EUROPEAN COMMISSION
DIRECTORATE GENERAL
JOINT RESEARCH CENTRE
Directorate F - Health, Consumers and Reference Materials
Food & Feed Compliance

# 13th WORKSHOP OF GMO NATIONAL REFERENCE LABORATORIES REGULATION (EC) No 882/2004 and 28th ENGL PLENARY MEETING

## 20-21 September 2017, Ispra, Italy

## Session: National Reference Laboratories and Official Control Laboratories

### 1) Welcome and approval of the agenda

The Chairman welcomed the participants and introduced the new DG SANTE contact person for the EURL. The agenda (see Annex 1) was approved without modifications.

### 2) Approval of the 12th workshop report

The report of the last meeting was adopted without modifications.

### 3) Tour de table: issues/opinions/training needs from NRLs (each NRL is invited to report orally)

The participating National Reference Laboratories (NRLs) designated by their Member States (MS) under Regulation (EC) N. 882/2004 were invited to report orally on activities, issues and future training needs.

The laboratories analysed a range of samples, but only few resulted to be non-compliant. Some laboratories monitored the presence on the market of GM petunia, GM salmon and GloFish® and highlighted the absence of related reference materials and control samples. Some NRLs declared expanding their activities to species identification and allergens, while others are working on enlarging the scope of accreditation to detection methods for GM animals, allergens and sequencing. Some are developing new strategies (e.g. sequencing) for detecting non-authorised GMOs.

Participants expressed the need of having more information sharing platforms and provision of CRMs at 0.1% GM level. The NRLs found the workshop on DNA extraction offered by the EURL GMFF and LGC very useful and suggested organising it again in the coming years. They expressed appreciation also for the provision of pre-spotted plates (PSP) for GMO screening and event-identification.

Laboratories suggested providing training on digital PCR, on optimisation of screening approaches for GMO detection, multiplexing, genome sequencing and bioinformatics. The harmonisation of screening procedure at EU level was highlighted as one of the most pressing need by the laboratories, given the challenge in managing the high number of GM events approved in EU, the increasing diversity of elements to be monitored and the lack of human resources for carrying out all the tests.

Some members commented the findings of few non-compliant samples and their representability of the food market and suggested improving the risk-based monitoring approach. A participant informed that NGO organisations asked about capability of detecting GM salmon, now legally distributed in Canada, and pressed for urgent actions.

The Chairman proposed collecting all issues on GM petunia and reminded that the case was not involving food safety. He informed that a new edition of standard ISO/IEC 17025 will be published at the end of the year. In the new version there are minor modifications of the technical requirements but more substantial changes regarding management aspects. He proposed screening the document for detecting in advance possible problems for the own laboratory and remarked that guidance will be provided by accreditation bodies etc. to all laboratories.

The Secretary reminded that a new ENGL working group on multiplexing will be soon activated.

The Chair further commented that the issue of flexible scope accreditation still experienced by some NRLs should be pursued more vigorously on different directions. Moreover, he explained that under the current EU legislative system it is not possible to regulatory enforce the production of CRMs for non-authorised GM events.

**4) Update on comparative testing activities (EURL GMFF)**

The JRC presented the results of Comparative Testing (CT) rounds CT01-17 and CT02-17 and announced that the reporting on the latter will be published soon. He informed that for evaluation of laboratory performance the consensus value was used, calculated as the robust mean of the results obtained by all NRLs (on log scale). For the maize flour sample containing the GM event VCO-1981, a CRM, the certified value was used as the assigned value. He informed that the performance evaluation was calculated by z and zeta scores and that the scoring limits had been changed by defining z score ≥3 as unsatisfactory. The $\sigma_{PT}$ value pre-set to 0.15 in CT01 was changed to 0.10 in CT02 to be more in line with the approach of the previous years. Some results were quite above or below the satisfactory level while for the maize VCO-1981 sample no questionable or unsatisfactory results were observed. He remarked that the EURL GMFF had plotted the z scores against the DNA extraction method used by the NRLs without finding a correlation between the two parameters.

The EURL GMFF will organise 2 CT rounds in 2018, NRLs will be invited to the first and second CT in April and June, respectively.

**5) Estimation of measurement uncertainty in Comparative Testing (P. Robouch, JRC)**

P. Robouch (JRC) acknowledged the quality and importance of the "*Guidance document on Measurement uncertainty for GMO testing laboratories*" by Trapmann et al. (vers. 2, 2009). In the two examples provided in the Annexes the authors suggest to estimate measurement uncertainty using either (i) the standard deviation of *reproducibility* derived from interlaboratory comparisons for method validation (when available); or (ii) the *intermediate precision* standard deviation obtained in the frame of the single-laboratory validation study. It was noted that the definition of the limit of detection (LOD) given in the guide defers from those set in other international documents; this issue will be taken into account during the revision of the guidance.

The second topic of the presentation related to the recommendations set by ISO 13528:2015 "*Statistical methods for use in proficiency testing (PT) by interlaboratory comparison*", including the assignment of the reference value (and corresponding standard uncertainty) and the selection of the criteria for PT assessment. The results of the last GMO PT organised by the EURL GMFF were used to support the discussion. The speaker explained that the z-score answers to the questions "*Does my result (value) belong to the expected distribution? Does it fall in the expected concentration range?*", while the zeta score provides another information: "*Does my range (result ± uncertainty) overlap with the assigned range ($x_{pt}$ ± 2* u ($x_{pt}$))?*" This question requires proper estimation of the measurement uncertainty by the participating laboratories. An additional evaluation is therefore required to identify over or under-estimated uncertainties. Such a thorough scrutiny of reported ranges allows laboratories (with negligible bias and realistic uncertainty) to be confident when assessing compliance of relevant food or feed commodities.

The speaker announced that starting from the following year the laboratories will need to declare compliance of samples analysed with the EU labelling legislation.

**6) Outcome of the DNA extraction workshop and way ahead**

The JRC acknowledged the contribution of LGC (UK) in organising the DNA extraction workshop held in Ispra on 7th-9th of June 2017. He presented the main outcome of the training and remarked that the following actions were agreed: 1) Share participants' presentations; 2) Update a table with information on DNA extraction methods; 3) Propose the creation of a WG to the ENGL Steering Committee; 4) Prepare a comprehensive guidance document; and 5) repeat the workshop in two-three years' time.
The speaker announced that the information provided at the workshop and the results of the EU survey to which laboratories had contributed are available on the ENGLnet (see https://englnet.jrc.ec.europa.eu/ForumDNAext/default.aspx).

The UK reppresentative remarked that a template presentation had been circulated to participants before the workshop for sharing problems encountered and their solutions. The organisers were encouraged by the level of engagement. More than 40 examples of issues and troubleshooting were presented by the participants during the interactive part of the workshop. The speaker presented a database detailing the issues and solutions collected which were categorized according to e.g. "method", "solutions", "contributor" or by keyword (topic) or

sample type. Users could select a specific solution or aspect and topic by using a filter. The database is available on the ENGLnet website mentioned above. The speaker suggested the following actions:

A. Asking participants to check accuracy of individual entries
B. Including additional information to make the database more usable
C. Considering preparing a short summary publication "discursive briefing" as a result of the workshop.

**7. AOB**

The JRC summarised the issues and training needs presented during the tour de table by the participants. He announced that a training workshop on digital PCR (dPCR) combining hands-on experience and general presentations will be organised by the EURL GMFF and the Slovenian National Institute of Biology on 29th of November - 1st of December 2017. Another workshop on cost-effective screening strategies for GMO detection will be organised by the EURL GMFF in 2018 (probably first half).

## Session: 28th ENGL Plenary meeting including continuation of the NRL 882 Workshop

**8. Welcome and approval of the Agenda**

The Chair welcomed the participants, the draft agenda was approved.

**9. Approval Report 27th ENGL plenary**

The report of the last meeting was approved without modifications.

**10. Dynamic Action List (DAL) of 27th ENGL plenary**

The Secretary reviewed the open points of the action list. He announced that an open forum on research topics and another one for sharing issues on implementation of Regulation (EU) No 619/2011 will be soon activated on ENGLnet.

**11. Outcome of the 33rd ENGL SC meeting (June 2017)**

The Secretary summarised the main points discussed by the Steering Committee (SC) during the last meeting in June 2017.

The difficulty in finding guidance documents on the ENGL website was highlighted; it was requested placing the documents in a more visible section. The Secretary explained that the ENGL website will be soon migrated to another webpage environment.

***12. Progress reports ENGL working groups:***

**12.1 WG Update of Methods – Presentation of definitions proposal**

The WG chair informed that the group had a web meeting on the 13th of September 2017 and is planning to finalise the document after the ENGL plenary. A final version will be provided by the end of 2017. The group applied the ISO 16577:2016 definition of measurement principle, measurement method, and measurement procedure to the GMOs area and proposed the following definitions:

- PCR measurement method = generic description of a method based on in vitro enzymatic reaction
- PCR measurement procedure = detailed description of a PCR measurement method
- Updated PCR measurement procedure = modified procedure of a PCR method.

The Chair explained that in a note of the new ISO 17025 edition the term "measurement method" is considered synonymous with the term "measurement procedure" as defined in ISO Guide 99. He suggested maintaing consistency in terminology in accordance with international standards. The members agreed in using the robustness of the method as criteria for defining whether the modifications introduced in a measurement procedure create a new method or not. In this sense, modifications could be introduced as long as the performance characteristics of the methods are maintained within an acceptable range.

**12.2 WG Digital PCR**

The WG chair informed that many corrections and amendments were introduced in the document and that the main text had been finalised. Since there were still discussions on the examples provided in the Annex he suggested submitting at least the main text of the document to the SC. The publication of the guidance is foreseen for the end of the year. The speaker presented the structure of the document and some open issues

such as the application of generic conversion factors for the calculation of the mass/mass percentages in event-specific multiplex dPCR reactions.

The Chair suggested increasing the uncertainty estimate to offset the problem of generic conversion factors covering different zygosity values for the female or male donor of the target GM events.

The Chair suggested combining the main text with the Annex before submitting to the SC and recommended speeding up the publication of the entire document.

**12.3 WG-Mvrf2 (Method Verification) - Presentation of the final document**

The WG chair explained that the group had updated the method verification guideline according to the second version of the guidance on Method Performance Requirements (MPR) and ISO 16577. As a result the introduction and the section on DNA extraction were rewritten and the terminology and the general considerations updated. She announced that Annex 2 will be available on the EURL GMFF homepage after finalisation of the WG-DNA document. Additional Excel sheets for performing calculations in the laboratory were provided by Rikilt and BVL. The document has been already reviewed by the SC and it will be circulated again among ENGL members before publication.

The Chair congratulated the group for finishing the work.

**12.4 WG-Proc (ENGL Procedures)**

The WG chair explained that the group has been working on a new draft covering four procedures, i.e. publications, functioning of working groups, participation of non-ENGL members to meetings and establishment and functioning of ENGL e-discussion forum. They will be collecting comments from the WG members in the following week. The group could consider incorporating other procedures.

The Chair suggested including a procedure about confidentiality and copyrights issues.

**12.5 AG SMV (Advisory Group on Selection of Methods for Validation) - N. Papazova**

The WG chair reported that the WG was evaluating two proposals, one on detection of a potato endogenous gene for which it is collecting information from the developer to assure that the method complies with the MPR criteria, the other on a multiplex digital PCR. The group decided waiting for the publication of the guidance on digital PCR before evaluating the multiplex PCR proposal. He requested participants to submit proposals for methods filling analytical gaps by compiling the form available on the GMOMETHODS database web page. The WG will meet by the end of the year to perform a gap analysis and evaluate the proposals. The group is planning to finalise the taxon-specific table and to discuss methods for detecting GM fish. The Secretary invited ENGL members in participating to the WG activities.

**12.6 New WGs on sequencing data and multiplex PCR: presentation of mandates**

The Chair commented that three WGs could finalise soon their activities. He remarked that a WG needs sufficient participation and support from ENGL members and that it should deliver in a maximum of two-three years.

The Chair proposed a mandate for a new WG on DNA extraction since many laboratories have highlighted the importance of this issue. Members commented that the mandate was quite extensive and could be realistic only with a sufficient number of contributors but accepted the proposal and the expected deliverables.

The Chair presented the mandate for a new WG on PCR multiplexing. He remarked that the development of methods should not be a task of a WG and suggested modifying the phrasing of the mandate accordingly. Also defining applicability to analytical areas other than GM food, feed, and seed was considered outside of the ENGL expertise. The participants approved the proposal.

The Chair proposed a mandate for a new WG on good practice/quality of DNA sequencing data. Regarding the timeline he questioned if it is realistic the goal of finalising the task within four meetings. Other members regarded the topic to be strategically important and considered it necessary progressing the work started within the Decathlon project.

The Chair suggested writing a milestone document instead of guidance. The proposal was accepted by the ENGL members.

The Chair informed that a call for members for each WG will be sent by email and that it will be critical to have a sufficient number of participants.

**13. Update from DG SANTE**

DG SANTE informed that an explanatory note on new techniques in agricultural biotechnology from the Scientific Advice Mechanism (SAM) has been published at https://ec.europa.eu/research/sam/pdf/topics/explanatory_note_new_techniques_agricultural_biotechnology.pdf.
A high-level conference on "Modern Biotechnologies in Agriculture" has been organised by the Commission for the 28th of September in Brussels to provide an overview on the topic.

DG SANTE also informed that a method for detecting GM salmon has been provided by AquaBounty but without reference material or positive control samples. Regarding GM Petunia, Member States were asked to submit a report on the results of the analysis.

***14 Scientific and technical session 1***

**14.1 GloFish and GM salmon (F. Debode)**

F. Debode (BE) reported that no GM animals or derived products are authorised in the EU market and that applications for authorisation have not been presented so far.

**a) GM salmon**

The speaker remarked that among the different GM fish developed for human consumption, a GM Atlantic salmon (*Salmo salar*) developed by the company AquaBounty has been already approved in Canada and it may be commercialised now at large scale. To detect the GM salmon, classical DNA extraction methods as CTAB have been shown to be effective and many methods have been published for detecting endogenous targets in salmon genome. These targets, however, are not specific to *Salmon salar* but to different Salmonidae species. He informed that the CRA-W laboratory has developed two methods for detecting the GM salmon transgenic sequences. The methods were first tested on plasmid DNA as no real genomic DNA or positive controls were available. The methods were further verified in 2017 by BVL (Germany) on genomic DNA provided by AquaBounty. Only one of the two methods performed successfully on the GM DNA samples tested. A detection method has been submitted by AquaBounty to the US Food and Drug Administration (FDA) and DG SANTE but no information is publicly available since the method is covered by a confidentiality agreement.

The speaker remarked that several EU Member States are considering control plans and accreditation of methods for detecting GM salmon and offered to share the method and the control plasmid developed by the CRA-W laboratory.

**b) GM ornamental fish**

The speaker informed that, in addition to GloFish®, new ornamental GM fish are being developed. For their detection DNA extraction is performed from single fish. He presented existing methods for detecting red, yellow and green colours and informed that his laboratory has developed an additional real-time PCR method, not yet published, for detecting these three traits. The speaker remarked that some GM fish coming from Sri Lanka have appeared illegally in the EU market and announced that the CRA-W laboratory could share with the ENGL community some positive material of GloFish bought from the USA market and information on the detection methods.

**14.2 Update on GM Petunia (L. Grohmann, T. Prins)**

The presentation was divided in two parts, given by L. Grohmann (DE) and T. Prins (NL), respectively.

L. Grohmann explained that after the announcement of orange non-authorised GM petunia on the EU market by the Finnish Food Safety Authority (Evira) in2017, official control checks were performed in several German Federal States as well in EU member states. A large range of GM positive varieties, covering at least 67 GM trade names (experimentally confirmed), was found. An insert sequence very similar to the GM petunia first described in a 1987 article by Meyer et al. was detected by using different EU reference and in-house validated methods to confirm the presence of the described elements in the collected GM petunia samples. In a second group of GM petunias some of these elements could not be detected and a different gene, probably from another construct, could be amplified. The speaker presented a screening strategy for detecting different GM petunia varieties and announced that molecular data of GM petunias and elements detected are provided in the

EUginius database. The attempt is now to determine how many events are present on the market to help clarifying the situation.

T. Prins informed that P-35S, T-35S, P-nos and nptII elements were confirmed in GM petunia samples, very similar to the GM event described by Meyer et al., but that some discrepancies were found. The sequence of the Meyer et al. GM petunia event does not contain a T-ocs primer annealing site, yet T-ocs sequences were detected in the GM petunia samples found on the market. The presence of construct-specific sequences was investigated in those GM petunia plants. He proposed a GM petunia decision support system defining two groups according to the detection of P-35S, P-nos, T-nos and T-ocs elements. Group 1 GM petunia (consistent with the construct described by the Finnish research institute) contain the maize A1 gene and the T-ocs primer binding sites not present in the sequence of event RL01-17 that was provided by P. Meyer; group 2 GM petunia contain a petunia F3'5H gene. No further information is available on this group so far. The speaker remarked that further analyses are planned for characterising the two groups.

The Chair commented that thanks to the work performed so far, official control laboratories could have a good set of molecular markers for identifying GM petunia.

***15 Scientific and technical session 2***

**15.1 GMO activities in Japan (K. Kitta)**

The speaker provided an update on regulatory activities, GMO testing and the legal situation in Japan. She informed that after the findings in Finland, inspections in Japan by the Ministry of Agriculture, Forestry and Fisheries (MAFF) have identified more than 50 varieties of GM petunias. For the screening they used conventional PCR methods targeting the P-35S-CAMV, CS-dfr-maize, P-nos, CS-F35H-PETHY and CS-nptII elements. Regarding food and feed testing strategies in Japan, quantitative screening analysis is performed and individual event-specific quantification is only carried out for samples with GM content higher than 4.5%. The increased number of stack varieties is creating problems for quantification, therefore for seeds/grains testing the sub-sampling approach is more widely used. A study panel was established for the labelling policy on GM foods and a discussion is ongoing on lowering the legal GM threshold (currently set at 5%). In that case, however, the detection methods would also have to be modified.

**15.2 CRISPR/Cas9 editing of carotenoid genes in tomato (C. D'Ambrosio)**

Tomato is an ideal model organism to demonstrate the power of the CRISPR/Cas9 technology. The study focused on morphological markers that could be detected by visual inspection such as corolla and ripe fruit colours; genes in the carotenoid biosynthesis pathway were selected for CRISPR editing. There are two well characterized natural mutants of this pathway which produce white flowers and yellow fruits instead of yellow flowers and red fruits, respectively. The experimental goal was to edit the plant genome to obtain an equivalent of natural mutants. A single guide RNA along with the Cas9 variant was inserted under a promoter in a suitable plant binary vector and inserted by agrobacterium transformation in cotyledon. Transgenic plants were screened by PCR/RE genotyping and the mutation was characterised by sequencing. These transformed plants were phenotypically identical to the natural mutants. In the last phase of the strategy they performed negative selection by PCR analysis for offspring plants exhibiting the mutant phenotype but no longer containing the sgRNA/Cas9 cassette (exogenous DNA) and characterised by sequencing their mutation. It was found that the edited alleles were inherited following Mendelian laws.

Participants asked whether it would be possible to detect mutations induced with the CRISP/Cas9 system: apparently it is still impossible to distinguish in the progeny the natural from the induced mutant.

**15.3 Experiences of the EU accreditation bodies with GMO accredited labs (Ionanis Sitaras)**

The Chair announced that the speaker could not be present at the meeting but that the EURL GMFF would nonetheless collect information from the laboratories.

**15.4 The European Commission's Knowledge Centre for Food Authenticity: activities related to species identification (F. Ulberth, V. Paracchini, JRC)**

F. Ulberth (JRC) explained that according to the new Regulation (EU) 2017/625 (Art. 9) on official controls not only the safety but also the integrity of food needs to be ensured. Key characteristics of food fraud are the violation of EU food law with intention, economic gain and customer deception. Proving food fraud is however difficult since the intention needs to be demonstrated but it can be minimized by having good control systems and means of objectively determining the product. He introduced the new EC Knowledge Centre for Food Fraud

Detection and Prevention and presented the tools and services offered. They include incident reporting (MedISys) monitoring media covering fraud cases, a methods database designed by a food integrity project and a compositional database of authentic products for wine. The speaker listed the analytical services offered and informed that a network of laboratories has been created.

V. Paracchini (JRC) presented the results of a project for fish species identification of *Common sole* and *Atlantic code*, the two species more commonly subject to mislabelling. She explained that current approaches using mitochondrial DNA barcoding and Sanger sequencing cannot be used on processed food. She proposed two approaches: 1) a LAMP assay targeting a mitochondrial sequence 2) a meta-barcoding approach where different targets are simultaneously amplified and then sequenced. To allow species identification they developed a database of reference sequences by sequencing the genomes or by retrieving them from existing genetic data with a bioinformatics approach. The strategy was tested on flesh mixtures of *Solea solea* and *Solea senegalesis* and on 40 samples containing mixtures of Solea species. They were able in the first case to quantify the two species according to the original proportion and in the second case to identify the correct species. The work was recently published (see article). Another publication is in preparation for the LAMP assay. A new project will develop a new barcode for the thunnus and code species since in thunnus the mitochondrial DNA sequence is not sufficient for identifying the correct species.

The work was appreciated and the importance of producing own reference databases was underlined since public collections may contain mistakes in species reference sequences. Hopefully the database will be soon available.

## *16 Scientific and technical session 3*

### 16.1 NGS-based species identification (M. Staats)

M. Staats (NL) presented a DNA meta-barcoding approach combining NGS sequencing with DNA barcoding. It is a novel methodology already used for environmental monitoring, diet studies and authentication of plant/animal ingredients. The project is directed to customs authorities that require a single assay highly sensitive for identifying endangered species (CITES) in traditional medical products and other complex food/medicinal supplements. The method used a panel of 12 universal DNA barcodes designed to target a wide range of animal and plant species, allowing PCR amplification from samples containing fragmented DNA and providing improved resolution for identification and quality assurance. They assessed the DNA meta-barcoding procedure and its reproducibility in a collaborative trial involving 16 laboratories where 160 samples with complex mixtures of plant and animal tissues were tested. To analyse the sequencing data they used a dedicated bioinformatics workflow with an intuitive and user friendly web interface. All animals could reproducibly be identified at the species-level while plants could mainly be identified at the family level.

The speaker informed that users could freely access the web interface at "CITESspeciesDetect". The output provides conservative assignment of species and prevents erroneous taxon identification.

### 16.2 Update on CEN and ISO (L. Grohmann)

L. Grohmann (DE) presented a summary of the activities of CEN TC (technical committee) 275 on GM foodstuffs and species analysis (WG 11). He explained that WG11 has a new enlarged scope now covering also nucleic acid based methods for species analysis, not including microorganisms. To coordinate the activities of different CEN Technical Committees related to species detection methods, a CEN Food Authenticity Coordination Group was established. A kick-off meeting was organised in June 2017. WG11 had other meetings on September 2016 and June 2017 where new projects were taken on board for (i) providing guidelines on validation of qualitative real-time PCR methods applied to food testing and (ii) a method for fish species identification by DNA barcoding using mitochondrial cytochrome b and c oxidase I gene segments.

L. Grohmann presented also a summary of the activities of ISO/TC 34/SC 16 on horizontal methods for biomarker analysis. He informed that the 7$^{th}$ Plenary Meeting of SC16 was organised in Washington DC on 6$^{th}$-8$^{th}$ September 2017 in Washington DC and listed the activities of the different working groups.

Within ISO TC 276 on "Biotechnology" two projects have been proposed on analytical methods by WG3: ISO/AWI 20395-1 a "guidelines for evaluating the performance of targeted nucleic quantitative methods Part. 1 qPCR and dPCR" and ISO/AWI 20397-2 on "General requirements for massive parallel sequencing –Part 2: methods to evaluate the quality of sequencing data".

**16.3 SPECENZYME: A Belgian project designed to evaluate the purity of food enzymes including GMO (N. Roosens)**

N. Roosens presented a multidisciplinary project called SPECENZYM, aiming at studying the purity of Food Enzymes (FE) for the development of general purity criteria and increased consistency in their approval. The project involves several scientific institutes and partners (14), all specialised in different fields covering GM aspects, allergens, production of mycotoxins, antibiotics and heavy metals. The speaker provided a general overview and description of the different work packages. Based on the list of 300 FE submitted to EFSA for safety evaluation, the project will consider the main chemicals and microbiological impurities/contaminations potentially present, identify which are produced by GM microorganisms and collect information on the genetic modification for detecting the presence of GM DNA in the food preparation. On this basis a description of acceptable purity criteria for each FE will be provided. Regarding FE produced by GMM, the project will collect all information on constructs and sequences in a database, will define strategies for their isolation and extraction of the DNA. As a final deliverable a GMM screening workflow and a monitoring approach should be developed.

**16.4 Update on allergen detection labs network (H. Emons, JRC)**

The Chair provided a summary of a workshop organised by the JRC for establishing a laboratory network on harmonisation of food allergen measurements. The meeting had 36 participants from 21 Member States. The agenda included 3 main sections: 1) Current status of EU allergen measurements, 2) Collaborative approaches (where the ENGL example was given) and 3) Review on the advantages and pitfalls of current technology to achieve comparable measurements. Communication and harmonisation needs were highlighted, in particular training in the fit for purpose criteria of allergen measurements to support quantitative risk assessment procedures. For achieving that measurements results are comparable all actors need to target the same measurement scale. A pitfall of current technology is the existence of diverse measurements methods in the allergen areas, thus making it challenging to express the results in the same measurement scale. It was agreed for the purpose of risk assessment to express the results as mg of allergen protein/kg of food. The Chair asked ENGL members to contact the EURL GMFF in case of interest in joining the network.

**17 *Break-out Groups***

1) Recommendations for GM fish analysis
2) Sample homogenization
3) (GM) DNA in feed additives

**18 Reports of break-out groups**

**1) Recommendations for GM fish analysis:**

Among participants to the discussion, some had experiences in DNA extraction and analysis of GloFish®, two laboratories performed analysis on GM salmon.

For detection of GM salmon a real-time PCR method developed by CRA-W and one additional method from the Norwegian Veterinary Institute will be published soon. Other methods were published by a Japanese laboratory or provided by the company AquaBounty to FDA and DG SANTE but no further information is available at the moment. German laboratories have obtained limited amounts of GM salmon genomic DNA from the company but the material is covered by a Material Transfer Agreement (MTA). CRA-W informed that a plasmid has been developed to be used as a control and offered sharing information on GM salmon detection and controls with other ENGL members.

CRA-W notified the development of three methods for detecting red, green and yellow GloFish® which will be published soon and be made available on the Euginius database. Members remarked the existence on the market of different GM events and the possibility of using other fluorescence-based methods for detecting GM fish. CRA-W offered to share control material for detecting red GloFish® while no material seems to be available for the other colours.

**2) Sample homogenization**

The group had limited awareness on the ENGL "Guidelines for sample preparation procedures in GMO analysis" (http://gmo-crl.jrc.ec.europa.eu/ENGL/docs/WG-SPP-Final-Report.pdf) providing practical guidelines on validation processes to ensure homogeneity & sample reduction (representativeness). Members recommended giving a clear structure for the publication of guidelines on the web. They commented that a validation procedure is

missing in the document and suggested including an example. It was further commented that with many samples the time required for cleaning the equipment is very time consuming and that the measurement of particle size is not feasible in the laboratory. To prove homogeneity they advise performing duplicate/triplicate measurements and verify if the variation is lower than 25%. A general update of the ENGL document was not considered necessary but it was recommended adding a concrete example of grinding and sampling validation procedures that could be easily implemented in a laboratory.

**3) GM DNA in feed additives**

The Commission is planning to revise the information to be provided by the applicant for authorisation of feed additives under Regulation (EC) No 429/2008. DG SANTE asked to receive assistance in drafting guidance on the detection of recombinant DNA in feed additives produced through fermentation. This guidance should define specific performance requirements for the DNA detection methods provided by the applicant in compliance with the EU Regulation. The group expressed the need to clarify the target (any DNA, only DNA from the production strain, also DNA from other strains), and the definition of DNA presence (number of molecules, nucleotides). qPCR seems to be the best technology for this purpose.

**19. Meeting conclusions, AOB and DAL ENGL 28th**

The Secretary reviewed the updated dynamic action list (Annex 2).
The 2018 ENGL Plenary will be organised the first week of October.
Based on the input provided during the meeting, the EURL GMFF will organize a workshop on screening for GMO analysis in 2018.
The EURL GMFF reminded that a training workshop for NRLs on dPCR is planned for the end of November 2017 in Slovenia.

The Chair thanked the participants for their valuable contributions and ideas and closed the meeting.

## ANNEX 1: Agenda

EUROPEAN COMMISSION
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**13th WORKSHOP OF GMO NATIONAL REFERENCE LABORATORIES**
**REGULATION (EC) No 882/2004 and 28th ENGL PLENARY MEETING**

**20-21 September 2017, Ispra, Italy**

**Draft Agenda**

**Day 1: 20th September 2017**

Session: National Reference Laboratories and Official Control

| AP | Time | Topic | Documents/comments |
|---|---|---|---|
| 1 | 9:15 | ▪ Welcome and approval of the agenda | Draft agenda |
| 2 | | ▪ Approval of the 12th workshop report | Report |
| 3 | | ▪ Tour de table: issues/opinions/training needs from NRLs (each NRL is invited to report orally) Follow-up discussion | Oral input from NRLs |
| | *10:45* | *Coffee Break* | |
| 4 | 11.15 | ▪ Update on comparative testing activities (W. Broothaerts, EURL GMFF) | Presentation |
| 5 | 11.45 | ▪ Estimation of measurement uncertainty in Comparative Testing (P. Robouch, JRC) | Presentation |
| 6 | 12.15 | ▪ Outcome of the DNA extraction workshop and way ahead (M. Burns, W. Broothaerts) | Presentation |
| 7 | 12.45 | ▪ AOB and conclusions | |
| | *13:00* | *End of session NRLs* | |
| | *13:00* | *Buffet lunch* | |

**Day 1: 20th September 2017**

Session: NRL 882 WS and 28th ENGL Plenary meeting

| AP | Time | Topic | Documents |
|---|---|---|---|
| 8 | 14:00 | ▪ Welcome and approval of the Agenda | Draft agenda |
| 9 | | ▪ Approval Report 27th ENGL plenary | Report |
| 10 | | ▪ Dynamic Action List (DAL) of 27th ENGL plenary | DAL ENGL27 |
| 11 | | ▪ Outcome of the 33rd ENGL SC meeting (June 2017) | Report SC33 |
| 12 | | *Progress reports ENGL working groups:* | |
| 12.1 | 14:20 | ▪ WG Update of Methods – Presentation of definitions proposal - R. Onori | Presentation |
| 12.2 | 14:40 | ▪ WG Digital PCR - S. Pecoraro | Presentation |
| 12.3 | 15:00 | ▪ WG-Mvrf2 (Method Verification) - Presentation of the final document – L. Hougs | Presentation |
| 12.4 | 15.20 | ▪ WG-Proc (ENGL Procedures) - M. Mazzara | Presentation |
| | *15:30* | *Coffee Break* | |
| | | *Progress reports ENGL working groups:* | |
| 12.5 | 16:00 | ▪ AG SMV (Advisory Group on Selection of Methods for Validation) - N. Papazova | Presentation |
| 12.6 | 16.15 | ▪ New WGs on sequencing data and multiplex PCR: presentation of mandates | |
| 13 | 16:30 | ▪ Update from SANTE | |
| 14 | | *Scientific and technical session 1* | |
| 14.1 | 16:50 | ▪ GloFish and GM salmon (F. Debode) | Presentation |
| 14.2 | 17.15 | ▪ Update on GM Petunia (L. Grohmann, T. Prins) | Presentation |
| | *17:40* | *End of day 1* | |
| | *19:30* | **Networking dinner at "Villa Borghi"** | |

**Day 2: 21st September 2017**

Session: NRL 882 WS and 28th ENGL Plenary meeting

| AP | Time | Topic | Documents |
|---|---|---|---|
| 15 | | *Scientific and technical session 2* | |
| 15.1 | 8:45 | ▪ GMO activities in Japan (K. Kitta) | Presentation |
| 15.2 | 9:20 | ▪ CRISPR/Cas9 editing of carotenoid genes in tomato (C. D'Ambrosio) | Presentation |
| 15.3 | 9.55 | ▪ Experiences of the EU accreditation bodies with GMO accredited labs (Ionanis Sitaras) | Presentation |
| 15.4 | 10:30 | ▪ The European Commission Knowledge Centre for Food Authenticity: activities related to species identification (F. Ulberth, V. Paracchini, A. Maquet, JRC) | |
| | *11:05* | *Coffee Break* | |
| 16 | | *Scientific and technical session 3* | |
| 16.1 | 11:30 | ▪ NGS-based species identification (M. Staats) | Presentation |
| 16.2 | 12:05 | ▪ Update on CEN and ISO (L. Grohmann) | Presentation |
| 16.3 | 12:30 | ▪ SPECENZYME : A Belgian project designed to evaluate the purity of food enzyme including GMO (N. Roosens) | Presentation |
| 16.4 | 12.55 | ▪ Update on allergen detection labs network (H. Emons, JRC) | Presentation |
| | *13:15* | *Buffet lunch* | |
| 17 | 14:15 | *Break-out Groups*<br>1) (GM) DNA in feed additives<br>2) Sample homogenisation<br>3) Recommendations for GM fish analysis | <br>Mandate<br>Mandate<br>Mandate |
| | *15:30* | *Coffee Break* | |
| 18 | 16:00 | *Reports of break-out groups* | |
| 19 | 16:40 | *Meeting conclusions* | |
| 20 | 16:50 | *AOB* and DAL ENGL 28th | |
| | *17:00* | *End of meeting* | |

Meeting documents available at: https://englnet.jrc.ec.europa.eu/28thENGLPlenary-13thNRLsWorkshop/default.aspx?InstanceID=1

# ANNEX 2: Action list

| 28th ENGL PLENARY ACTION LIST 21/9/2017 | | | | |
|---|---|---|---|---|
| **ACTIONS** | **Resp.** | **Timelines** | **Status** | **Comments** |
| **ENGL MEETINGS** | | | | |
| Make available on ENGLNet report and presentations of 28th ENGL Plenary | SEC | Oct-17 | Open | |
| **ENGL WORKING GROUPS** | | | | |
| **WG dPCR** | | | | |
| Finl draft for the SC | WG | Oct-17 | Open | |
| **WG Mvrf2** | | | | |
| Send doc to ENGL for comments | SEC | 30/09/2017 | Open | |
| **WG Procedures** | | | | |
| Organise next meeting | SEC | 31/10/2017 | Open | |
| **WG UpMeth** | | | | |
| finalise the report for the SC comments | SEC | Oct-17 | Open | |
| Template data submission | EURL | 30/6/2017 | Open | |
| **AG Method Selection for Validation** | | | | |
| Organise next meeting | SEC | 30/12/2017 | Open | October/November |
| Finalise the list of taxon-specific modules | WG + EURL | 31/12/2017 | Open | |
| Review existing methods for endogenous genes for animals | WG | 31/12/2017 | Open | To be started after finalisation of the task above |
| **OTHERS** | | | | |
| Edit mandated of the 3 WGs and call for WG members | SEC | 15/10/2017 | Open | |
| Oganise WS on screening | SEC + EURL | 31/3/2018 | Open | |
| Update the Guidance on flexible scope of accreditation | SEC | ? | Open | Minor amendments, contact task force |
| Planning update of the MU doc | SEC | ? | Open | Include dPCR. Add to the agenda of the SC June |
| Forum on research topics | SEC | 31/12/2017 | Open | J. Ovesna moderator |
| Position document identyfying issues with 619 and suggesting possible solutions, including approach for MU | SEC | 31710/2017 | Open | Moderator N. Papzova |
| TASK force on GMM in FA members call | SEC | 30/09/2017 | Open | |