories of FAO and IAEA Member States in meeting the need for effective and appropriate monitoring methods for residues of selected antibiotic and anthelmintic veterinary medicines. Therefore, the importance of completing the development and validation of robust and transferable analytical methods through the preparation of Standard Operating Procedures (SOPs) and publishable information was stressed, as was the need to develop viable solutions to potential challenges.

During the meeting the CRP Work plan and the Logical Framework Matrix were used to assist discussions.

The outputs of the meeting were summarized as follows:

- Work done according to the 3rd RCM plan and in the context of overall program of work over the project’s duration presented and feedback provided;
- Solutions to challenges identified/discussed;
- Final report, SOPs and TECDOC drafted.
3. **RCM Presentations.**

The presentations by Research Contract Holders focused on results, constraints and application/transfer of technology developed. These were followed by extensive discussions especially on the quality of the work done and how relevant it would be to end users. Working with the Agreement Holders and other participants, each Research Contract Holder drafted and revised their SOPs and technical reports.

Agreement Holders also presented their current work on veterinary drug residues and analysis of other food contaminants as well as emerging areas of food traceability, biomarkers and metabolomics among others. Presentations were delivered on the analysis of unknown substances/contaminants in foods as well as increasing concerns on natural sources of certain contaminants that present international trade barriers to foodstuffs from some IAEA Member States. Effective development and application of isotopic analytical techniques including strategies to transfer such technology to many other Member States was also discussed.

4. **Conclusions and Recommendations**

**Conclusions:**

1. A research network was established under this CRP comprising of 17 research organizations from 15 IAEA Member States for 5 years. It provided an opportunity to share methods, process samples and compare results and to strengthen the analytical capabilities of the participant laboratories to develop and validate methods for monitoring contaminants.

2. New multi-residue screening/confirmatory methods including use of state-of-the-art LC-MS/MS techniques with stable isotopes (e.g. $^{13}$C labelled sulfa compounds) for drug residue analysis in animal products and environmental samples were developed.

3. Flumequine pharmacokinetics studies were conducted in Sea Bream using $^{14}$C Flumequine involving whole body autoradiography and liquid scintillation counting. The study demonstrated that Flumequine has a kinetic profile suitable for use in Sea Bream and assures consumer safety as the distribution and elimination of the drug were rapid.

4. Natural occurrence of the antibiotic Chloramphenicol was investigated in animal products and environmental samples (pasture and soil) using a tailored rapid screening technique supported by an LC-MS/MS method. The method was been applied in Mongolia.

5. A radioimmunoassay technique was developed, validated and used to screen residues of florfenicol and its amine analogue in fish muscle in Brazil. A radioimmunoassay kit was also developed. Method confirmation was done using an LC-MS/MS.

6. LC-MS/MS and HPLC-FLD methods were developed, validated and applied to test ten benzimidazoles and avermectin anthelmintic residues in Peru. Innovative extraction of milk samples was employed. A related efficient and sensitive LC-MS/MS method was developed for determination of benzimidazole residues in animal tissues including milk, using $^{13}$C-thiabendazole as an internal standard. This method was applied in China.

7. A multi-screening thin layer chromatographic method for testing sulfonamide drug residues in chicken tissue was developed and applied in Sri-Lanka.
8. A robust confirmatory and quantitative LC-MS/MS method was developed for simultaneous determination of sixteen Aminoglycoside antibiotics in porcine tissues. This method was also applied in China.

9. A multi-parametric Dot ELISA was developed for the detection of enrofloxacin and ciprofloxacin in chicken meat. Specific factors contributing to successful production of antibodies were studied. A complementary accurate, reproducible and highly sensitive LC-MS/MS method for testing enrofloxacin and ciprofloxacin in chicken matrices was also validated. Broiler chickens were successfully used to determine the depletion of enrofloxacin in muscle, kidney, liver and plasma.

10. As an offshoot of work that involved use of streptavidin-HRP and $^{125}$I-labelled streptavidin as tracer, a direct competition ELISA test was developed and validated for monitoring tetracycline residues in edible animal products with a limit of detection of 10ng/ml plasma.

11. A number of publications in peer review journals were produced through this CRP and technical documents prepared.

12. Some of the technology developed in the CRP has already been disseminated to laboratories in other Member States through expert missions, fellowships and scientific visits under IAEA's Technical Cooperation program and as SOPs/protocols.

13. The CRP also identified new pertinent areas of research such as untargeted analyses that could be filled through future projects. There were a number of spill-overs from the CRP such as attaining ISO accreditation by some participating laboratories and completion of MSc/MPhil degrees through participation under a couple of projects.

14. Producing of good antibodies suitable for immunoassays presented challenges

**Recommendations:**

1. The need for stable-isotope analogues remains very high. However, they are very expensive. Future CRPs could explore the possibility that one participant purchases the standard(s) and others would obtain sub-portions of it.

2. Nuclear and isotopic methods developed (including well defined SOPs) should be made available to other Member States e.g. through the Joint FAO/IAEA database for veterinary drugs, the Food Contaminants Residue Information System.

3. It would be helpful to some research contract holders, if exceptional additional financial support is considered in form of technical visits to certain laboratories for instance to perfect a certain technique or conduct specialized tests; follow-up missions by an agreement holder to the CSI holding the research contract; since some contract holders encounter challenges procuring minor consumables that may not have been included in a proposal submitted to the IAEA, such visits could provide an alternative avenue to deliver/share small quantities of materials (including test kits/consumables).

4. The technology developed in the CRP should be transferred to Member States e.g. through hosting fellowships and scientific visits or fielding expert missions under IAEA's Technical Cooperation projects.

5. Since producing good antibodies suitable for immunoassays presented challenges, appropriate, commercially available but easily accessible antibodies should henceforth be considered as another viable option in addition to dependence of technical contracts with appropriate suppliers.