EUROPEAN COMMISSION
JOINT RESEARCH CENTRE

Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

25th ENGL PLENARY MEETING

13-14 April 2016, Ispra, Italy

Meeting Report

1. Welcome

The Secretary welcomed the participants on behalf of the President and apologized for his absence. He informed that representatives from Estonia, Latvia, Norway, Malta, Ireland and Slovenia were excused. Finally he welcomed the speakers invited for the scientific and technical sessions.

2. Approval of the Agenda

The Chair asked postponing at 12:00 the update from SANTE organized via video-link and switching the first two scientific presentations in the afternoon section. The agenda (Annex 1) was approved with those amendments.

3. Approval Report 24th ENGL plenary

The report previously circulated for comments was approved without modifications.

4. Outcome of the 30th ENGL SC meeting (February 2016)

The Chair summarized the main outcome of the ENGL Steering Committee meeting, held in February 2016. Participants requested an update on the JRC reorganization and were reassured on the future of the ENGL and the continuation of its activities.

5. Dynamic Action List (DAL) of 24th ENGL plenary

The Secretary reviewed the open points of the list.

6. Update from SANTE

SANTE provided an update on Directive (EU) 2015/412 amending Directive 2001/18/EC that offers the option to Member States of prohibiting/restricting GMO cultivation on their territory. Nineteen Member States have used the new Directive's provisions and have launched the procedure to adopt national opt-out measures to ban/restrict cultivation of already authorized GMOs or have requested to be excluded from the geographical scope of the GMO applications. These requests will be reflected in the related Decisions.

SANTE informed that the Commission is updating Annex II and III of Directive 2001/18/EC and that the process should be completed by April 2017. The Commission is also analyzing the new breeding techniques (NBT) and working on a legal interpretation of the GMO legislation.

The work of the three Scientific Committees on synthetic biology has been finalized and has been published on the European Commission web site at (http://ec.europa.eu/health/scientific_committees/all_opinions/index_en.htm).
The experts considered the new approaches to be covered by GMO legislation, although future applications may challenge this conclusion.

SANTE informed that two peer-review reports on the outcome of an Open-ended Online Forum and an ad hoc technical expert group on synthetic biology established under the Convention on Biological Diversity (CBD) had been published at

http://bch.cbd.int/onlineconferences/#portals (?). The Commission is preparing an orientation document on synthetic biology within the Cartagena Protocol on Biological Diversity.

SANTE reported that sequencing errors were identified in the documentation of GMOs already authorized that were submitted by the applicant within the framework of Directive 2001/18/EC and Regulation (EC) No 1829/2003. The Commission requested the EURL GMFF to update to the latest technological and methodological standards the guidance document on submission of sequence information. The JRC has provided the document in February 2016.

SANTE informed that the report of the ENGL WG on seed testing and sampling was presented to Member States. The document was appreciated and will be further discussed in future regulatory meetings.

SANTE also informed that following notification of non-authorized GM event OXY-235 being present in conventional oilseeds lots, Member States were requested to provide a monitoring plan.

The ENGL was informed that the Commission recently published a report on an audit performed in China to assure compliance with EU legislation on GMOs. Chinese authorities were able to demonstrate that a number of measures were put in place to guarantee an efficient surveillance system. A participant from Germany added the comment that according to the audit report, safety certificates for 88 GMMs have been issued in China, but no GM *Bacillus subtilis* production strain is authorized. It remains therefore unclear how the vitamin B2 from China have been produced.
Concerning the purity of GMM derived products, SANTE added the information that work is undergoing on the subject and that for GMM products authorization the applicant is required to provide a purification protocol. For feed additives the EU legislation requires the provision of methods for detecting the additives, but not the GMM used for its production.

7. Update on rapeseed Oxy-235 (EURL GMFF and SANTE)

The Chair reported that seed lots produced in France and to be used for confined trials were found to be contaminated with Oxy-235 seeds and asked if laboratories were testing for this GM oilseed rape. A member informed that small contaminations were detected in some fields cultivated with oilseed rape by screening with P-35S and T-nos methods. Another participant confirmed similar results although using a different approach. It was remarked that GM oilseed seeds can survive in the fields for more than 10 years. No other action is needed at this stage as the laboratories have been provided with all tools to conduct testing.

8. Update on the Network for Species Identification (JRC)

The Chair informed that a workshop on species identification had been organised by the JRC to foster activities in the field. A clear need for standardization of detection methods and work towards the creation of a network similar to ENGL has emerged from the meeting. It was suggested that a formal request from competent authorities could win internal support to prioritize and sustain actions in this area. Some members remarked that the term species identification is too broad and that the subjects covered should be clarified, but confirmed the need for harmonization and standardization activities in these areas.

9. Update on CEN and ISO activities (Dr. L. Grohmann, BVL, DE)

The CEN TC 275 WG 11 held a preparatory meeting in March in view of the ISO TC 34 SC16 meeting. WG11 members recommended extending the scope to molecular biology methods for plant and animal species identification, in particular for meat, fish, honey and milk. The WG also supported the new approach presented by German experts for validation of qualitative real-time PCR methods (both for single-laboratory validation or collaborative studies) and suggested their submission to ISO.

Regarding ISO, TC 34 SC16 (horizontal methods for biomarker analysis) held the plenary meeting in Shanghai in March. SC16 activity is expanding to cover detection of animal-derived material (meat speciation). In addition, three consolidated ISO draft documents on real-time PCR screening methods for GMO detection (P-FMV; P-nos and P-nos-nptII; cry1Ab/Ac and Pubi-cry) have been circulated for final decision/vote to all committee members. The Chair confirmed the involvement and the support of the JRC and the EURL GMFF for standardization efforts and encouraged ENGL members in getting more involved in CEN and ISO committees.

10. Progress reports ENGL working groups

10.1 AG SMV (Advisory Group on Selection of Methods for Validation)

The collaborative studies for validation of the methods previously selected, T35S-pCambia and event-specific pentaplex, are ongoing. No new method was proposed in 2015.
WG members suggested broadening the possibility to submit methods also to non EU countries, defining first the analytical gaps from the authorization pipeline described in the report of JRC IPTS (Seville) and then requesting submission of related method proposals.

Work is ongoing to define a list of best reference gene method for each crop.

The Chair suggested contacting the Decathlon project to be updated on methods developed and invited participants in providing more support to the activities of the AG, including submission of new method proposals. In was also suggested to consider a dPCR method, therefore the AG is requested to evaluate available dPCR methods for validation in a ring-trial.

10.2 WG on unit of measurement

The WG report is in progress and comments from the members are currently being collected. A final draft providing a table with conversion factors linking the events to the CRMs used is expected by June 2016, for consideration by the Steering Committee.

10.3 WG on digital PCR

The draft WG report was recently revised; the final draft should be ready for consideration by the Steering Committee in June 2016.

10.4 WG on update of methods

The WG has already agreed on actions needed when a method is modified in one or more of its components. The WG has also reviewed different international standards and guidelines to provide a definition of “method” and “protocol”. The WG agreed on considering the primers and probes as core elements of a method, while the protocol/procedure as the detailed description of the method. Future activities will concern the design of a template for providing validation data to the WG, the establishment of contacts with other relevant ENGL WGs and the finalization of the guideline document by May 2016.

11. Scientific and technical session

11.1 Activities of the Custom Laboraratories European Network (CLEN) (Dr M. F. Filippi, Italian Customs Agency-Chemical laboratory of Turin, Italy)

Dr. F. M. Filippi, of the Italian Custom Agency described the organization and the analytical activities carried out by the Custom Laboratories European Network (CLEN). The principal mission of the Custom Laboratories relates to Tax Provisions and Tariff Nomenclature, but covers also other fields (e.g. Common Agricultural Policy, narcotics and psychotropic drugs, product quality and fraud detection). The samples more frequently tested according to 2012 figures were animal and vegetable products and prepared foodstuff (31%), mineral products (27%) chemical or industrial products (19%), textiles (3,2%) and many others in minor percentages on which the laboratories mainly performed microbiological, genotyping and

radioactivity analysis. Twenty-three MS have their laboratories accredited under ISO 17025 and for almost half of them the accreditation covers more than 40 methods. The speaker described the activities of the six CLEN actions covering e.g. laboratory inter-comparison (proficiency testing) and method validation, giving finally examples of activities that could require DNA analysis. It can be concluded, also considering the discussion after the presentation, that custom laboratories still have little experience in DNA analysis and that a more structured collaboration between ENGL and CLEN would be very beneficial for both networks.

11.2 Recent developments under the Cartagena Protocol on Biosafety and the Biosafety-Clearing House (Dr A. Bowers, Dr M. Pessoa de Miranda, Biosafety Clearing House)

Dr A. Bowers provided a demonstration of the functionalities of the Biosafety Clearing House (BCH) database and in particular of the new tool National Report Analyzer. This on-line interactive tool offers statistical and trend analysis by CBD region or country on the responses provided in the second and third national reports. He invited members in supplying information on biosafety experts in the BCH section Roster of Experts.

Dr. M. Miranda provided an introduction on the Cartagena Protocol on Biosafety, an international legally binding instrument setting the minimum requirements for the trans-boundary movement of Living Modified Organisms. Parties to the Protocol requested the establishment, through the BCH, of an electronic network of laboratories involved in LMOs detection and identification. The network agreed to develop a set of technical tools and providing resource information and guidance for sampling, detection and identification of LMOs on the BCH online portal. A new portal on synthetic biology has been established on the BCH website including transcripts of the Open-ended Online Forum and recommendations of the Ad Hoc Technical Expert Group on synthetic biology.

11.3 Single lab validation of qualitative qPCR methods on the basis of POD modelling (Dr L. Grohmann, BVL, DE)

Dr. L. Grohmann presented the guidelines for single-laboratory validation of qualitative real-time PCR methods developed by the German WG §64 LFGB. A new procedure for testing $LOD_{95\%,}$ on the basis of POD modeling was presented. A Web-Tool is available at https://quodata.de/content/validation-qualitative-pcr-methods-single-laboratory to calculate the $LOD_{95\%}$ and perform a plausibility check with the results (positive/negative) obtained from 12 PCR replicate measurements on six dilution levels with defined target copy number. Practical suggestions for performing specificity and robustness tests and relative performance criteria were also provided. The approach was published by Uhlig et al. in Accred. Qual. Assur. (2015). Participants appreciated the information provided.

12. *Break-out Groups*

The following break-out discussion groups were organized:

1) NGS data quality control and regulatory use

2) GM animals

During this session the members of the WG on ENGL Procedures met for the WG kick-off meeting

13. *Scientific and technical session (2)*

13.1 New Breeding Techniques: Opportunities and Regulatory Challenges (Prof. H. Brinch-Pedersen, Aarhus University, Denmark)

Concepts and characteristics of new breeding techniques (NBT), including Cis-/Intra-genesis and genome editing, were introduced. The first two techniques induce in-vitro rearrangements in the genome or generate extra copies of genetic elements derived from plants of the same sexually compatible genetic pool. Genome editing techniques, instead, introduce new genetic variations by generating a DNA double strand break (DSB) followed

by repair with a donor DNA sequence either by homology directed repair (HDR), inducing precise alteration /corrections (knock-in), or by non-homologous end joining (NHEJ) inducing insertion/deletion mutations (knock-out). The DSB is not random but targeted and unique and can be programmed at specific sequence sites. The molecular tools used for genome editing are DNA sequence-specific binding proteins with nuclease activity, in particular 1) Zinc Finger Nucleases (ZFNs) currently phased out, 2) Transcription Activator - Like Effectors Nucleases (TALENs) where the target nuclease recognition specificity needs to be previously designed in the protein sequence and 3) CRISPR/Cas (Clustered Regulatory Interspaced Short Palindromic Repeats/CRISPR- Associated) systems forming a complex with a synthetic single-guide RNA (sgRNA), whose specificity is depending on the oligonucleotide rather than the protein sequence. The CRISPR/Cas system can be modified to induce a single strand break (nicks) to increase specificity. A different approach for genome editing is using oligonucleotide-directed mutagenesis (ODM) with the plant native enzymes and a gene repair oligonucleotide (GRON). The speaker provided different examples of stable transformation and transient expression systems. The discussion focused on the fact that NBTs can make genome variations indistinguishable from natural variations and from variations introduced by conventional breeding and chemical or physical mutagenesis; thus, should these techniques lead to the formation on a GMO, their detection will pose new challenges for the control laboratories. In this sense, the ENGL can contribute by raising awareness on this issue and by sharing ideas and information available.

13.2 CRISPRing plant genomes (Prof. B. Mueller-Roeber, Potsdam Univerisity, Germany)

The CRISPR/Cas9 system can modify the genome at multiple sites and generate heterozygous, homozygous or biallelic (where both alleles are mutated at different loci) progenies. Only sites adjacent to specific sequences can be targeted by the CRISPR/Cas9 system and therefore not all sequences in the genome can be modified. A database of such sequences is available and can be used to favour optimal design of the target site. After the genome editing step the desired mutations are screened by sequencing the related PCR amplicons. Larger genomic fragments up to 1 Mb long can be deleted with this technique but the most frequently used option is the replacement of a gene with its modified version. The discussion focused on the potentialities of the technique and more specifically on the possibility to detect the modifications introduced; the conclusion was that the traceability of such modifications can only be done putting in place costly strategies, most probably based on sequencing.

13.3 Research and development of GM animals in China (Prof. L. Yang, Shanghai Jiao Tong University, Shanghai, CN)

In the last years China has funded research on GM technologies, including GM animals, investing a considerable amount of resources. Since the beginning of the project in 2008 many GM fish, cattle, swine and goat/sheep have been developed; major traits of interest are increase growth rate, disease resistance, food conversion rate, muscle mass, nutritional quality or wool quality. Some of these GM animals have been already approved for field trial but none have been commercially approved yet for human consumption. The GM animals that will be probably approved soon are those for production of pharmaceuticals. Techniques of genome editing are also applied in the development of these GMO. National standards for GM animal detection, one for a GM carp fish and three as endogenous reference genes for cattle, sheep/goat and swine, are available but only in Chinese language.

14. Reports of break-out groups and discussion

1) NGS (next generation sequencing) data quality control and regulatory use

No much experience exists yet on NGS characterization of non-authorized GMOs, though more papers are being recently published. No clear benefits were envisioned at the moment in using the technology for GMO detection but laboratories are employing NGS for analysis in other fields. It was concluded that given the limited experience the times were too premature for creating an ENGL working group on the subject. Participants reminded that

quality criteria and performance parameters for NGS are being discussed within the Decathlon project.

2) GM animals

Members agreed that GM animals are a reality and eventually they will enter the market but that only three laboratories have developed methods for their detection. The major concern is the knowledge of the products in the pipeline and therefore the targets to be used for the screening. The moderator reminded a recent JRC publication on the subject in Trends in Food Science & Technology (2015) (see http://www.sciencedirect.com/science/article/pii/S0924224415001223).

15. AOB

Dr. J. Kreysa announced that starting from July he will be transferred to Brussels as Advisor for Bio-Economy. He thanked all ENGL members for the fruitful collaboration and for the fine work done in the last years.

16. DAL ENGL 25th

The Secretary announced that the following ENGL plenary will be organized in September and that a summary of the actions points following the discussion (Annex 2) will be provided on ENGLnet. He thanked the participants and closed the meeting.

Annex 1 - Agenda

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Institute for Health and Consumer Protection
Molecular Biology and Genomics Unit

25th ENGL PLENARY MEETING

13-14 April 2016, Ispra, Italy

ROOM 11 (Auditorium) – Building 58C

Draft Agenda

Day 1: 13th April 2016

AP	Time	Topic	Documents
1	9:15	▪ Welcome	
2		▪ Approval of the Agenda	Draft Agenda
3		▪ Approval Report 24th ENGL plenary	Report 24th ENGL
4		▪ Outcome of the 30th ENGL SC meeting (February 2016)	Report SC30
5		▪ Dynamic Action List (DAL) of 24th ENGL plenary	DAL ENGL24
6		▪ Update from SANTE	
7		▪ Update on rapeseed Oxy-235 (EURL GMFF and SANTE)	
8		▪ Update on the Network for Species Identification (JRC)	
9		▪ Update on CEN and ISO activities (Dr. L. Grohmann, BVL, DE)	Presentation
	10:45	*Coffee Break*	
10	11:15	*Progress reports ENGL working groups*	
10.1		▪ AG SMV (Advisory Group on Selection of Methods for Validation)	Presentation
10.2		▪ WG on unit of measurement	Presentation
10.3		▪ WG on digital PCR	Presentation
10.4		▪ WG on update of method	Presentation
	12:45	*Buffet lunch*	
11	14.00	*Scientific and technical session (1)*	
11.1		▪ Activities of the Custom Laboratories European Network (CLEN) (Dr. M. F. Filippi, Italian Customs Agency-Chemical laboratory of Turin, Italy)	Presentation
11.2		▪ Recent developments under the Cartagena Protocol on Biosafety and the Biosafety-Clearing House (Dr. A. Bowers, Dr. M. Pessoa de Miranda, Biosafety Clearing House)	Presentations
11.3		▪ Single lab validation of qualitative qPCR methods on the basis of POD modelling (Dr. L. Grohmann, BVL, DE)	Presentation
	16.00	*Coffee Break*	

12 12.1 12.2	16:30	*Break-out Groups* 1) NGS data quality control and regulatory use 2) GM animals *During this session the members of the WG on ENGL Procedures will meet for the WG kick-off meeting*	 Mandate Mandate
	17:45	*End of day 1*	
	19:30	**Social dinner at Restaurant "La Playa"**	

Day 2: 14th April 2016

AP	Time	Topic	Documents
13 13.1 13.2 13.3	09:15	*Scientific and technical session (2)* ▪ New Breeding Techniques: Opportunities and Regulatory Challenges (Prof. H. Brinch-Pedersen, Aarhus University, Denmark) ▪ CRISPRing plant genomes (Prof. B. Mueller-Roeber, Potsdam University, Germany) ▪ Research and development of GM animals in China (Prof. L. Yang, Shanghai Jiao Tong University, Shanghai, CN)	 Presentation Presentation Presentation
	11:00	*Coffee Break*	
14	11:30	*Report of break-out groups and discussion*	
15	12:15	*AOB*	
16	12:30	DAL ENGL 25th	
	12:45	*End of meeting and sandwich lunch*	

Annex 2 – Action list

25th ENGL PLENARY ACTION LIST 14/04/2016				
ACTIONS	**Resp.**	**Timeline**	**Status**	**Comments**
ENGL MEETINGS				
Make available on ENGLNet report and presentations of 25th ENGL Plenary	SEC	Apr-16	Open	
ENGL WORKING GROUPS				
WG Detection Interpretation Reporting				
Publish final report	SEC	Apr-16	Open	
WG UoM				
Final draft to the SC	WG	May-16	Open	
WG dPCR				
Organise 4th meeting	SEC	May-16	Open	Set for 7 June, will be done only if necessary
WG UpMeth				
Organise 4th meeting	SEC	May-16	Open	Date to be decided in consultation with WG members
Final draft to the SC	WG	May-16	Open	
AG Method Selection for Validation				
Contact Decathlon to get info on new methods	SEC	Apr-16	Open	
Consider/seek the submission of a dPCR method	SEC + AG	Apr-16	Open	
Finalise the list of taxon-specific modules	AG	May-16	Open	
Review existing methods for endogenous genes for animals	AG	Jun-16	Open	To be started after finalisation of the task above
OTHERS				
Consider a letter from the EURL to ENGL members on the establishment of a network on "species identification"	SEC	Jul-16	Open	porposed during the meeting
Provide table on DNA extraction methods	SEC	May-16	Open	Prepare webform
Send SOP for PCR inhibition	SEC	Apr-16	Open	Proposed by BOG on DNA extraction
Position document identyfying issues with 619 and suggesting possible solutions, including approach for MU	EURL/ENGL	May-16	Open	electronic forum? WG?