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

29th ENGL MEETING

& 14th WORKSHOP OF GMO NATIONAL REFERENCE LABORATORIES UNDER REGULATION (EU) 2017/625 (Part 2)

2-4 October 2018, Ispra, Italy

Report

- **Welcome and approval of the Agenda**

The Chairman welcomed the participants. The Agenda was approved without modifications.

- **Approval Report 28th ENGL Plenary**

The report previously circulated was approved with no amendments.

- **Dynamic Action List (DAL) of 28th ENGL plenary**

The Secretary reviewed the open points of the action list. The item regarding Regulation (EU) No 619/2011 was previously integrated in the discussion on CRMs for the 0.1% mass fraction level. The Secretary agreed to mention the issue in the note to DG SANTE.

- **Outcome of the 35th ENGL SC meeting (June 2018)**

The Secretary summarised the main points discussed by the Steering Committee during the last meeting. This included a note to be prepared by the EURL GMFF requesting clarification to DG SANTE on the expression of results for samples potentially containing stacked GM events. As a follow-up to the screening workshop the SC decided for the ENGL plenary to propose a discussion on screening and to invite representatives of regional networks from Africa. Members at the SC meeting further discussed the survey on CRMs and availability of reference material for identifying the bacteria strain producing vitamin B_2. The Secretary finally informed that event-specific methods for three alfalfa GM events and control samples were made available by the German laboratory network.

- **Proposal on how to sum up GM events detected in the sample**

The EURL GMFF presented a draft request for clarification from the ENGL to DG SANTE regarding the reporting of results for samples containing more than one EU authorised GM event per (plant) species. Stack events should not be distinguished under Regulation (EU) No 619/2011 since according to Annex II, section B.2, the analytical results should be reported for one measured transformation event. Expression of results however, is not defined in such straightforward detail under Regulation (EC) No 1829/2003 where Art. 12(2) prescribe the limit (threshold) for the GM content of food or feed above which GMO labelling is mandatory. In that case the results should be expressed at ingredient level. The ENGL is asking if the results of quantification analysis (GM content with measurement uncertainty) should be always reported per single GM event for each (plant) species or if they could also be reported as the sum of the contents of all single GM events (with appropriately estimated measurement uncertainty) per species.

Participants appreciated the possibility of discussing the text and receiving guidelines from DG SANTE. It was suggested deleting the sentence "interpreting the analytical results" from the text since interpretation is a legal competence of the Competent Authority, not of the official control laboratories. It was further clarified that when using a screening approach it

is also possible to report the total GM content without specifying the single GM event contribution. It was also requested a clarification on expression of analytical results for seeds containing stacked GM events. DG SANTE suggested not including seeds in the request because the subject is under discussion with MS. As a result all participants agreed in using the term "grains" instead of "seeds" in the document.

Discussion of Progress reports of ENGL WGs:

- **WG Update of Methods**

The speaker expressed satisfaction for the work done and confidence in the clarity of the instructions for the renewal of the applications. He explained that the group needed only one meeting to finalise the document and that it will be able to provide the final draft to the ENGL by the end of the year.

- **WG Digital PCR**

The WG chair reported that the group had three meetings and two video conferences. They decided to have a drafting group to separate the tasks. The draft document provided to the ENGL members received many comments which were regarded and assessed by individual members of the drafting group and the chair. The document has been distributed to the WG members to receive approval by the 5th of October. It should then be submitted for final approval to the SC members. The participants agreed to delete the part on the "summing up of GMOs for the same species" from the text since it was regarded as not necessary for the purpose of the document.

- **WG ENGL Procedures**

The WG chair explained that the group collected requests where internal rules were still missing on the functioning of the ENGL. It included text on approval of documents, participation of external members to the ENGL plenaries and functioning of web discussion fora. The document has been completed and will be reviewed first by the SC because it is not of scientific nature.

- **WG multiplex PCR methods**

The WG chair remarked that the mandate of the group was entailing the preparation of a guidance and review document on multiplex methods, the definition of relevant performance requirements, modularity and transferability on different platforms including digital PCR. He informed that a kick-off meeting was organised in March 2018 where the structure of the document and the tasks were identified and subgroups and respective leaders defined. Comments on a first draft compiled by the drafting subgroup were discussed in a web-meeting in September 2018. The drafting subgroup is planning to review the comments and to distribute the revised document to the WG members by the end of November. After a new revision by the drafting team the WG is expected to approve the final text in the first quarter of 2019. The Chair asked to verify the scope of the document and the intended readership.

- **WG good practice/quality of DNA sequencing data**

The WG chair explained that the WG was recently established and is aiming at defining minimum requirements and recommendations for the generation and provision of sequencing data for the EU authorisation of GM events. The WG is composed by 20 members and started its activities before summer. In the first meeting they reviewed the mandate and elaborated the structure of the document. They focused on different scenarios for detection and identification of GMOs also in complex matrices. The document will include a chapter on quality control of sequence reads and validation of bioinformatics pipelines. The Chair informed that similar activities exist at international level.

- **WG DNA extraction**

No presentation

- **WG AGSMV**

The WG chair remarked that in 2018 the WG had two meetings to discuss the method proposals previously submitted. The method developer provided the experimental data requested on robustness and copy number stability for the method detecting a potato reference gene. They will be the need for an additional meeting to further discuss the other method submitted, a multiplex ddPCR assay for quantitative identification of five soybean GM events approved in EU. The WG is awaiting finalisation of the WG document on ddPCR for a definition of the acceptance performance criteria for validation of ddPCR methods. The group also discussed analytical gaps and whether it should consider new screening strategies. Some members suggested improving decision making supporting tools such as i.e. the JRC GMO-Matrix application provided on the EURL GMFF web site. In particular, they requested to include information on the EU authorisation status of the GM events presented in the matrix. Participants to the ENGL meeting agreed in considering this information useful for the analytical interpretation of the data or the experimental design of the screening approaches.

The Chair welcomed Elke Anklam, Director of JRC-F.5. In her speech the Director remarked the historical importance of the ENGL and requested support on the challenges posed by the recent European Union Court of Justice (EUCJ) ruling on new mutagenesis techniques.

- **Update from SANTE**

DG SANTE informed that an updated guidance for environmental risk assessment (ERA) was adopted[1]. The repeal of the "old" ERA guidance notes[2] will be submitted for a vote to the next Regulatory Committee under Directive 2001/18/EC due to take place on 18 October, 2018. As regards pending cultivation applications, information is not available yet on the timing of the final adoption.

MSs have requested to establish a WG to discuss pragmatic converging of seed testing. If this request is confirmed during the Regulatory Committee on 18 October, work could possibly start in 2018.

The Commission invited the AquaBounty Company to provide a reference material for detection of its commercial product GM salmon but had no reaction. A new GM trout appears to be also under development by this company. As regards the contamination incident with unapproved GM wheat in Canada, the Commission was satisfied with the efforts of the laboratory and Canadian authorities in collaborating with the EURL GMFF.

DG SANTE informed the participants of the recent ruling of the Court of Justice of the European Union (CJEU) on new mutagenesis techniques (case C528/16). DG SANTE explained that according to the CJEU ruling all new mutagenesis techniques are covered by the GMO legislation while the conventional mutagenesis techniques remain exempted because of their long safety record. The same ruling authorises the MSs in implementing national legislation on conventional mutagenesis techniques as long as this is not interfering

[1] Commission Directive (EU) 2018/350 of 8 March 2018 amending Directive 2001/18/EC of the European Parliament and of the Council as regards the environmental risk assessment of genetically modified organisms (OJ L 67, 9.3.2018, p. 30–45,
available at https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32018L0350)

[2] Decision 2002/623/EC establishing guidance notes supplementing Annex II to Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC (OJ L 200, 30.7.2002, p. 22–33,
available at https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32002D0623)

with EU legislation and Treaties, especially regarding free circulation of goods in the EU. The CJEU ruling did not request any action from the Commission and therefore there is no legal requirement for amending the legislation.

In the Standing Committee (11 September 2018), the Commission explained to the MSs that from the 25th of July 2018 onwards, all enforcement of the GMO legislation needs to include products generated with these new techniques.

The EURL GMFF explanatory note draft submitted for comments to the ENGL members was regarded as useful for indicating the analytical challenges in the enforcement of the EU legislation following the new CJEU ruling.

The participants expressed regret for missing the benefits of these new technologies in agriculture for the achievement of the sustainability goals and for not integrating published scientific data (i.e. from the SAM report[3]) in the final ruling. DG SANTE encouraged the participants in voicing their difficulties and concerns not only to the Commission, but also to their Competent Authorities.

The Chair noted in the last Standing Committee a huge discrepancy between the opinions of the scientific experts and the consequential political voting. He urged the participants in raising the implementation issues of the new CJEU ruling to their Competent Authorities. The JRC Director supported the request.

- **Discussion on consequences of the ruling C528/16ECJ for GMO detection**

The Chair reminded that the EURL GMFF had previously circulated a draft explanatory note addressing future possibilities and limitations for the detection and identification of genome-edited products and requested ENGL members to express their opinions.

Some participants considered the document and especially the executive summary as too optimistic and suggested a modification of the latter. Other comments regarded the need to provide a clear definition of "event" and event-specificity, the possibility of distinguishing natural mutations from those introduced by conventional or new mutagenesis techniques and finally the real feasibility and cost-effectiveness of the pan-genome database approach mentioned in the note. Quite a few members stressed that with the new CJEU ruling the EU legislation on GMOs is no longer enforceable and that monitoring approaches for genome edited products would be too costly. It was further remarked that traceability and labelling of the GM products is required in the EU legal framework and that quantification of single point mutations introduced by new mutagenesis techniques is not feasible. Difficulties in the full characterisation of varieties' populations were underlined. It was finally remarked that the section on the pan-genome database approach should be rewritten to avoid interpreting it as a feasible solution.

- **EFSA sequencing guidance (JRC)**

Following a DG SANTE request, the JRC published in 2016 a guideline for the submission of GMO DNA sequences and associated annotations under Directive 2001/18/EC and Regulation (EC) No 1829/2003. Sequencing information included in the dossier for GMO authorisation had to be submitted to the JRC and is verified for its compliance to the established quality requirements. Given the EFSA needs for risk assessments and the advancement in sequencing technology, DG SANTE has recently requested EFSA to

[3] New techniques in Agricultural Biotechnology
(see https://ec.europa.eu/research/sam/index.cfm?pg=agribiotechnology,
outcome available at
https://ec.europa.eu/research/sam/pdf/topics/explanatory_note_new_techniques_agricultural_biotechnology.pdf#view=fit&pagemode=none)

include in one guidance document the requirements for the characterisation of the transgenic insert(s) and flanking sequences, the insertion site analysis and verification of the genetic stability of the GM event. An EFSA working group with external experts and two JRC contributors has addressed this request over the past twelve months. A technical note on the quality of DNA sequencing for the molecular characterisation of GM plants has been published on the EFSA website in July 2018 (https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5345). Starting from the 1st of October 2018, EFSA will perform the compliance check of the information on sequencing newly submitted by the applicants, while the JRC will continue the task on checking the information on sequencing for the applications already submitted. The EURL-GMFF will be informed on any new sequence corrections or editing provided in the meantime by the applicants. The Technical Note ensures a high level of harmonisation with respect to the JRC Guideline.

- **Report of the workshop on GMO analysis in Singapore (June 2018) (JRC)**

The JRC provided an update on a workshop organised in Singapore in June 2018 in cooperation with ASEAN members, an association of 10 countries in Southeast Asia cooperating on harmonisation of legislation and scientific approaches. At the workshop representatives of the EURL GMFF and of the BVL from Germany provided a three-day course with hands-on activities and theoretical training on PCR quantification. They also participated to the annual meeting of the ASEAN network where they presented the tasks of the EURL GMFF as defined in the new Regulation (EU) 2017/625 for official controls, the EU databases on GMOs and the ENGL working group activities. Other presentations from invited speakers included low level presence (LLP) initiatives at global level for grains trading and harmonisation of risk assessment procedures. The workshop was hosted by the Agri-Food and Veterinary Authority of Singapore.

Discussion on specific topics:

The Secretary welcomed the participants, excused the absence of the Chair and explained the new organisation of the ENGL plenaries. He presented the program of the meeting and encouraged the members in participating actively to the debates. He finally introduced the moderators of the two discussion sections for which a predefined set of questions had already been prepared.

- **Screening strategies (Moderator: F. Debode)**

The moderator presented a summary of the workshop organised on GMO screening in Gembloux (BE) on the 22nd-25th of May 2018 and the results of a survey previously launched by the EURL GMFF on the use of GMO screening methods for official controls on food/feed/seeds in the Member States.

Discussions at the screening workshop highlighted the lack of common screening elements in new GMOs, the use of event-specific tests to complete the coverage and the need for novel screening elements for ensuring complete coverage of all authorised GMOs. The participants also highlighted the lack of harmonisation in screening approaches and of CRMs for low level presence detection. A discussion followed between ENGL participants on a predefined set of questions.

1) Which screening strategy to adopt in the lab?

The participants remarked that the screening strategy depends on the purpose of the testing and the scenario, the type and number of samples to be analysed and the quantity of DNA available. Therefore, they all agreed that a single EU screening approach would not fit all Member State needs.

2) Should the screening be harmonised in EU?
Many members suggested having harmonisation at least for the most common screening methods such as those detecting e.g. the 35S promoter. Few disagreed claiming that laboratories may have increased chances of detecting contamination at EU level when using different assays or that harmonisation may be unnecessary as new GM events present non-common transgenic elements. Few laboratories were already using the JRC PSP and therefore implementing harmonised assays conditions. Some members recognised the importance of a matrix approach in screening and suggested providing information on EU authorisation for the different GM events in the JRC GMO-matrix web interface.

3) Where are the needs and gaps?
4) What should the ENGL do?
Participants did not request a specific action but suggested maintaining workshops or discussion sections for new screening approaches at the ENGL plenaries. The moderator invited providing information on new analytical gaps or method proposals to the WG AGSMV. It was suggested by participants that the EURL should review the GMO methods database by using the GMO matrix tool in order to extract those screening methods (e.g. the P-35S method) which provide the best (highest) coverage. The output of such a search should be published e.g. by a note. The Secretary acknowledged the usefulness of the proposal and a modification suggested for the JRC GMO-Matrix tool and informed evaluating its implementation in collaboration with DG SANTE.

- **Genetically Modified Microorganisms (Moderator: L. Grohmann)**

The speaker informed that recombinant DNA or even living unauthorised GMM strains were detected in feed additive products and that their Competent Authority requested to provide methods for detecting GMMs. He clarified that feed additives are authorised under Regulation (EC) No 1831/2003 and that this legal frame also applies to feed additives produced "with" or by means of a GMM. Feed additives containing GMM are covered also by Regulation (EC) 1829/2003. EFSA has published a revised guidance on the characterisation of microorganisms used as feed additives or as production organisms. The speaker then presented the three questions to be discussed:

1) What could be the analytical strategy of the official control labs for properly testing the above mentioned food/feed products, if requested?
Some members deplored the lack of information especially on imported feed additive products and the resulting difficulty in designing proper screening approaches. A member of the EURL GMFF suggested using PCR assays commonly employed in microbiology for the detection of bacterial ribosomal genes. Further characterisation of the bacterial strains could be achieved by PCR amplification from variable ribosomal regions.

2) What are the difficulties and gaps, possibly already experienced for such analyses? What are the minimal requirements necessary for the labs, if GMM testing is demanded?
Members wondered whether the detection strategy should focus on the detection of a living organism, a host or recombinant DNA and on how it could distinguish a normal bacterial contamination from the one originating from the production strain. Many complained on the confidentiality of the legal dossier regarding the bacterial strain used for the production of the feed additive and the related method of detection. It was also remarked that reference materials are not available for performing the analysis. A strategy whereby each official laboratory develops its own method of detection was regarded as not feasible. The Secretary informed that more stringent acceptance criteria for the method detecting the production strains were being developed in collaboration with DG SANTE. He remarked however, that the method submitted by the applicant will remain confidential and therefore not available to official control laboratories.

3) What is analytically feasible and/or required with respect to obtain test results clearly showing that a food/feed product produced "from" or "with" a GMM is not compliant (e.g. testing for presence of the production strain, marker gene detection, identification of the genetic modification)?

Members were generally interested in finding an analytical feasible solution to the testing for GMM contamination in a food/feed product even though they did not considered an immediate need at the moment. A speaker from Germany informed having recently published an official method providing guidance for control laboratories on the detection of GMMs. The document includes a collection of methods for detecting the different targets relevant for GMM detection, the list will be published soon. Similar initiatives have been proposed in other MSs and could be considered as a basis for a larger project.

The Secretary proposed to include in the agenda of the next SC meeting the possible establishment of a new WG. The SC will evaluate if ENGL members have sufficient expertise for addressing the analytical gaps highlighted in the discussion on GMM detection.

The Secretary welcomed the representatives from regional offices and underlined the importance of maintaining contact and foster collaboration with regional representations and related networks. He announced that a colleague from Azerbaijan could not participate to the meeting and presented the following speakers.

Session: Open Science Day

- **Presentation: Nevena Alexandrova (FAO, Regional Office for Europe and Central Asia)**

FAO provides policy advice and technical assistance on agriculture and biotechnology to different countries and offers a neutral world forum for mutual discussions. FAO is supporting several projects which are small interventions having catalytic effects on investments attractions. The organisation also offers training to increase capacity and expertise and is developing guidance for policy formulation, compatibility of national biosafety systems, particularly legislation on LLP and New Breeding Techniques (NBT). FAO covers 53 member countries with different agricultural sectors in Europe and Central Asia. In particular FAO is supporting in the Eurasian Economic Union (EAEU), including the Russian Federation, Belarus, Armenia, Kazakhstan and Kyrgyzstan, three regional programs 1) Empowering family farms; 2) Improved agri-food trade; and 3) Sustainable natural management under climate change. The challenges faced by those countries cover the lack of policy on agri-biotechnology at national level, minimal capacity, funding and experiences. FAO started a project in Tajiki on capacity building and GMO monitoring. The organisation is also supporting initiatives on harmonisation of GMO legislation in EAEU.

- **Christopher Viljoen, GMO Testing Facility, University of the Free State, Republic of South Africa**

A representative from a GMO laboratory in the Republic of South Africa reviewed GMO detection activities, needs and challenges in the different regions of Africa. He explained that laboratories involved in GMO analyses have developed regional networks quite frequently at informal level, without funds and on the base of personal interests in establishing and sharing information. The networks operating in the different regions of Africa were 1) Southern Africa: SANGL, (since 2010), 2) Western Africa: WAEMU and WANGL, 3) Eastern Africa: EAGL and 4) Central Africa: CAN-DETECT. Countries in Africa import GMOs and perform GM detection routine at different levels. GMO analyses cover mainly ELISA tests but also conventional or real-time PCR and are performed by existing laboratory infrastructures. Many countries have research activities on plant-genomics and develop their own GMOs.

General challenges in GM detection:
The major challenges described were the possibility of obtaining accreditation in some countries, the lack of specific technological expertise in GMO detection, the difficulty in participating to training workshops (distances are challenging) and proficiency tests (PTs). Supply of reagents at a reasonable cost and lack of coordination between regulatory authorities and GMO detection laboratories in many countries were also underlined. Finally the need for financial support for regional activities in GMO detection and the lack of CRMs for indigenous crops were highlighted.

Training needs
In general the different African networks expressed the need for training on GMO detection, on using specialised equipment, on method verification and quality management.

Needs and gaps in networking
Due to frequent electrical power failures and different spoken languages. communication between networks was considered to be challenging.
An ENGL member suggested sharing information on new genetic constructs and market developments in African countries since in some regions companies developing GMOs do not have the legal requirement of providing the related data and control material. Another participant suggested participating in internal PT schemes to prove that the laboratory is proficient in detecting GMOs, especially if there are no accreditation bodies.

- **Regulatory aspects of genome edited organisms in non-EU countries (L. Grohmann, BVL, DE)**

The speaker presented the regulatory decisions regarding new genome-edited organisms (GE) in other regions of the world. The survey was based on work performed by another German agency (JKI) in 2018 on 14 countries/regions including i.e. Canada, USA, South America, Australia and New Zealand. The regulatory system of the US and Canada were explained which are based on the product-based assessment of genome-edited plants. USDA/APHIS has published a list and decision letters at their website under the "Am I regulated?" process. Currently ten entries can be found if this list is searched for 'CRISPR'. In Canada decisions on GE plants are taken on a case-by-case basis and refer to products with different or new traits as 'plants with novel traits'. The Cibus canola event 5715 was mentioned as example relevant for EU countries. Several countries (Russia, China, Japan, India etc.) have not taken final decisions on how to regulate GE products, although research and developments are highly granted and supported in their countries. A German study noted a huge increase of articles describing genome editing in model plants and identified about 60 new related developments. It was noted by the speaker that based on the foreseen increase of soybean trade between US and the EU there may be a risk that GE soybean enters the EU market soon. The speaker also informed that soon information on relevant genome-edited crops will be introduced in the existing EUginius GMO database.

A participant from Poland commented that in addition to the problem of asynchronous authorisation, EU Member States will also face asymmetric production of GE organisms specifically designed for those internal markets. A participant from Norway informed that a proposal is under evaluation in its country on a product-based assessment with four risk categories.

- **New approaches for and characterisation of genome-edited fruit trees (L. Dalla Costa, Edmund Mach Foundation, San Michele all'Adige, Italy)**

The speaker presented the strategies and challenges for the generation of grapevine and apple varieties by a CRISPR/Cas9 genome editing approach. The process required the selection of a target site in the gene of interest and its sequence confirmation, the design of sgRNA, co-cultivation of the embryogenic callus with *Agrobacterium tumefaciens* carrying

a vector with the desired sequences and a resistance marker gene, followed by embryo germination in selective conditions and plant regeneration. Common limiting factors for applying genome-edited approaches to perennial fruit trees are 1) the long time required for the procedure (8 to 13 months); 2) the low transformation efficiencies of the embryogenic cells; and 3) the chimerical integration of the exogenous DNA (T-DNA) in the plant tissues. The selected regenerated plants were characterised by qualitative PCR for the presence of T-DNA sequences and then subjected to acclimatisation and green house cultivation. The authors tried to remove the exogenous DNA by using an flp enzyme that recognises FRT sequences positioned at the end of the transgenic insert which is inducible with a heat-shock treatment. They were able to remove the cassette in the selected apple lines but not in the grapevine plants. The authors also tried to develop a method for detecting T DNA border sequences remaining in genome edited plants. The speaker also explained that for perennial fruit trees plant regeneration has a very low efficiency when the transfection is performed on protoplasts.

- **Nanopore sequencing technology: a new route for the fast detection of unauthorized GMO Sequences with MinIon (M.A. Fraiture, Sciensano, Belgium)**

The speaker explained that current GMO detection strategies applying a first screening step may be able to cover more than 95% of authorised GMOs with only four genetic markers. Since many genetic elements are present in both authorised and no-authorised GMOs, the detection of a transgenic sequence does not allow discrimination between those two possibilities. The speaker proposed modifying the current workflow by introducing in the strategy a new step of DNA fragment sequencing and by performing a three-step approach covering 1) DNA walking for recovering the DNA fragment of interest; 2) NGS sequencing; 3) Sequence analysis by comparison to sequence database.
In the first step primers specific for the transgenic target previously observed in qPCR screening are combined with degenerated random tagged primers to perform PCR amplification. In the second step the PCR products are sequenced on a NGS platform (Nanopore) compatible with long reads and heterogeneous libraries. The authors evaluated the feasibility of the strategy on a pure unauthorised Bt rice sample (100%) with P35S, Tnos and t35SpCambia primers. By comparison with available published sequence databases they confirmed the presence of the GM event in the sample. This approach could allow high throughput and wide monitoring of the GMOs present on the market. The authors will now test the approach with more difficult and less known GM samples.

- **The EURL for Feed Additives (C. von Holst, JRC)**

The speaker clarified that two types of legal systems apply to feed, one covering "feed materials" not requiring a pre-market authorisation and for which a negative list of forbidden products exists; the other regarding "feed additives" (FA) demanding a pre-market authorisation granted under Regulation (EC) No 1831/2003 and for which a positive list of authorised products is made available in a Register managed by the Commission. As for the GMO food & feed products, a comitology system at EU level is involved in the decision of FA authorisation. Regulation (EC) No 429/2008 specifies the information to be included in the application for authorisation which includes also a method of detection. A European Reference Laboratory for FA (EURL-FA) had been established under Regulation (EC) No 1831/2003. Its responsibilities are further detailed in Regulation (EC) No 378/2005 and include the provision of feed additive samples – how they are supposed to be placed in the market - and of evaluation reports on the method of analysis of the feed additive submitted by the applicants. Different from the GMO control system, analytical methods for FA are very diverse and they are subjected to only single-laboratory validation and verification by a second and independent laboratory. The applicant is responsible for the organisation of the validation and verification experiments. For some FA the laboratories use multi-analyte methods at least for screening, but for enforcing minimum, maximum or

labelled levels they have to use the analytical methods included in the legal act authorising the feed additive.

Amino acids, vitamins and enzymes are mainly produced by biotechnology but are not under the scope of Regulation (EC) No 1829/2003, provided that they do not contain the production strain or traces thereof. Only zootechnical additives or additives consisting, containing or produced from GMOs are obtaining a holder-specific authorisation under this Regulation, i.e. for such products only a specific company (the holder) that is included in the legal authorisation act can place the additive on the market. Feed additives produced by the means of GM microorganisms, such as vitamin B_2, are supposed to be free of any traces from the production strain and are authorised via a non-holder specific authorisation, i.e. they can be placed on the market by any business entity. This is also due to the fact that the target of authorisation is not the strain but the FA-active substance. Since the legal act of the authorisation of these products contains a link to the specific production strain, only companies that have access to this specific strain for the production of the target feed additive can make use of this authorisation. That is why it is a restricted non-holder related authorisation. Many FAs were placed on the market before Regulation (EC) No 1831/2003 came into force and must undergo a re-authorisation exercise to remain on the market. This process is not yet completed. The former legal frame did not foresee any authorisation criteria for vitamin B_2, thus allowing the industry to place vitamin B_2 from any production strain on the market. However, this situation will change – probably next year – when the re-authorisation of all submitted vitamin B_2 products is finalised. Then only vitamin B_2 produced by specific strains will be authorised.

Regulation (EC) No 429/2008 specifying the details to be included in the dossiers by the applicant is currently under revision and should provide in the future more detailed requirements for the submitted methods detecting "traces" from the production strain.

Participants requested clarification on the meaning of the word "traces" and on whether it was regarding a living organism or simply DNA, and in that case on the number of defined genome copies. The speaker clarified that FA containing recombinant DNA (not DNA only) derived from the production strain must be authorised under Regulation (EC) No 1829/2003 and that the word "traces" could mean anything. Questions were also raised on the criteria of purity and the reason for treating differently products generated by microorganisms or produced with plants. The speaker explained that in some cases the limits are established under the Regulation of undesirable contaminants and that the type of processing makes a difference in the evaluation.

- **Identification of single target taxon-specific reference assays for the most commonly genetically transformed crops (S. Jacchia, JRC)**

The study presented was aiming at determining the number of DNA target copies in the crop genome for the taxon-specific assays validated under Regulation (EC) No 1829/2003 and at compiling a list of best candidate taxon-specific methods possibly targeting only a single-copy gene. The GM crops examined were soybean, cotton, maize and oilseed rape. Given the challenges presented by the real-time PCR approach for this purpose, the authors decided to use a ddPCR approach. Moreover, the transferability of the validated methods from real-time PCR to ddPCR and the effect of DNA digestion on the ddPCR method performance were tested. The speaker presented the results of the ddPCR experiments and a summary list of best reference gene assays per crop species. For the maize, cotton and oilseed rape assays, respectively, the *hmg* and *ZmAdh1* (maize), *AdhC* (cotton) and *FatA(A)* (oilseed rape) target genes resulted to be present in a single copy; both soybean EU reference methods resulted to target only one copy of the *Le1* gene in the genome. It has to be taken into account that the detection method targeting the *FatA(A)* gene is not specific for *Brassica napus* and amplifies the same gene from two other canola species *Brassica rapa*

and *Brassica juncea*. Under the experimental parameters tested the real-time PCR methods could be transferred in most cases to the ddPCR platform without further optimisation and that for the studied conditions DNA digestion was not showing any significant effect on the performance of the ddPCR assay. The speaker reminded that the *Adh1* taxon-specific method validated for detection of the GM event GA21 was containing a mismatch in the forward primer and displayed reduced amplification efficiency in some maize varieties. In an EU survey performed in 2015 the method was still being used by 23% of the responding laboratories. She asked whether a ranking system should be assigned to reference assays present in the GMOMETHODS database. All participants regarded the proposal favourably but further remarked that the ranking systems should be based on objective criteria and dynamically updated. They asked to include in the GMOMETHODS database and in the EURL GMFF reports at least the information presented on the copy number of the target genes.
The Secretary commented that methods displaying a lower ranking in the list were still reliable for their performance criteria.

The Secretary asked if the ENGL members present would agree with the publication of their name and e-mail address in the list of participants. None expressed disagreement to the request.

- ***Meeting conclusions and AOB***

The Secretary announced that the action list (DAL, see Annex 2) will be compiled and published on the ENGL web page. He thanked the participants for the lively discussions and closed the meeting.

Annex 1 – Agenda

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14th WORKSHOP OF GMO NATIONAL REFERENCE LABORATORIES
REGULATION (EU) 2017/625 and 29th ENGL MEETING

2-4 October 2018, Ispra, Italy
Room 58C

Agenda

Day 1: 2nd October 2018

Session: ENGL

AP	Time	Topic	Documents
8 9 10 11	14:00	▪ Welcome and approval of the Agenda ▪ Approval Report 28th ENGL Plenary ▪ Dynamic Action List (DAL) of 28th ENGL plenary ▪ Outcome of the 35th ENGL SC meeting (June 2018)	Draft agenda Report DAL ENGL28 Report SC35
12	14:20	*Discussion of Progress reports of ENGL WGs:* ▪ WG Update of Methods ▪ WG Digital PCR ▪ WG ENGL Procedures ▪ WG multiplex PCR methods ▪ WG good practice/quality of DNA sequencing data ▪ WG DNA extraction	Progress reports
	15:30	*Coffee Break*	
13 14 15 16	16:00 16.20 17:05 17.25	▪ Update from SANTE ▪ Discussion on consequences of the ruling C528/16ECJ for GMO detection ▪ EFSA sequencing guidance (C. Savini, JRC) ▪ Report of the workshop on GMO analysis in Singapore (June 2018) (F. Gatto, JRC)	
	17:40	***End of day 1***	

Day 2: 3rd October 2018

Session: ENGL

AP	Time	Topic	Documents
17		*Discussion on specific topics:*	
17.1	9:30	▪ Screening strategies (Moderator: F. Debode)	Scoping doc in ENGLnet
	10:45	*Coffee Break*	
17.2	11:15	*Discussion on specific topics:* ▪ Genetically Modified Microorganisms (Moderator: L. Grohmann)	Scoping doc in ENGLnet
	12:30	*Buffet lunch*	

Session: Open Science Day

AP	Time	Topic	Documents
18	14:00	▪ Challenges in implementing national biosafety frameworks in countries from Eastern Europe and Central Asia: experiences and outcomes from FAO projects (Nevena Alexandrova, FAO, Regional Office for Europe and Central Asia)	
19	14:45	▪ Overview of GMO activities in Azerbaijan and Central Asia (M. Abbasov (Genetic Resources Institute of ANAS, Baku, Azerbaijan)	
	15:30	*Coffee Break*	
20	16:00	▪ Update from South Africa GMO Network (Christopher Viljoen, GMO Testing Facility, University of the Free State, Republic of South Africa)	
21	16:45	▪ Regulatory aspects of genome edited organisms in non-EU countries (L. Grohmann, BVL, DE)	
	17:30	***End of day 2***	
	19:30	***Dinner at Hotel Europa***	

Day 3: 4th October 2018

Session: Open Science Day

22 23	9:00 9:45	▪ New approaches for the development and characterisation of genome-edited fruit trees (L. Dalla Costa, Edmund Mach Foudation, San Michele all'Adige, Italy) ▪ Nanopore sequencing technology: a new route for the fast detection of unauthorized GMO Sequencing with MinIon (M.A. Fraiture, Sciensano, Belgium)	
	10:30	*Coffee Break*	
24 25	11:00 11:45	▪ The operation of the EURL for Feed Additives (C. von Holst, JRC) ▪ Identification of single target taxon-specific reference assays for the most commonly genetically transformed crops" (S. Jacchia, JRC)	
26	12:30	*Meeting conclusions and AOB*	
	12:45	***End of meeting***	

Meeting documents available at:
https://englnet.jrc.ec.europa.eu/29thENGLmeeting_14thNRLworkshop/default.aspx?InstanceID=1

Annex 2 – Action List

29th ENGL PLENARY ACTION LIST 04/10/2018			
ACTIONS	**Resp.**	**Timelines**	**Status**
ENGL MEETINGS			
Make available on ENGLNet report and presentations of 29th ENGL Plenary	SEC	Nov-18	Open
ENGL WORKING GROUPS			
WG dPCR			
Publish the document	SEC	Dec-18	Open
WG Multiplex			
Organise web meeting Drafting Team	SEC	Oct-18	Open
Organise 2nd meeting WG	SEC	Dec-18	Open
WG DNA extraction			
Organise meeting of task force leaders	SEC	Nov-18	Open
WG Sequencing			
Organise next meetings	SEC	Dec-18	Open
WG Procedures			
Send doc to the SC for comments	SEC	Nov-18	Open
WG UpMeth			
Organise next meeting	SEC	Nov-18	Open
finalise the report for the SC comments	SEC	Jan-19	Open
Template data submission	EURL	tbd	Open
AG Method Selection for Validation			
Organise next meeting	SEC	Dec-18	Open
Propose a ranking for taxon specific methods	WG + EURL		Open
OTHERS			
send letter to SANTE on AOCS CRMs issues	EURL	Nov-18	Open
send request to SANTE un summing up GM levels	SEC + EURL	Oct-18	Open
Ask SANTE for info on Russian legilsation on GMO	SEC	Nov-18	Open
Forum on research topics	SEC	Dec-18	Open