

The European Union Reference Laboratory for the detection of animal proteins in feedingstuffs

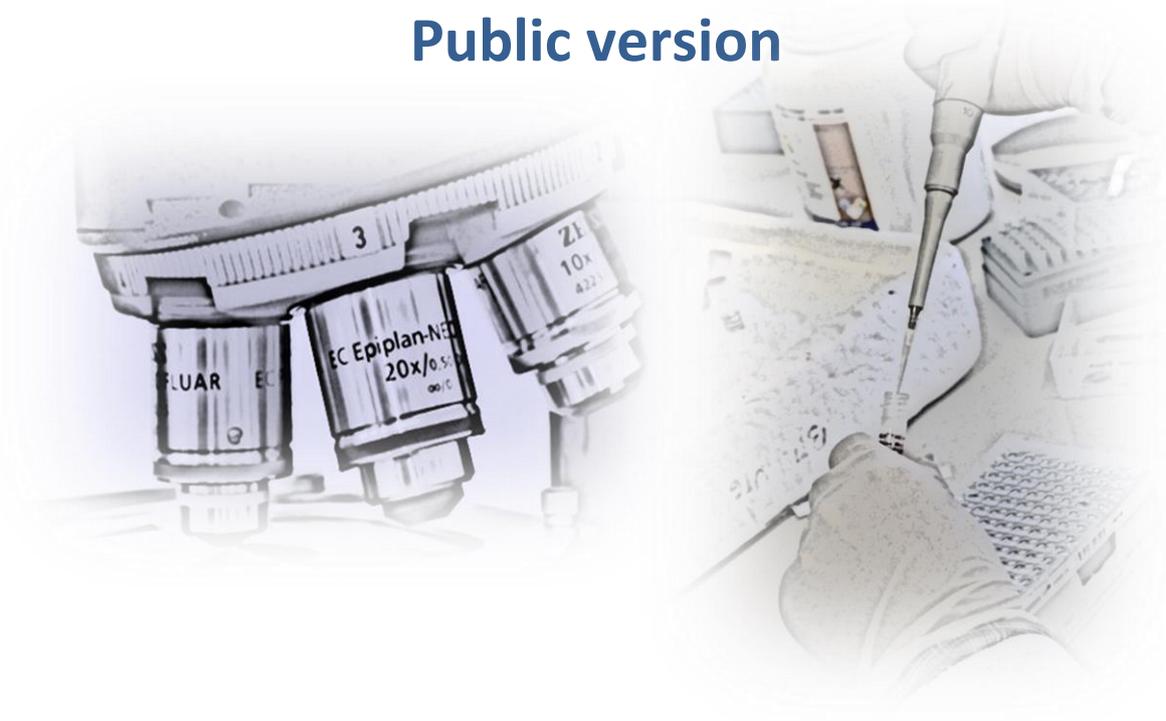


Walloon Agricultural Research Centre – CRA-W (Belgium)



Annual report on the 2014 activities

Public version



Activity 1 Scientific advice & support to the European Commission (105 m/d)

1.1 Scientific advice and support to the European Commission (60 m/d):

- *In relation to the development of EC feed legislation (in 2014 tuning of the revised Annex VI of EC/152/2009 is foreseen, extension of feed ban relaxation to pigs, preparation to poultry extension foreseen in 2015 [SOP preparation mainly]),*
- *Participation and scientific assistance to DG Sanco meetings (e.g SCoFCAH),*
- *Technical assistance on request of DG Sanco (e.g. scientific questions, comparison of methods, requested priority experiments, new feed e.g. insects). This assistance and related experiments concern issues which are not covered by other activities (Activity 4).*
- **Meeting with DG SANCO at the EURL-AP facilities (19th of November).**
- **DG SANCO requested from the EURL-AP an in-depth examination of RIKILT report 2014.011 presenting the results from the IAG ring test animal proteins 2014 and to state on the relevance of its conclusions revealing potential shortcomings of the microscopic and PCR methods. The EURL-AP issued a scientific opinion that analyses step by step the relevance of the shortcomings. The validity of the methods is confirmed and no justification of modification could be identified. However, different studies should be conducted in order to investigate possibility of improvements of the protocols in place.**
- **From 8th till 11th September 2014, the EURL-AP participated to a TAIEX organised in co-operation with the Ministry of Food, Agriculture and Livestock of Turkey on light microscopy and Polymerase Chain Reaction (PCR) methods for the determination of constituents of animal origin for the official**

control of feed (AGR IND/EXP 54667) in Ankara, Turkey.

- *The EURL-AP team was involved in the organisation and participation of the BTSF (Better training for safer food) platform on Prevention, Control and Eradication of Transmissible Spongiform Encephalopathies as coordinated by JVL Consulting. The EURL-AP was requested to prepare (1) a lecture on detection of PAP by light microscopy and PCR method, (2) practical exercises on application of the 2 different PAPs detection methods on feed according current legislation. In 2014, the EURL-AP presented these lectures twice in Utrecht, The Netherlands (June and October), and did the same exercise once in Ljubljana, Slovenia (November) and in Lisbon, Portugal (November). The total number of participants – mainly official control veterinarians from EU and third countries – reached about 70 persons during the 2014 sessions.*
- *Lecture held on 13th of October at the TSE Working group meeting in Brussels about the “State of play as regards validation by EURL-AP of a PCR test for detecting pig material in feedingstuffs “.*
- *Launching of the report “EURL-AP Inquiry on PCR analyses performed on feed or feed material for aquaculture in the European Union for the second half-year of 2013” based on an inquiry among the NRL network to understand the origin of the higher positive result rate for contamination by ruminant material with the introduction of PCR as an official method.*
- *On request of DG-Sanco a report was set up about the state of play regarding the performance of the NRL network of the EURL-AP. The report was based on the results of the NRLs to the proficiency tests organized by the EURL-AP for the two official methods (light microscopy and PCR). For light microscopy the gathered results went back up to 2006 while for PCR the only available data were of 2013.*
- *On the request of the DG SANCO, EURL-AP started to record data on morphological parameters which could be used for microscopy identification of insect meals. Several insects’ species were collected and processed into meals and micrographs were taken. After accurate selection of the pictures,*

the micrograph collection of the EURL-AP was enriched with about 70 news iconographic references allowing to identify insect fragments in feed by end of April 2014.

1.2 On the request of DG SANCO or the NRLs, performance of 22 counter-analyses on samples with disputed results (45 m/d).

- ***During 2014, several requests of official counter analysis were submitted by NRLs:***

- ***1 sample from Member State A, fish feed, February 2014***
- ***1 sample from Member State B, swine complete fodder, March 2014***
- ***1 sample from Member State A, fish feed, June 2014***
- ***1 sample from Member State A, fish feed, July 2014***
- ***1 sample from Member State C, fish feed, August 2014***
- ***1 sample from Member State D, glycerin liquid sugar, September 2014***
- ***1 sample from Member State D, glycerin liquid sugar, October 2014***

All samples were analysed both by light microscopy and PCR.

- ***The number of counter analyses decreased in 2014. This decrease seems to be linked to an improvement of the implementation of official methods and the improved skill of the laboratories.***

Activity 2 Scientific knowledge vigilance and dissemination (61 m/d)

2.1 Upon the request of the European Commission or in order to fulfil his role as EURL, participation in international scientific fora/meetings relating to the detection of animal proteins in feedstuffs (1 IAG meeting, 1 AOCS annual conference, 1 other [EFSA, EFPR, FEFAC...]) with eventual presentations and posters to prepare for it. (missions outside the EU will get the agreement of DG Sanco) (25 m/d)

- ***The EURL-AP was present at the IAG annual meeting held in Posieux, Switzerland, from the 10th till the 12th of June 2014. Three lectures were***

presented. The topics of the presentations were the i) report on the EURL-AP Proficiency test microscopy 2013, ii) the on-going research on alternative methods at EURL-AP and iii) proposals aiming at modification of the IAG functioning

- *In the framework of the project SAFE-PAP about the use of terrestrial animal by-products in Atlantic salmon production and funded by the Research Council of Norway, the EURL-AP was present at the annual meeting of the consortium that took place in Bergen the 8th of May. Participation to this project is of importance to be informed of the developments of alternative techniques such as LC-MS/MS for feed analysis and facilitates close collaboration with expert labs.*

2.2 Participation in the annual EURL Directors co-ordination meeting (2 m/d)

- *Participation to the EURL Directors meeting of 17th of January 2014 where information was provided about novelties of the procedures in view of the new Common Financial Framework as well as additional information was given about the performance indicators.*
- *A second EURL Directors meeting was held on 4th of July with once again emphasis on the financial aspects and the performance indicators. An additional item was the feedback of the EURLs on the performance of the NRLs.*

2.3 Valorisation of EURL-AP activities (production in journals, books, help to other EURL's...) (30 m/d)

- *The manuscript related to the development of a FISH protocol for the specific detection of ruminant processed bones were accepted and published in the online and open access journal "Scientific reports". The paper is entitled "Determination of the ruminant origin of bone particles using fluorescence in situ hybridization (FISH)".*
 - *Lecrenier, M.C., Ledoux, Q., Berben, G., Fumière, O., Saegerman, C., Baeten,*

V., Veys, P. (2014). Determination of the ruminant origin of bone particles using fluorescence in situ hybridization (FISH). *Scientific Reports*, 4, art. no. 5730.

- Tena, N., Fernández Pierna, J. A., Boix, A., Baeten, V. & von Holst, C. (2014). Differentiation of meat and bone meal from fishmeal by near-infrared microscopy: Extension of scope to defatted samples. *Food Control*, 43, 155-162.
- Pinotti, L., Krogdahl, A., Givens, I., Knight, C., Baldi, A., Baeten, V., Raamsdonk, L.V., Woodgate, S., Marin, D.P., Luten, J. (2014). The role of animal nutrition in designing optimal foods of animal origin as reviewed by the COST action feed for health (FA0802). *Biotechnology, Agronomy and Society and Environment*, 18 (4), pp. 471-479.

2.4 Experts meeting at EURL-AP (EU experts invited for scientific discussions on current issues on PAP detection. Expert meetings are also needed for SOP management [cf 5.4]) (4 m/d)

- ***An expert meeting on complementary methods for the identification of animal by-products was held on 14-15 October 2014. Nine experts coming from BfR (Germany), CER, CRA-W, University of Namur (Belgium), FERA, University of York (UK) and NIFES (Norway) were invited to attend to the meeting in Gembloux. The aim of this meeting was to share the experience of the participants to identify the most promising strategies to fill the current analytical gaps for the determination of animal by-products nature and the detection of unauthorised ingredients. After the presentation by the EURL-AP of legislative aspects, the actual analytical situation and the analytical ways already under investigation, the experts shared their personal experience. The second day was structured around an open-discussion regarding the possible analytical solutions and the design of an action plan. The follow up of the meeting consisted of numerous scientific exchanges and discussions by mail. This also includes enrichment of the sample bank (a.o. gelatines).***

Activity 3 Maintenance of EURL-AP technical skills (255 m/d)

3.1 Daily and long-term management of the EURL-AP (65 m/d):

- *Team organisation and administration tasks,*
 - *Maintenance of the competences of the EURL-AP team and formation of the new recruits,*
 - *Preparation of the reports of activities according to the report guidelines transmitted by DG SANCO,*
 - *Preparation of the new workprogramme with its budget.*
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- ***The EURL-AP attended training on mass spectrometry in proteomics in Paris, France, in December 2014. This training was spread over 5 days and consisted of both theoretical and practical course. The objectives were to acquire theoretical and practical basis for the acquisition and the interpretation of mass spectra from protein material and for the use of sequences databases for the identification of proteins. This training was very instructive especially because it allowed an open discussion on the various protocols, ions source and analysers and their advantages and disadvantages depending on the desired application. Some tips and tricks were also exchanged.***
 - ***In link with other Belgian and international projects, the EURL-AP went during three days (2 – 4 September 2014) in the BfR facilities to be familiarized with the ELONA (Enzyme Linked OligoNucleotide Assay) technique using aptamers for the detection of ruminant thrombin.***
 - ***In link with the development of new PCR targets, the EURL-AP participated to formation in bioinformatics (from the 3rd till the 7th November 2014). Formation entitled “Les différents domaines de la Bioinformatique”, organized by the Pasteur Institut in Lille, France.***
 - ***From the 19th till the 21th of November 2014, the EURL-AP went in JRC Ispra, Italy, for the Training on digital PCR and droplet digital PCR. The objectives were to see the various experiments already realised with the digital PCR***

exposed in presentations and to increase our knowledge on this technique.

- *Finalizing the annual report of 2013 in the beginning of 2014 and filling in the form of the ex-post performance indicators related to the 2013 workprogram.*
- *Preparation of the 2015 workprogram with its budget, the deliverables and the ex-ante form of the performance indicators.*

3.2 Maintenance and development of the sample bank (190 m/d):

- *Establishment of the specification for new material or for renewal of existing material of the EURL-AP sample bank,*
 - *Composition analyses (PCR ,microscopy, NIR,...) of the collected samples,*
 - *Maintenance of informatics tools for the appropriate management of the samples (SMS system),*
 - *Collection/production of samples of animal origin in support to EURL-AP method development experiments,*
 - *Storing of the samples and production of microscopic image representative of the particles,*
 - *Maintenance of the sample bank.*
- *In 2013, the need of single species industrial PAPs was underlined, notably as a requirement for the development of complementary PCR and alternative methods. Industries accepted to produce such meals for EURL-AP. The production started in April 2014. Five pure-species PAPs, of porcine, chicken, turkey, ovine and bovine origin respectively, were prepared. To avoid cross-contamination, the different species were treated one by one and produced according to a specific sequence. The planned production sequence was:*
 - *Porcine meal*
 - *Chicken meal*
 - *Turkey meal*
 - *Bovine meal*
 - *Ovine meal*

Porcine and chicken PAPs were received at the end of April but the first analyses of these meals revealed an abnormal high fat content and so these meals had to be produced again. Bovine meal was received in May. The defatted batch of porcine meal (porcine meal B) was received in September. The meals were analysed by light microscopy and PCR. PCR analyses were not possible on the first porcine meal due to high fat content. The chicken meal was positive for the chicken and poultry (chicken/turkey) target and not completely free of pig DNA. Bovine meal was positive with the ruminant and cattle targets and contained pig and poultry traces. The second porcine meal was positive with the pig target but showed trace signals with the ruminant target. These results give clear evidence of the difficulties to avoid cross-contamination. The ovine meal was analysed by the industry. Porcine DNA was not detected but it contained chicken DNA.

- *During the expert meeting, the identification of species origin of gelatine was discussed. In order to study this potential contamination source, EURL-AP contacted the gelatine manufacturers of Europe (GME). Nine gelatine samples produced from different species (pig, bovine and fish) and tissues (skin, bones and hides) as well as obtained according to different processes (limed and acid treatment) were collected through members of the European Chemical Industry Council (Cefic). They were received at EURL-AP in November. EURL-AP also received six gelatines from one participant of the expert, FERA (UK).*
- *Some of the new samples were analysed by NIRM and NIR imaging techniques in order to increase and update the existing databases.*
- *The application for the samples management system (SMS) has been updated in order to improve relevant search queries on the PCR results and to integrate use of bar codes on the samples.*

Activity 4 Development and evaluation of analytical methods (460 m/d)

4.1 Development and research on PAPs detection and characterization by light microscopy or PCR (180 m/d):

- *Contribute to the development on PAPs detection and characterization and improvement of existing methods of analysis (combination of light microscopy and PCR, complementary species specific PCR targets, fine-tuning of the cut-off for the validated ruminant PCR method),*
- *Calibrants for targets in development.*
- ***The specificity test of poultry targets was completed with the analysis of lesser black-backed gull DNA (*Larus fuscus*). This test consolidates the choice for the PCR target allowing the simultaneous detection of DNA of 2 poultry species (chicken and turkey) but does not interfere with the sea gull DNA. The other targets detect either only chicken or turkey or many birds including the sea gull.***
- ***During 2014, the sensitivity tests of pig PCR target continued on new mixes at a content of 0.1 % (m/m) or less in porcine PAP. The robustness of the method was checked.***
- ***Participation to a characterization study by digital and droplet digital PCR of ruminant and porcine calibrants organized by JRC-IRMM. The EURL-AP received the material the 1st of July 2014. The results obtained with our digital PCR device (Biomark™ HD system, Fluidigm, California, U.S.A.) were transmitted before the deadline of the 8th of September.***
- ***The plasmid containing the target DNA sequence for chicken and turkey was given to JRC-IRMM in December for the production of calibrants.***
- ***The validation through a collaborative study of the PCR method for the detection of pig DNA was delayed so as to be able to be organized it directly with the calibrants delivered by the JRC-IRMM. The EURL-AP received this valuable material during December 2014. In the meantime, the EURL-AP prepared the design and the material for this collaborative study. The announcement and the invitations were sent to the participants before the***

end of the year in order to allow the study to take place in January and February 2015.

4.2 Development and evaluation of complementary methods (180 m/d):

- *Contribute to the development of complementary analytical methods necessary to assure the correct implementation of official methods and explorative or alternative methods (e.g. authorised ingredients vs prohibited PAP, influence of feed matrices, identification of sediment origin).*
- *Evaluation and testing of the sequential combination of the selected successful complementary methods with a view to the SOP operational diagrams completion.*
- *Performing EURL-AP available methods or adapting them on outbreak material to make them available for the NRLs network.*
- ***The development of mass spectrometry for the detection of bovine blood meal and blood products initiated in 2013 continued in 2014. Thirty-eight new samples were analysed in the laboratory of cellular biochemistry and biology (URBC) of the University of Namur (UNamur). In contrast to the previous year, EURL-AP has contributed to the sample preparation (extraction, cleaning and digestion) but mass spectrometry analyses were still subcontracted to UNamur. In the first half of 2014, the analyses were focused on bovine and porcine blood meal and blood products as well as feed adulterated with bovine plasma powder. In November, avian blood samples were analysed in order to increase the database. The analysis of bovine blood meal and feed adulterated with bovine haemoglobin powder has also completed this database. Commercial compound feed (fish feed) known to contain blood meal were also analysed in order to test the first peptides selection on real samples. At the end of 2014, the analysis of the results started and 18 bovine blood biomarkers were pre-selected. The biomarkers selection and the enhancement of the database will be continued in 2015.***

- *Concerning the development of the Fluorescence in situ hybridization, an agreement was reached with the industry for the production of new batches of PAPs. The production started in April 2014. Five pure species PAPs, of porcine, chicken, turkey, ovine and bovine origin respectively, were prepared and the bovine and porcine meals (defatted one) were received in May and September respectively. Due to the time needed for the meals production, the FISH development has been postponed to 2015 and efforts on complementary methods has been focused on the development of mass spectrometry method.*
- *NIR method was transferred in 2013 to the new Bruker NIRM platform. As a continuation of this transfer, in 2014 numerous samples were analysed by NIRM (fish feeds and fishmeals principally). This work will be continued in 2015 for achieving new standardized and calibration equations. Among the use of the NIRM platform the homogeneity study for the EURL-AP proficiency tests is a key element. All sediments were checked by NIRM to ensure the rapid detection of adulterating animal material.*
- *EURL-AP also started a mid infrared (MIR) study on gelatines in order to try to identify the species origin of gelatines by MIR. The first step was to collect gelatines samples. The analysis protocol was then established and analyses will begin in early 2015. Samples will be characterized by NIRM, NIR, MIR, fluorescence and Raman spectroscopy.*

4.3 Organization and preparation of collaborative studies for validation of methods from 4.1 (PCR pig/poultry targets) and 4.2 (when those methods are validated internally) (100 m/d)

- *The collaborative study for the validation of a pig PCR assay was designed and prepared in 2014. The official invitation letter planning the study during February 2015 was sent to 20 laboratories in December 2014.*

Activity 5 NRL network management and support (475 m/d)

5.1 Management of the Network and scientific advice (145 m/d):

- *Supply information and scientific advices (including non-official experiments or analyses of results upon NRL submission [excluding counter analyses which are included in 1.2]),*
- *Audit NRLs, coordinate on-site training on methods of analysis and assist staff from NRLs if proficiency testing reveals limited experience (on agreement of DG Sanco),*
- *To help to develop, extend and keep in the NRLs the highest standard of technical skill and quality management under accreditation on analytical methods for detection and identification of animal proteins in feed ingredients and in feedingstuffs.*
- ***In 2014, as a direct consequence of the results obtained from the proficiency test microscopy 2013 which showed that 6 NRLs were underperforming, one visit of a NRL had to be organised by the EURL-AP. This visit took place in June 2014. Actions towards other underperforming NRL did not requested on-site visits. The visit allowed to close the case of underperformance. A report was communicated to the involved NRLs and to DG Sanco. This report included also recommendations and requests for the future either in terms of equipment or in terms of implementation of the methods.***
- ***As a support to the NRL Ireland for the training of its own lab network, the EURL-AP shared the slide show files used during its PCR trainings.***
- ***The EURL-AP was solicited by the Belgian Federal Agency for the Safety of the Food Chain to participate to information sessions of its inspectors. A lecture on detection of PAP by light microscopy and PCR method was presented and followed by practical case studies on the application of the 2 different PAP detection methods in feed according current legislation.***

5.2 Communication and maintenance of information tools (35 m/d):

- *Maintenance and update of EURL website (internet/intranet) to*

disseminate and share information with NRLs and others stake holders,

- *Preparation and sending of newsletters for NRLs.*
- ***During the 12 months period, 26042 web pages from the public site were visited through 6226 sessions by 2558 users coming from 104 countries and 1005 network locations throughout the world. The most visited webpage after the homepage is the webpage about the legal sources and SOPs (2290).***
- ***Regarding the intranet, 966 connexions were recorded during this period. 3 new NRL members, 1 IAG member and 1 new EU member joined the network. 5 NRL members left the network.***
- ***Monthly updates were carried out. Maintenance tasks were provided to maintain security and confidentiality systems and to operate backup.***
- ***First issued newsletter of 2014 was prepared and posted on the intranet on June 2014. The 17th newsletter's content consists in the minutes of the 8th EURL-AP Annual Workshop held in Riga from the 21st till the 22nd of May 2014. This newsletter represents a workshop summary, including comments, decisions and brief abstracts of the lectures.***
- ***A second newsletter was published in December 2014. This 18th newsletter announced the next edition of the annual EURL-AP workshop that will take place in Celbridge, Ireland in April 2015. It exposed the last development realised by the EURL-AP since the 2014 annual workshop and also explained the planned 2015 program.***

5.3 Organization of proficiency tests (1 for microscopy and 1 for PCR skills) and follow-up (175 m/d):

- *Definition (with the collaboration of the DG-Sanco) of the objectives,*
- *Preparation of the proficiency tests (including homogeneity study),*
- *Invitation of the NRLs to participate,*
- *Packaging and sending of the samples,*
- *Collection of the results,*
- *Redaction of the reports.*

- *The European Union Reference Laboratory for animal proteins in feedingstuffs (EURL-AP) organised the PCR proficiency test for assessing the ability of the NRL network with respect to the detection of ruminant proteins in feed using PCR according the Commission Regulation n°51/2013 and the version 1.0 of the EURL-AP SOPs “DNA extraction using the Wizard® Magnetic DNA purification system for Food kit” and “Detection of ruminant DNA in feed using real-time PCR”. Total number of participants was 31 (27 NRLs and 4 labs outside the NRL network). The study was based on a set of 6 blind samples consisting of feed samples (blanks, feed matrices fortified with terrestrial processed animal proteins or contaminated feed) sent to the participants the 21st of April 2014. In order to be in line with the reintroduction of non-ruminant PAPs in aquafeed, five out of the 6 samples were aquafeed.*

Official announcement of the study was made on the 21st of February 2014 through a letter. On the 21st of March 2014, the material for the test (a set of 6 blind samples) was provided to the participants by express shipment. The 24th of March 2014 all the participants had their set of samples. On the same date the Excel result report file containing the instructions, a recording sheet and a report summary was posted on the intranet part of the EURL-AP website.

All NRLs provided results in due time (deadline: 28th of April 2014). As an innovation for this year, all the participants received after the closure of the results (29th of April 2014) an individual table giving them a feedback of their results. Five labs reported one false result out of 6 analyses to be carried out per lab (3 labs with one false positive result and 2 labs with one false negative result) and 3 labs had 2 false results (gathering the three possible combinations for 2 false results) on their six analyses. The results of the EURL-AP Proficiency Test PCR 2014 were compiled in a report.

Corrective actions (sending of a new set of 3 samples) were taken with the participants having 2 false results. Two of the three labs sent correct results.

The investigations continued with the third lab giving continuously a false positive result with the same sample. This individual problem seems to be due to a cut-off that must be reset. Nevertheless, it was impossible to find any outlier data using the developed statistical tools to check the pertinence of the cut-off in use in the lab.

- *The results of the EURL-AP Proficiency Test Microscopy 2013 were analysed at the beginning of 2014. A first draft version of the report was diffused to the NRLs for revision. Upon reception of the comments a final version was published in April 2014 before the EURL-AP annual workshop. The total number of participants involved in the study was 33 of which 27 NRLs and 6 foreign participants. A set of 8 blind samples consisting of compound feeds and various fish feeds fortified or not with processed animal proteins (PAPs) was to be analysed. Adulteration levels by PAPs were all performed at 0.1% w/w. Overall results were satisfying although a major problem of specificity was noted for the identification of hydrolysed feather meal with merely 22% of correct detection. The present study also demonstrated that new interpretation rules of Annex VI of regulation EC/152/2009 regarding the presence of low levels of animal particles were reducing the number of total errors by 18% compared to the former ones. Follow up of the underperformance cases was organized.*
- *In autumn 2014, the EURL-AP designed and prepared the proficiency test for microscopy. The official announcement was made on the 4th September 2014. The preparation of the samples as well as the homogeneity study took place in September and October 2014. The homogeneity study was realised by microscopy, NIRM and PCR. All the analyses were finished before sending of the sample set on the 7th November 2014. The sample set was mainly focussed on fish feeds and compound feed with adulteration of PAPs around 0.1%. Some special cases of adulteration with almost microscopically undetectable materials were insured (a.o. blood meal, milk and a bovine PAP without bones). The number of samples to analyse was of nine. The deadline*

was the 29th November 2012 and all the NRLs sent their results. The study of the results was initiated in December and will continue in 2015 with the publication of the report. As for previous years, next to the 27 NRLs, some non-European countries were invited to participate (Australia, Japan, Norway and Serbia).

- *In link with the organization of proficiency tests, the EURL-AP began the writing of procedures for an ISO 17043 accreditation. These procedures were followed for the first time during the organization of the EURL-AP PT microscopy 2014.*

5.4 Production of SOPs, assistance support and samples dedicated to NRL network (30 m/d):

- *Revision of public SOPs: definition of the objective, development, expert meeting, redaction and diffusion on internet,*
- *Coordinate the preparation, reception, storage, maintenance and distribution to national reference laboratories (NRL) of samples containing animal proteins derived from different species and in particular from fish, poultry, pigs and ruminants to be used as reference materials or to carry out comparative testing,*
- *Production of technical network SOPs (redaction and posting on intranet),*
- *Production of support (e.g. excel files for cut-off determination,...).*
- ***Two SOPs dealing with instructions related to the PCR method were updated (version 2.0) to be in line with remarks received from NRLs since their publication in June 2013. In addition, the Excel file for the determination of cut-offs was upgraded (version 2.0) to allow an automatic detection of outliers in the calibration replicates.***

5.5 Organisation of the EURL-AP workshop (35 m/d):

- *Preparation of the agenda,*
- *Invitation of the attendees,*

- *Realisation of the workshop,*
- *Minutes of the workshop,*
- *Handling of participants' cost claims.*

Note: For 2014 it is proposed, as for the last 4 years, to organise the annual workshop outside Belgium. In 2014 the Latvian NRL proposed to host the workshop in Riga.

- ***In 2014, the annual workshop was organized in Latvia. The 8th EURL-AP workshop was held the 21st and 22nd of May 2014 in Riga. Once again, as in 2013, the EURL-AP didn't have to deal with the logistic organisation (meeting room renting, catering, hotel, etc) which was taken in charge by the Latvian NRL.***
- ***The program of the first day of meeting included the following items of presentation and discussions: The day began with the presentations of the organisation and activities of the Institute of Food Safety, Animal Health and Environment – BIOR and the Latvian NRL, a summary of the EURL-AP 2013 activity report, a presentation of the JRC-IRMM about the production of certified materials to implement EU regulation on PAPs, the changes in SOPs for PCR and the results of EURL-AP Proficiency test PCR 2014. The session continued with the presentation of the results of the EURL-AP Proficiency test Microscopy 2013, a presentation of NRLs' performance evolution from 2007 to 2013 and a presentation of the IAG proposals for improvement of microscopic method. The day concluded with a presentation of the Irish NRL about the effect of sample grinding on PAPs detection by microscopy, a presentation of the British NRL about the declining BSE epidemic in Europe and an open discussion which focused on 3 topics: PCR PT and method, Microscopy PT and method and the Microscopy and PCR operational scheme. For the second day of the workshop, the presentations were firstly focused on legislative aspects with a presentation on the impact on laboratories of the future Regulation on Official control presented by the DG Sanco and the presentation of a summary of the survey official control results on fishmeal in***

2013. The progress in the in-house validation of the pig target was then presented as well as a presentation on the DNA methylation analysis. After the break, the morning continued with a presentation of the German NRL about the aptamers and its first results with bovine thrombin and with a presentation of the last EURL-AP development regarding the complementary methods. The day and the workshop finished with the activity program for 2013, and the workshop conclusions. The localisation of the workshop 2015 will be in Celbridge (Ireland). This workshop will take place over three days instead of 2 to respond to the NRL request to have more time for discussion.

- **In 2014 again, representatives of both, the microscopy method and the PCR method were invited to the workshop. In most cases, the same person was the representative of the 2 methods. Minutes of the workshop were recorded in the 17th EURL-AP newsletter.**

5.6 Organization of specific training for microscopy and PCR (preparation, realisation at EURL-AP facilities) and other educational activities (CD, DVD, training notes update...) (55 m/d)

- **One training in microscopy was also organised this year in September. It involved 3 NRLs coming from Cyprus, Czech Republic and Denmark. All the participants came at their own costs as these NRLs already attended a similar training before.**